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Insect Parasitoids: Biology and Ecology

Edited by Eline B. Hågvar & Trond Hofsvang

Agricultural University of Norway – Advisory Service, Ås, Norway

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Norway, 24-28 May 1994

Edited by Eline B. Hågvar & Trond Hofsvang

Agricultural University of Norway – Advisory Service, Ås, Norway

Preface

The 5th European Workshop on Insect Parasitoids was arranged at Honne Conference Center, Biri, Norway 24-28 May 1994. These rather informal meetings have previously been arranged in Leiden (1981), London (1985), Lyon (1987) and Perugia (1991).

At Honne, about 90 participants joined the meeting, and most of them gave a lecture or a poster. All the talks and posters are published in this supplement edition of Norwegian Journal of Agricultural Sciences. The talks are arranged according to the topics, whereas the posters are arranged alphabetically according to author.

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Transgenic arthropod natural enemies for pest management programs

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Genetic manipulation of beneficial arthropods requires methods for efficient and stable transformation, and knowledge of appropriate promoters and other regulatory elements to obtain effective expression of the inserted gene in both space and time. Few genes are cloned and of potential value for pest management programs at this time. Eventually, we will have genes that code for high or low tolerances to temperature and relative humidity, or alter sex ratios or developmental rates and fecundities. Before transgenic arthropods can be developed with these complex traits, we must understand the underlying mechanisms and identify the critical genes involved. One factor hindering the genetic manipulation of beneficial arthropods is the lack of a «universal» transformation system that will provide a rapid and general method for introducing exogenous DNA into species for which little genetic information is available. Maternal microinjection may provide one mechanism to deliver DNA to diverse arthropod species.

Key words: biological control, genetic improvement, maternal microinjection, parasitoids, transgenic

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Advances in molecular genetics provide opportunities for employing genes from a wide array of species to modify beneficial arthropod species for use in pest management programs (Beckendorf & Hoy 1985). The goal is to *enhance* the natural enemy so that improved biological pest control is achieved. Competitiveness and ability to function effectively under field conditions usually are required. A significant constraint on genetic manipulation of most beneficial arthropods is the anticipated difficulty of maintaining quality in mass-reared populations. One of the significant benefits of recombinant DNA techniques may be that it will be easier to maintain «quality» in transgenic arthropods.

Arthropod natural enemies have been modified by artificial selection. Hybridization of different strains to achieve heterosis or the use of mutagens to obtain a specific trait have been proposed, but these approaches have been employed only rarely (Hoy 1990a). Recombinant DNA techniques could make genetic improvement of arthropod natural enemies more efficient and less expensive because, once a useful gene has been cloned, it could be inserted into a number of beneficial species in a relatively short period of time. Recombinant DNA methods broaden the number and type of genes available for use, because a gene from a prokaryote or other organism can be used if provided with an appropriate promoter. Because long selection

programs are unnecessary, there is less likelihood that laboratory adaptation will occur during the manipulation.

Traditional genetic manipulation projects involving artificial selection or mutagenesis by irradiation typically have three phases: conceiving and identifying the problem, developing the genetically-manipulated strain, and evaluating and implementing the new biotype (Hoy 1990a). This paper will describe the state of the art of genetic manipulation of beneficial arthropods by recombinant DNA techniques from the point of view of improving pest management programs and discuss the issues surrounding the assessment of risks of releasing transgenic arthropods into the environment.

WHAT SHOULD BE MANIPULATED?

Genetic improvement of arthropod natural enemies for biological control of pest arthropods by traditional genetic methods has involved selecting for resistance to pesticides, lack of diapause, and increased tolerance to temperature extremes. Developing a flightless strain of the parasite *Microplitis croceipes* has been proposed for releases against cotton bollworms, *Helicoverpa zea*, because the wasps would be unable to leave the cotton fields where they are released (USDA 1993). Most genetic manipulation projects have involved selection of predatory mites (Acari: Phytoseiidae) for resistance to pesticides (Hoy 1990b). Pesticide-resistant predatory mites have been evaluated in the field and are being implemented in integrated pest management (IPM) programs in apples, pears, almonds, grapes, greenhouses, and strawberries (Hoy 1990b). Genetic manipulation has proven to be practical and cost effective when the trait(s) limiting efficacy can be identified, the improved strain retains its fitness, and methods for implementation have been developed (Headley & Hoy 1987).

Traits primarily determined by single major genes, such as pesticide resistance, are most appropriate for manipulation at this time because methods for manipulating and stabilizing traits that are determined by complex genetic mechanisms are not yet available. Genetic improvement can be useful when the natural enemy is known to be a potentially effective biological control agent except for a limiting factor, the limiting trait primarily is influenced by a single major gene, the gene can be obtained by selection, mutagenesis or by cloning, the manipulated strain is fit and effective, and the released strain can be maintained by some form of reproductive isolation.

The outcome of releasing genetically-modified natural enemies is determined by the goals of the pest management program. The genetically-manipulated natural enemies can be released in large numbers in order to have a significant impact on the pest population over a short interval, perhaps only one growing season (augmentative releases). Alternatively, smaller numbers of genetically-manipulated natural enemies can be released with the goal of permanently establishing them in the environment so that their effect can be maintained over several growing seasons in orchards and vineyards. The outcome of the releases will be different depending on whether there are «native» populations of the genetically-manipulated natural enemy present or not.

To date, most releases have involved pesticide-resistant natural enemy populations. If susceptible native populations are present, pesticides are applied to reduce these populations, and the resistant populations could become established in

two different ways; 1) the resistant strain could completely replace the susceptible native population, especially if few or no natives remained after pesticide applications (the replacement model). 2) Alternatively, the resistant and susceptible populations could interbreed and the resistance gene could be selected for, with the resultant hybrid population becoming resistant (the introgression model). If no susceptible population is present, as is the case when genetically-manipulated strains are released into greenhouses or in new geographic regions, the population can be maintained with the desired trait intact (Hoy 1990a, 1993).

STEPS IN GENETIC MANIPULATION BY RECOMBINANT DNA METHODS

Genetic improvement by recombinant DNA techniques involves several steps (Figure 1). A successful outcome generally requires that we have a thorough knowledge of the biology, ecology and behavior of the target species. Identifying one or more specific traits that, if altered, potentially would achieve the goals of the project is a critically important first step. Next, suitable genes must be identified and cloned. Appropriate regulatory sequences must be identified so that the inserted gene will be expressed at appropriate levels in the correct tissues and at a relevant time.

Stable transformation involves incorporating the genetic information into the germ line so that the information is transmitted to succeeding generations. Usually several transformed lines are developed and evaluated to determine which lines are most fit and stable. It is likely that transgenic lines will next have to be evaluated in a contained greenhouse before they can be released into the environment. If the laboratory and greenhouse tests indicate that the transgenic strain is relatively fit and the trait is stable and appropriately expressed, the transgenic strain(s) should be evaluated in small field plots to confirm their efficacy and fitness.

Before any field tests can occur, however, permission to release the transgenic strain should be obtained (Hoy 1990a, 1992a, b). At least initially, it will be easier to obtain permission from regulatory agencies to release transgenic arthropod natural enemies than to release transgenic *pest* species.

Research is essential in all aspects in this sequence (Figure 1). First, we need to identify and clone useful genes. We need to ensure that the inserted genes will be expressed in specific tissues and at the appropriate time. Identifying appropriate regulatory sequences is nearly as important as identifying functional coding sequences. Effective germ line transformation methods are needed so that stable transformation of organisms can be achieved in an efficient and predictable manner.

Inserting cloned DNA into pest or beneficial arthropods could be accomplished by several different techniques (Table 1). The effects of the inserted DNA could be transient and short term, or stable and long term. If the inserted DNA is incorporated into the chromosomes in the cells that give rise to the germ line, the foreign genetic material should be transmitted faithfully and indefinitely to successive generations. Cloned DNA can be isolated from the same or other species, and it is technically feasible to insert genes from microorganisms into arthropods and have the DNA transcribed and translated. However, DNA coding sequences isolated from microorganisms must be attached to promoters (controlling elements) and other regulatory DNA sequences derived from a higher organism so that the gene will be expressed in arthropods. These regulatory sequences determine when a gene will be

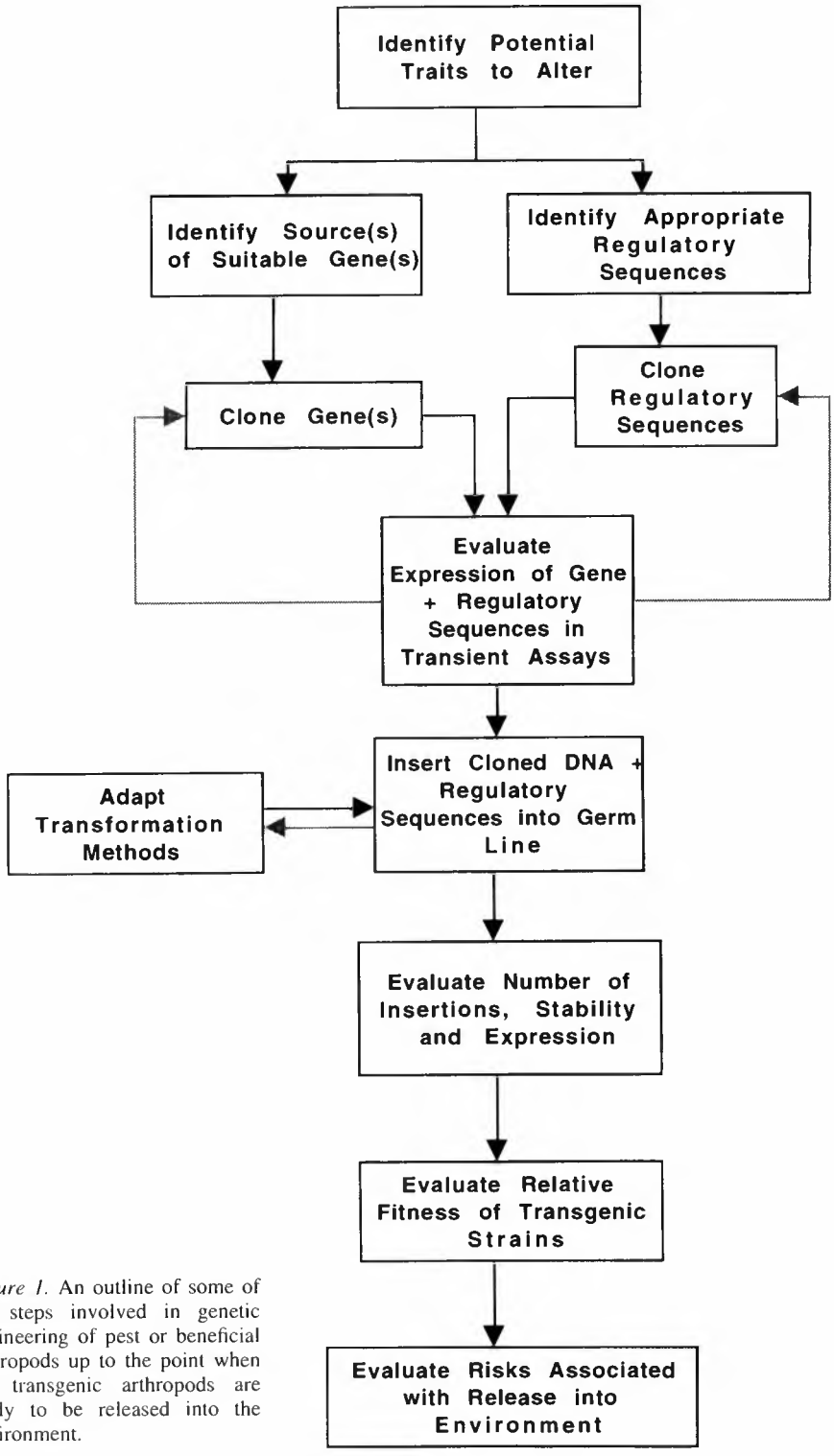

Figure 1. An outline of some of the steps involved in genetic engineering of pest or beneficial arthropods up to the point when the transgenic arthropods are ready to be released into the environment.

transcribed, at what level, in what tissues, and how long the messenger RNA can be used for translation. The following discussion reviews the status of these components of genetic manipulation.

Table 1. Some potential methods for stably transforming arthropods.

Technique	Example(s) Available	Reference(s)
Artificial chromosomes	None in arthropods; feasible with yeast & mice	Schedl et al. 1992
Baculovirus vectors *Autographa californica* *Bombyx mori*	Primarily for protein expression in larvae or cell cultures; lethal to infected host unless additional genetic modifications are conducted	Iatrou & Meidinger 1990, Miller 1988
DNA delivered by microprojectiles	Transient expression only in *Drosophila* embryos	Baldarelli & Lengyel 1990
Electroporation	Transient transformation of *Drosophila* only	Kamdar et al. 1992
Maternal microinjection	*Metaseiulus occidentalis* (Acari: Phytoseiidae)	Presnail & Hoy 1992
Microinjection of eggs	Three mosquito species; P element apparently not functional	Miller et al. 1987 McGrane et al. 1988 Morris et al. 1989
P element vectors	*Drosophila* species only	Handler & O'Brochta 1991
Soaking dechorionated eggs in DNA solution	*Drosophila*	Walker 1989
Sperm as vectors of DNA	*Lucilia cuprina* *Apis mellifera*	Atkinson et al. 1991 Milne et al. 1989
Transfection of cultured cells	*Aedes albopictus*	Fallon 1991
Transformation of insect symbionts	Bacterial symbiont of *Rhodnius prolixus* engineered; symbionts inserted into symbiont-free insects were transmitted to successive generations and *Rhodnius* survived antibiotic treatment.	Beard et al. 1992

Transplant nuclei and cells	*Drosophila*	Zalokar 1981
Transposable element (TE) vectors from target species	None at this time, but TEs are known from several, including *Anopheles gambiae* & *Bombyx mori*	Michaille et al. 1990 Besansky 1990
Yeast recombinase (FLP)- mediated recombination on specific target DNA sequences (FRT)	*Aedes aegypti*	Morris et al. 1991

POTENTIAL GERM LINE TRANSFORMATION METHODS

Most research on stable (germ line) transformation methods has been accomplished with *Drosophila melanogaster* (Table 1). Initial efforts to genetically engineer *D. melanogaster* were rarely successful until Spradling and Rubin discovered that a transposable element called the P element could be genetically manipulated to serve as a vector to carry exogenous genes into the chromosomes of germ line cells (Rubin & Spradling 1982, Spradling & Rubin 1982). The DNA carried by the P-element vector became stably integrated into the chromosomes of the fly and was expressed. This pioneering work has elicited immense amounts of research on fundamental analyses of gene structure, function, and regulation in *Drosophila* and has given us a broad understanding of how flies develop (Lawrence 1992). Many genes have been identified, isolated, and cloned from *Drosophila*. Presently, only a few of these genes appear to be potentially useful in genetic manipulations of either pest or beneficial arthropods.

P element vectors

The P element was genetically engineered to serve as a vector for inserting exogenous DNA into *D. melanogaster*. Since the pioneering research of Rubin & Spradling (1982), P-element vectors have been investigated as possible carriers of exogenous DNA into the chromosomes of other arthropods (Handler et al. 1993). P-element vectors have been used effectively with other *Drosophila* species such as *D. similans* and *D. hawaiiensis*. Other insects, including three mosquitoes and the Mediterranean fruit fly, *Ceratitis capitata,* have received microinjected DNA cloned into P-element vectors. *Aedes aegypti* (Morris et al. 1989), *Anopheles gambiae* (Miller et al. 1987), and *Aedes triseriatus* (McGrane et al. 1988) have been transformed in a stable manner. Unfortunately, the rate of transformation was very low (less than 0.1% of the microinjected embryos) and there is no evidence that the process of transformation was P-element mediated. It appears that P element-mediated transposition may be limited to *Drosophila* species (Handler & O'Brochta 1991, Handler et al. 1993). There is no firm evidence that integration of any exogenous DNA in an insect outside the genus *Drosophila* has been P-element mediated. As a result, a variety of other methods for achieving transformation have been considered and evaluated (Table 1).

Other transposable element vectors

P elements are only one family of transposable elements that are found in *Drosophila melanogaster* and other arthropods. Transposable elements are commonly found in all organisms (including bacteria, yeast, plants, nematodes, mice, humans, *Bombyx mori*, mosquitoes) whenever they have been sought, but they have been less well studied in other arthropods (Berg & Howe 1989). It is possible that species-specific transposable elements, such as *mariner* and *hobo*, could be isolated and genetically modified for use as vectors in specific insects. The *mariner* element is found in a wide variety of insects (Robertson 1993a, Atkinson et al. 1993), but it is unclear whether an active element can be engineered for use as a vector. However, engineering vectors is not a rapid nor inexpensive process and this approach may be limited to those arthropod species that are of major economic importance. Furthermore, because transgenic arthropods being released into the environment should be stably transformed, such transposable element vectors must be incapable of additional subsequent movement after the first, targeted transformation. Thus, issues of risk assessment should be considered in designing a genetic manipulation project involving «native» transposable element vectors (Hoy 1992a, b).

Microinjection

Microinjecting exogenous DNA carried in Pelement vectors into *Drosophila* eggs is a well developed technique (Santamaria 1986). These microinjection methods had to be modified for mosquito eggs, with slightly different injection methods required for different genera (McGrane et al. 1988, Miller et al. 1987, Morris et al. 1989). Milne et al. (1988) developed a method for microinjecting early honey bee, *Apis mellifera*, embryos. Presnail & Hoy (1992) found that eggs of the phytoseiid predator *Metaseiulus occidentalis* were extremely difficult to dechorionate and dehydrate and that the needle tip had to be modified for this species. Based on results to date, it appears that the *Drosophila* microinjection methodology will have to be adapted empirically to each insect species and will not be feasible with all. Variables to consider include whether to dechorionate or not, whether to dehydrate and for how long or under what conditions, what age/stage to inject, what holding conditions to implement after injection, and what size and shape of needle to use.

It may be feasible to microinject exogenous DNA into insect embryos without using any transposable element vector. It is known that transformation of a number of organisms, including *Drosophila*, can be achieved by such a method, although at a relatively low rate (Walker 1989).

Maternal microinjection

Another potential target for DNA-mediated transformation are the developing eggs of gravid female insects or mites. For example, early preblastoderm eggs present within adult females of the predatory mite *Metaseiulus occidentalis* were microinjected by inserting a needle through the cuticle of gravid females. This technique, called «maternal microinjection», resulted in relatively high levels of survival and stable transformation without the aid of a transposase-producing helper plasmid (Presnail & Hoy 1992). In this example, the bacterial *lacZ* reporter gene regulated by the *Drosophila hsp70* promoter was expressed in larvae developing from the injected eggs and in subsequent generations of mites arising from the transformants. Stable transformation was confirmed in the sixth generation by polymerase chain reaction

(PCR) amplification of a region spanning the *Drosophila/E. coli* DNA sequences inserted into the mite. Microinjection of another colony (COS) of *M. occidentalis* resulted in four transformed lines and stable transformation was confirmed after about sixteen generations both by PCR and by Southern blot analysis of genomic DNA (Presnail, Jeyaprakash & Hoy, unpubl.).

Maternal microinjection of *M. occidentalis* is less laborious than microinjection of eggs that have been laid by females, because the eggs do not need to be dechorionated or dehydrated prior to the injection. Survival rates of injected females were comparable to survival of microinjected *Drosophila* eggs. The transformation rate was approximately 1/10th the efficiency of P element-mediated transformation of *Drosophila*, but comparable to techniques employed for species in which transformation is achieved without a P-element vector (Presnail & Hoy 1992).

Maternal microinjection may provide a DNA delivery system for other arthropod species. Presnail & Hoy (unpubl.) found that DNA could be injected into multiple eggs of *Metaseiulus occidentalis* and a different predatory mite species, *Amblyseius finlandicus*, as well as the parasitic wasp *Cardiochiles diaphaniae*. It should be possible to adapt needle diameter and tip structure so that it can be inserted into the region of the ovary(ies) of many arthropods. Injection may be facilitated by inserting the needle into membranous regions between sclerotized segments after preliminary dissections have been made to determine the locations of the ovaries (Presnail & Hoy 1992).

Soaking dechorionated eggs in DNA may result in transformation

Before the development of P-element vectors, exogenous DNA was introduced into *Drosophila* embryos by soaking them in DNA solutions after dechorionation (reviewed by Walker 1989). Some of the adult flies produced exhibited somatic mosaicism, which appeared to be due to incorporation and expression of exogenous DNA in somatic cells. However, the method has not been pursued because of low uptake (<2%), variable phenotypes, and the difficulty in establishing stably-transformed lines of flies. Most of these experiments used total genomic DNA, and Walker (1989) speculated that soaking embryos in specific cloned gene sequences rather than total DNA could result in higher rates of stable transformation.

Sperm as vectors of DNA

The use of sperm as carriers of exogenous DNA has been evaluated for honey bees and the Australian sheep blowfly *Lucilia cuprina* (Atkinson et al. 1991). If sperm are used to transfer genes, the exogenous DNA should bind to the sperm so that it be carried into the nucleus of the egg. This approach to gene transfer would probably be limited to species such as the honey bee, for which semen can be collected and used for artificial insemination.

DNA delivered by microprojectiles

A novel method for delivering DNA into living plant cells involves coating microprojectiles with DNA or RNA, then shooting them into a plant cell with a gun. The so-called «gene gun» has been used successfully to transform major crop plants, yeast, and cultured cells. Its use with arthropod eggs is, however, limited.

Baldarelli & Lengyel (1990) obtained transient expression of DNA in *Drosophila* embryos after ballistic introduction with DNA-carrying tungsten particles.

The embryos were incubated overnight and tested the next day. The authors suggest this method may, with some modification, be suitable for stable germ line transformation. This technique would be especially useful with arthropods whose eggs may be difficult to dechorionate. Also, this technique may be particularly useful for species that deposit large numbers of eggs. It would not be advantageous for species, such as parasitic Hymenoptera, that deposit their eggs into the body of their insect host because obtaining large numbers of eggs by dissection would be extremely tedious. However, if an artificial ovipositional medium were available for the parasitic wasps, it might be possible to obtain reasonable numbers of eggs at the appropriate developmental stage.

Electroporation

Introducing DNA into insect embryos using an electric field pulse allows many more embryos to be transformed at one time than does microinjection into individual dechorionated eggs. With short electric impulses above a certain strength, membranes are made more permeable for a short interval. This allows material to cross the perturbed membrane. Non-dechorionated *Drosophila* embryos, incubated in a solution of DNA, can take up and transiently express this DNA (Kamdar et al. 1992). Efforts to use electroporation for germ line transformation of insects has been investigated, but stable transformation has not yet been reported.

Engineered chromosomes

Engineered artificial chromosomes have been constructed in yeast which behave much like the natural ones do. Apparently, the essential components needed for chromosomes include genes, centromeres, autonomously-replicating sequences that serve as origins of chromosome replication, and telomeres. In yeast, artificially-constructed chromosomes that were only 10 to 15 kb long did not behave like natural chromosomes (full sized yeast chromosomes are about 150 kb long), but artificial chromosomes about 50 kb long were more stably inherited. The larger artificial chromosomes passed through meiosis and were present in a few copies per cell. Although the longer chromosomes performed better, they were lost about twice as often as normal chromosomes, perhaps because the spacing between different DNA segments is incorrect or the chromosomes still are not long enough.

It may be possible to use yeast artificial chromosomes to transform arthropods. Transgenic mice have been obtained by injecting a yeast artificial chromosome (YAC) into fertilized mouse oocytes (Schedl et al. 1992). These mice carried the YAC DNA and expressed the YAC-encoded tyrosinase gene, which caused the albino mice to become pigmented. The YAC integrated into the mouse genome and the presence of yeast telomeric sequences apparently did not reduce the efficiency of integration.

These results suggest that it may be possible to construct artificial chromosomes for arthropods that can confer useful traits in a stable manner. Such artificial chromosomes may be particularly useful for situations where it is desirable to insert a number of genes that are linked.

Transplanting nuclei and cells

Zalokar (1981) reported methods for injecting and transplanting nuclei and pole cells into eggs of *Drosophila*. Thus, it might be possible to genetically transform insect

cells in cell culture, isolate the nuclei, and transplant them into the region where the germ line cells (pole cells) will develop in embryos.

Transformation of an insect symbiont

Richards (1993) suggests that the insects that transmit the infectious agents of malaria, trypanosomiasis, filariasis, dengue fever and viral encephalitis to humans, as well as the vectors that transmit hundreds of viral, fungal, and bacterial diseases to plants could be managed by genetically altering the symbionts of the insect vectors. Many symbionts supply nutrients that are essential for their hosts and are carried as inclusions within their host's cells. Many bacterial symbionts resemble *Escherichia coli*, which is relatively easy to genetically engineer. After the symbiont has been transformed, it could be inserted back into a symbiont-free arthropod host, which could be reared and released. The symbionts would be vehicles for expressing foreign genes that would reduce or enhance the fitness of their arthropod hosts or reduce their ability to vector a disease (Richards 1993).

Beard et al. (1992, 1993) demonstrated that genetic engineering of insect symbionts is feasible by transforming a bacterial symbiont, *Rhodococcus rhodniia*, of the Chagas disease vector *Rhodnius prolixus*. The symbiont lives extracellularly in the insect gut lumen and is transmitted from adult to progeny by egg shell contamination or by contamination of food with infected feces. The symbiont was genetically engineered to be resistant to an antibiotic and the resistant symbionts were transmitted to insects lacking any symbionts. The insects containing the resistant symbionts subsequently were treated with the antibiotic and survived, transmitting the transformed symbiont to successive generations of insects.

Much research must be conducted to learn how to isolate and transform these symbiont species. Likewise, extensive research is needed on how to obtain effective expression and export of proteins, as well as identification of appropriate genes for transformation. A method for deploying the transgenic symbionts or transgenic symbiont-containing insects must be developed.

Baculovirus vectors

Nuclear polyhedrosis viruses (NPV), or baculoviruses, have double-stranded, circular DNA genomes contained within a rod-shaped protein coat. Baculoviruses infect a number of pest insects and several have been used as biological pesticides, including *Autographa californica* nuclear polyhedrosis virus (AcNPV) and *Lymantria dispar* NPV (LdNPV). These NPV and the *Bombyx mori* nuclear polyhedrosis virus (BmNPV) also have been exploited as vectors to carry exogenous DNA into insect cells (Miller 1988, Iatrou & Meidinger 1990, Yu et al. 1992). However, because insect cells or larvae die from their infection, baculovirus vectors are not suitable for producing stably-transformed insects.

Baculoviruses have been engineered to be superb expression vectors, and it is possible to produce a very wide array of proteins from different species in insect cells or larvae. Genetic manipulation of baculoviruses has been directed toward two major goals: improved commercial production of proteins of biomedical or agricultural importance (Miller 1988), and improved efficacy of baculoviruses as biological pesticides. If baculovirus vectors that are nonlethal to their hosts could be developed, they could also serve as vectors for stable transformation.

Yeast (FLP)-mediated recombination

The ability to introduce cloned and modified DNA into the germ line at a predictable chromosomal site is especially desirable, as it reduces the likelihood of position effects on gene expression. One method for accomplishing this is based on a system found in the yeast *Saccharomyces cerevisiae*. A gene for yeast recombinase, FLP recombinase, was found on a plasmid in *S. cerevisiae*. This plasmid also carries two inverted recombination target sites (FRTs) that are specifically recognized by the FLP recombinase. In yeast, FLP recombinase catalyzes the recombination of DNA between the FRT sites in the plasmid, inverting the sequences between them (intramolecular recombination). FLP will also catalyze intermolecular recombination between homologous sites on two different chromosomes (intermolecular recombination).

The FLP-FRT system has been modified to insert exogenous DNA into a specific site in a *Drosophila* chromosome. The FLP and FRT sequences were cloned from yeast and the FRT sites were inserted into the *Drosophila* chromosome through P element-mediated transformation (Konsolaki et al. 1992, Simpson 1993). When yeast recombinase is added to the transformed lines, exogenous DNA could be inserted into a specific location in the genome, e.g. between the FRT sites.

If the FRT sites can be inserted into other insects, the system may be useful for their site-directed modification. Morris et al. (1991) showed that FLP-mediated, site-specific intermolecular recombination occurred in microinjected embryos of the mosquito *Aedes aegypti*. The results of these experiments did not allow them to determine whether the mosquitoes were stably transformed. However, this technique may be useful for assessing the effect of specific gene constructs on competence to vector disease or development.

The FRT-FLP system could provide a rapid method of inserting different DNA sequences into a specific chromosomal site (where the FRT site is). However, because a stable FRT site must be present in the genome, different lines carrying FRT sites in different chromosomal locations will have to be evaluated to determine which site permits better expression of the foreign genes. Thus, the FLP system may be best suited for those species undergoing intensive and long term genetic analysis and manipulation.

WHAT GENES ARE AVAILABLE?

Genes theoretically can be isolated from either closely- or distantly-related organisms for insertion into arthropods by recombinant methods. It may also be possible to isolate a gene from the species being manipulated, alter it, and reinsert it into the germ line. Assuming that a transformation method is available so that either transient or stable transformation can be achieved, the major issue then becomes whether the exogenous gene is expressed appropriately and effectively. Expression requires an appropriate promoter and other regulatory elements.

Many genes have been cloned from *Drosophila* and other species and inserted into *Drosophila* by P element-mediated transformation. Most of the projects were directed at understanding gene regulation or in developing a selectable marker for identifying transformants. These cloned genes may not be particularly useful for genetic manipulation of beneficial or pest arthropods. Genes used to identify

transformants include microbial genes (neomycin or G418 resistance, chloramphenicol acetyltransferase [CAT] and β-galactosidase) and *Drosophila* eye color genes such as *rosy* or *white*. Additional genes cloned from *Drosophila* could be used directly for transforming pest or beneficial insects, or they could serve as probes for homologous sequences in other insect species. Cloned genes also could be modified by *in vitro* mutation to achieve a desired phenotype.

For the foreseeable future, resistance genes will probably be the most available and useful for transformation of arthropods. Potentially-useful resistance genes have been identified in *Drosophila* (Morton 1993). A number of genes have been cloned from insects and other organisms, including a parathion hydrolase gene *(opd)* from *Pseudomonas*, a cyclodiene resistance gene ($GABA_A$) from *Drosophila*, β-tubulin genes from *Neurospora crassa* and *Septoria nodorum* conferring resistance to benomyl, an acetylcholinesterase gene *(Ace)* from *D. melanogaster* and the mosquito *Anopheles stephensi*, a glutathione transferase gene (GST1) from *Musca domestica*, a cytochrome P450-B1 gene *(CYP6A2)* associated with DDT resistance in *Drosophila*, the knockdown resistance gene associated with resistance to DDT and pyrethroids in *Musca domestica*, and the amplification core and esterase B1 gene isolated from *Culex* mosquitoes that are responsible for organophosphorus insecticide resistance (Table 2).

Table 2. Some cloned resistance genes possibly useful for genetic manipulation of pest and beneficial arthropods

Gene (abbreviation) (Resistance)	Source(s)	Reference(s)
acetylcholinesterase *(Ace)* (pesticide resistances)	*D. melanogaster* *Anopheles stephensi*	Hall & Spierer 1986 Hall & Malcolm 1991 Hoffmann et al. 1992 Fournier et al. 1989, 1992a
ß-tubulin (benomyl resistance)	*Neurospora crassa* *Septoria nodorum*	Orbach et al. 1986 Cooley et al. 1991
catalase (H_2O_2 resistance)	*D. melanogaster*	Orr & Sohal 1992
γ-aminobutyric acid A ($GABA_A$) (dieldrin resistance)	*D. melanogaster*	ffrench-Constant et al. 1991, 1993 a, b ffrench-Constant & Rocheleau 1993
cytochrome P450-B1 (DDT resistance)	*D. melanogaster*	Waters et al. 1992
cytochrome P450	*Musca domestica*	Reyereisen et al. 1989

esterase B1 amplification core (organophosphate resistance)	*Culex* species	Mouches et al.1986, 1990
glutathione S-transferase (DmGST 1-1) (DMGST-2) (DDT resistance)	*D. melanogaster*	Toung et al. 1990, 1993
glutathione S-transferase (MdGST1) (organophosphate resistances)	*Musca domestica*	Wang et al. 1991 Fournier et al. 1992b
knockdown resistance *(kdr)* (DDT & pyrethroids)	*Musca domestica*	Williamson et al. 1993
metallothionein genes *(Mtn)* (copper resistance)	*D. melanogaster*	Theodore et al. 1991
multidrug resistance (Mdr49, Mdr50 & Mdr65) (colchicine resistance)	*D. melanogaster*	Wu et al. 1991 Gerrard et al. 1993
neomycin phosphotransferase *(neo)* (resistance to kanamycin, neomycin, G418)	Transposon Tn5	Beck et al. 1982
parathion hydrolase *(opd)*	*Pseudomonas diminuta*	Serdar et al. 1989 Dumas et al. 1990 Phillips et al. 1990
	Flavobacterium sp.	Mulbry & Karns 1989
(parathion, paraoxon resistance)		

Metallothionein genes have been cloned from *Drosophila* and other organisms that appear to function in homeostasis of copper and cadmium and in their detoxification (Theodore et al. 1991). Perhaps these genes could provide resistance to fungicides containing copper in arthropod natural enemies. In many crops, fungicides may have serious negative impacts on beneficial arthropods such as phytoseiid predators.

A family of genes of potential importance for pest management are the multidrug resistance genes in mammals. These genes, *mdr* or *pgp*, become amplified and overexpressed in multidrug-resistant cell lines, resulting in cross-resistances to a broad spectrum of compounds, including those used in cancer chemotherapy. The

multi-drug resistance genes code for a family of membrane glycoproteins that appear to function as an energy-dependent transport pump. Recently, three members of this multigene family were isolated from *D. melanogaster* and these genes (*Mdr49* and *Mdr65*) could provide resistances to a number of exogenous chemicals (Wu et al. 1991, Gerrard et al. 1993). For example, *D. melanogaster* strains that were made deficient for *Mdr49* were viable and fertile, but had an increased sensitivity to colchicine during development. Whether the insertion of multi-drug resistance genes would provide a useful increase in tolerance to chemicals that arthropods might encounter in the environment remains to be determined.

Preliminary results suggest that microbial genes conferring resistance to pesticides can function in arthropods. The *opd* gene isolated from *Pseudomonas* and conferring resistance to organophosphorus pesticides has been inserted, using a baculovirus expression vector, into cultured fall armyworm *Spodoptera frugiperda* cells and larvae (Dumas et al. 1990). Phillips et al. (1990) also transferred the *opd* gene into *D. melanogaster*. The *opd* gene was put under control of the *Drosophila* heat shock 70 promoter, *hsp70*, and stable active enzyme was produced which accumulated with repeated induction. It is likely that this gene could be used to confer resistance to organophosphorus pesticides in beneficial arthropod species, as well as serve as a selectable marker for detecting transformation of pest species. If the *opd* gene were linked to the sex-determining system of pest species being reared for genetic control programs, and could be induced by a specific environmental stimulus such as heat shock, it is possible that the unwanted females could be eliminated, thereby reducing mass rearing costs. Whether a pest species containing a pesticide resistance gene should be released into the environment is subject to debate, unless they are fully-sterile males.

Increased freeze resistance in frost-susceptible hosts may be increased by gene transfer. Antifreeze protein genes cloned from the wolffish *Anarhichas lupus* and the winter flounder *Pleuronectes americanus* have been expressed in transgenic *Drosophila* (Rancourt et al. 1990, 1992, Peters et al. 1993) using the *hsp70* promoter and yolk polypeptide promoters of *Drosophila*. While additional work is required to obtain flies that are able to tolerate cold temperatures, the results suggest that subtropical or tropical species of arthropod natural enemies could become useful or adapted in a much broader range of climates.

Altering longevity of beneficial arthropods might result in more effective biological control of pests in some environments. Research on mechanisms of aging may provide useful genes for modifying longevity of arthropods. A cloned catalase gene inserted into *D. melanogaster* by P-element mediated transformation provided resistance to hydrogen peroxide, which is implicated in cell damage, although the catalase did not prolong the life span of flies (Orr & Sohal 1992).

As basic research progresses, other traits that might be important or useful to introduce into beneficial insects will become obvious. Shortening developmental rate, enhancing progeny production, altering sex ratio, extending temperature and relative humidity tolerances, and altering host or habitat preferences could enhance biological control (Hoy 1976). However, it is not simple to document that changes in one or more of these attributes would actually improve the performance of a biological control agent.

Most of the methods discussed above involve inserting exogenous DNA into random sites in the arthropod chromosomes. The recent success in achieving targeted

gene conversion in *Drosophila* suggests that it may be possible to use transposable elements as targets for gene conversion in insects and mites (Sentry & Kaiser 1992). Targeted gene conversion could improve the efficiency and specificity of inserting genes in both pest and beneficial arthropods.

THE IMPORTANCE OF APPROPRIATE REGULATORY SIGNALS

Genes consist of coding segments that determine the amino acid sequences in the enzyme or structural proteins produced. However, whether a coding region is transcribed and translated in a specific tissue is determined by a number of regulatory sequences in the DNA, including promoters and enhancers. Some of these regulatory structures are in close proximity to the coding region, while others may be located farther away. The stability of messenger RNA is influenced by a variety of signals in the RNA, including the polyadenylation (polyA) signals at the 3' end of the RNA, which can influence the amount of protein produced. It is crucial to obtain expression of the inserted gene at appropriate times, levels, and tissues. Another factor that may be important in maintaining the inserted DNA in the transgenic line over time is the presence of origins of replication that regulate DNA replication of the chromosomes. If exogenous DNA is inserted into a region of the chromosome far from a site where an origin of replication occurs naturally, the exogenous DNA could be lost over time because it is not replicated.

Regulatory sequences from *Drosophila* can be combined with a protein-coding sequence from a prokaryote such as *E. coli* to form a DNA construct that will function in a eukaryote. However, regulatory sequences from prokaryotes do not function in eukaryotic organisms. Because regulatory sequences may vary from species to species, the source of regulatory sequences chosen for cloning may be as important, or even more important, than the source of the protein-coding sequences (Figure 1). Furthermore, some regulatory sequences allow genes to be expressed only in particular tissues or in response to particular stimuli (such as heat shock), while other genes are expressed in most tissues most of the time. If it is important that the inserted gene function in a tissue- or stimulus-specific manner, it is essential to identify tissue- or stimulus-specific promoters.

Currently, the number of suitable regulatory sequences available for genetic manipulation of arthropods is limited. The heat shock *(hsp70)* promoter from *Drosophila* is commonly used as an inducible promoter. It is the strongest promoter known in *Drosophila* and appears to function in all cells. Heat shock proteins are present in all organisms subjected to high temperatures and, while the number of these proteins varies from organism to organism, all produce a 70-kilodalton sized protein encoded by an *hsp70* gene family member. It is likely that the *Drosophila hsp70* promoter can be used whenever an inducible promoter is required that will function in all cells.

While the *hsp70* promoter is highly conserved, it may perform differently in different arthropod species. For example, the mosquito *Anopheles gambiae* was transformed with a plasmid containing the *hsp70* promoter of *Drosophila* attached to a microbial neomycin resistance gene, which also confers resistance to the antibiotic G418 (Miller et al. 1987). Transgenic mosquitoes expressed the *neo* gene at a low level in adults at 26°C and a heat shock for 15 minutes at 37°C enhanced the level of

expression. Recently, Sakai & Miller (1992) found that survival of transgenic larvae exposed to G418 was increased after heat shock at 41°C, which is higher than the temperature (37°C) typically used to induce genes in *Drosophila*. McInnis et al. (1990) found that three heat shocks produced higher survival rates in Mediterranean fruit flies, *Ceratitis capitata,* transiently transformed with *neo* and treated with geneticin.

Other commonly-used regulatory sequences from *Drosophila* are the actin 5C promoter, the α1-tubulin promoter, the metallothionein *(Mtn)* promoter (Kovach et al. 1992, Angelichio et al. 1991), and the *Bombyx mori* cytoplasmic actin A3 gene, which was expressed transiently in embryos (Coulon-Bublex 1993).

The effects of three poly(A) signals isolated from mammals and arthropods was evaluated to determine their impact on stability of transcribed mRNA. Angelichio et al. (1991) compared the poly(A) signals of the SV40 early region, SV40 late region and the *Drosophila* metallothionein gene. The SV40 late poly(A) constructs yielded protein levels that were three- to five-fold higher than the SV40 early construct. The metallothionein poly(A) and SV40 early constructs produced nearly equivalent levels.

It is often important that genes be expressed in tissue- or cell-specific patterns. Learning how to achieve this type of targeted gene expression in transgenic arthropods might employ a method similar to that used by Brand & Perrimon (1993) to evaluate the impact of a gene on cell fate during development of *Drosophila*. They inserted a gene that encodes the yeast transcriptional activator GAL4 randomly into the *Drosophila* genome to drive GAL4 expression from one of a diverse array of genomic enhancers. This system allows rapid development of strains in which expression of the target gene can be directed to different tissues or cell types.

Chromosome replication in higher eukaryotes is not well understood, but it is known that origins of replication are located at intervals along each chromosome. Origins of replication involved in amplification of chorion genes in *D. melanogaster* were identified by Carminati et al. (1992). During follicle cell differentiation, chorion genes (which code for the egg shell proteins) are amplified by multiple rounds of DNA replication, which results in high levels of protein expression during a very short period of time. The ACE3 chorion «element» has been cloned and shown to be sufficient to regulate amplification of the chorion gene cluster on chromosome III of *D. melanogaster* (Carminati et al. 1992). During genetic manipulation of pest or beneficial species, it may be useful to insert ACE3, or similar, elements along with the exogenous genes to ensure that replication of this region of the chromosome occurs in order to increase the stability of the exogenous DNA in the transgenic strain.

Identification, cloning, or genetic modification of promoters and other regulatory sequences may increase the precision with which desired proteins are transcribed and expressed in transgenic arthropods. Research to understand the structure and function of regulatory sequences for use in transgenic arthropods should have high priority. Project goals will dictate what type of regulatory sequences are most useful. In some cases, a low level, constitutive production of transgenic proteins will be useful, while in other cases high levels of protein production will be required after inducement by a specific cue. Researchers will have to evaluate the trade-offs between high levels of protein production and the subsequent impact on relative fitness of the transgenic arthropod strain based on the specific goals of each program.

IDENTIFYING TRANSFORMED ARTHROPODS

After inserting the desired genes, the next issue is how to detect whether the exogenous gene has in fact been incorporated into the germ line. Because transformation methods are relatively inefficient, a screening method is needed to identify transformed individuals. This process is relatively simple in *Drosophila*, where there is a wealth of genetic information and visible markers can be used to identify transgenic individuals. Most pest or beneficial arthropods lack such extensive genetic information or markers.

Identifying transformed individuals could be achieved by using a pesticide resistance gene, such as the *opd* gene as the selectable marker. However, the release of pesticide-resistant pest arthropods into the environment may create concerns about risk. Another option is to use the neomycin *(neo)* antibiotic resistance gene. This prokaryotic gene has been shown to function in both *Drosophila* and mosquitoes and is less likely to provoke concern about risks of releasing transgenic arthropods into the environment. Another marker is the ß-galactosidase gene *(lacZ)* isolated from *E. coli* and regulated by the *Drosophila hsp70* promoter, which has been expressed in both *Drosophila* and the phytoseiid predator *Metaseiulus occidentalis* (Presnail & Hoy 1992). The ß-galactosidase gene can be detected by an assay that produces a blue color in the transformed immature and adult insects and mites. If an appropriate marker is not available, transformed lines can be identified with the polymerase chain reaction (PCR) and subsequent analysis by Southern blot hybridization, or an immunological procedure.

RISKS ASSOCIATED WITH RELEASES OF TRANSGENIC ARTHROPODS

Risk assessments will be somewhat different for pest and beneficial arthropods. Until recently, most practitioners of biological control asserted that biological control of arthropod pests or weeds by arthropod natural enemies was environmentally safe and risk free if carried out by trained scientists. However, questions about the safety of classical biological control have been raised, particularly where environmentalists are concerned about the preservation of native flora and fauna (Howarth 1991), and the era of accepting classical biological control as environmentally risk free appears to have passed (Ehler 1990, Harris 1985, Hoy 1992a).

Evaluating the risks associated with releasing parasitoids and predators that have been manipulated with recombinant DNA techniques will likely include, as a minimum, the questions or principles outlined in Table 3 (Hoy 1990a, 1992a, b, Tiedje et al. 1989, USDA 1991). Concerns can be summarized as questions about: 1) whether the transgenic population is stable, 2) whether its host or prey range has been altered, and 3) whether its potential to persist in the environment (geographic distribution and climatic tolerances) has been altered. For the foreseeable future, releases will be evaluated by regulatory agencies on a case-by-case basis. Initial permits for releases will be for short term releases in controlled situations so that unexpected outcomes might be mitigated more readily.

Another concern involves questions about how far and how quickly the transgenic arthropod can disperse from the experimental release site. Less is known about dispersal behavior of many arthropod species than might be needed when

Table 3. Some issues to resolve relating to risks of releasing transgenic arthropods into experimental field plots

A. Attributes of the Unmodified Organism

* What is the origin of the transgenic organism (indigenous or nonindigenous) in the accessible environment?
* What is the arthropod's trophic level and host range?
* What other ecological relationships does it have?
* How easy is it to monitor and control?
* How does it survive during periods of environmental stress?
* What is the potential for gene exchange with other populations?
* Is the arthropod involved in basic ecosystem processes?

B. Attributes of the Genetic Alteration

* What is the intent of the genetic alteration?
* What is the nature and function of the genetic alteration?
* How well characterized is the genetic modification?
* How stable is the genetic alteration?

C. Phenotype of Modified Organism Compared to Unmodified Organism

* What is the host/prey range?
* How fit and effective is the transgenic strain?
* What is the expression level of the trait?
* Has the alteration changed the organism's susceptibility to control by natural or artificial means?
* What are the environmental limits to growth or reproduction (habitat, microhabitat)?
* How similar is the transgenic strain being tested to phenotypes previously evaluated in field tests?

D. Attributes of the Accessible Environment

* Describe the accessible environment, whether there are alternate hosts or prey, wild relatives within dispersal capability of the organisms, and the relationship of the site to the potential geographic range of the transgenic arthropod strain.
* Are there endangered/threatened species present that could be affected?
* Are there vectors or agents of indirect dissemination present in the environment?
* Do the test conditions provide a realistic simulation to nature?
* How effective are the monitoring and mitigation plans?

Modified from Tiedje et al. 1989; USDA 1991; and from a discussion held at a conference on «Risks of Releasing Transgenic Arthropod Natural Enemies», held November 13–16, 1993 in Gainesville, Florida.

releasing a transgenic arthropod. For example, Raymond et al. (1991) suggest that there has been a worldwide migration of *Culex pipiens* mosquitoes carrying amplified organophosphorus resistance genes. If migration, rather than independent selection on a conserved gene, is the basis for the widespread amplification of esterase genes in *Culex* mosquitoes, then dispersal of some transgenic arthropods could be more rapid and extensive than anticipated.

Another risk issue involves the possibility that horizontal transfer of genes may occur between one arthropod species and another (Houck et al. 1991, Plasterk 1993). The P element appears to have invaded *D. melanogaster* populations within the last fifty years, perhaps from a species in the *D. willistoni* group. The evidence for this hypothesis comes from the overlap in geographic ranges of *D. melanogaster* and *D. willistoni*, the strong similarity in DNA sequences of P elements from *D. melanogaster* and *D. willistoni,* the DNA sequence similarity among *D. melanogaster* P elements from diverse geographic locations, the absence of P elements from species closely related to *D. melanogaster*, the highly-infectious nature of active P elements when they are experimentally introduced into susceptible *D. melanogaster* populations, and geographic distribution patterns within *D. melanogaster*.

Controversy exists as to whether P elements may have been transferred between *Drosophila* species by the semiparasitic mite *Proctolaelaps regalis* (Houck et al. 1991). Horizontal transfer of P elements from *D. willistoni* to *D. melanogaster* must be a very rare event, requiring that two *Drosophila* females of different species lay their eggs in proximity so that a mite can feed sequentially on one and then on the other (in the correct order). The mite must carry the P element to the recipient egg which must be in a very early stage of embryonic development, the recipient embryo must incorporate a complete copy of the P element into a chromosome before it is degraded by enzymes in the cytoplasm, the recipient embryo must survive the feeding by the mite, and the adult that develops from the embryo must transmit the P element to its progeny. If each event is rare, and the combined probability is multiplicative, then the probability that horizontal gene transfer between different arthropod species will occur must be exceedingly rare.

Interspecific transfer of another transposable element *(mariner)* has been suggested as an explanation of their presence in the drosophilid genera *Drosophila* and *Zaprionus* (Maruyama & Hartl 1991). The *mariner* element occurs in five of eight species in the *D. melanogaster* species group, but is found only in the genus *Zaprionus* outside the *Drosophila* group even though *Zaprionus* is not closely related to *Drosophila*. DNA sequences indicate that the *mariner* elements in the two groups are 97% identical, although, by comparison, the nuclear gene *Adh* is not this close phylogenetically, suggesting that there has been horizontal transfer of the *mariner* element. A *mariner* sequence has been discovered in the genome of the lepidopteran *Hyalophora cecropia* (Lidholm et al. 1991) and Robertson (1993a, 1993b) found that the *mariner* element is present in many insects and several species of mites. Robertson (1993a) found sufficient diversity in *mariner* DNA sequences to classify them into several different subfamilies. The diversity of species containing *mariner* elements suggests that: 1) *mariner* elements have been present in arthropods for a long time, although some lineages have lost these elements, and 2) horizontal transfer of *mariner* elements has occurred. However, these horizontal transfers have occurred relatively infrequently on an evolutionary time scale. Many or most of the *mariners* discovered have become degenerated and inactive over time.

Horizontal transfer of genes may occur when bacterial endosymbionts move from species to species. DNA sequence data suggest that bacterial endosymbionts of mosquitoes, Coleoptera, and *Drosophila* may have been horizontally transferred. An analysis of the 16S rRNA genes specific to prokaryotes from *Culex pipiens, Tribolium confusum, Hypera postica, Aedes albopictus*, two populations of *Drosophila simulans* and *Ephestia cautella* indicated that their symbionts are all closely related (O'Neill et al. 1992). Horizontal transfer of symbionts may be more widespread than indicated above because they are involved in many examples of cytoplasmic incompatibility, in which certain crosses between symbiont-infected individuals lead to death of embryos or distortion of the progeny sex ratio. O'Neill et al. (1992) speculated that cytoplasmic incompatibility is due to infection with a specialized bacterium that infects a wide range of different arthropod hosts, including *Corcyra cephalonica, Sitotroga cerealella, Diabrotica virgifera, Attagenus unicolor, Rhagoletis pomonella, Rhagoletis mendax* and *Anastrepha suspensa*. While these species all carry the symbiont, cytoplasmic incompatibility has not been demonstrated in all of them.

Other potential vectors for horizontal transfer of DNA include the insect viruses. If horizontal transmission of DNA by transposable elements or microorganisms occurs, there is no absolute guarantee that genes inserted into any species are completely stable. Such naturally-occurring horizontal transmission of DNA between species may have provided some of the variability upon which evolution has acted. The extent and nature of this naturally-occurring gene transfer are just being determined (Plasterk 1993). It is unlikely, however, that the presence of a transgene in an organism will increase the very small probability that the transgene will be transferred to another species by horizontal transfer, unless the transgene was inserted using an active transposable element. Even then, the probability of horizontal transfer should be very small.

There are no clear guidelines for evaluating the risks of releasing transgenic arthropods for long term establishment in the environment. Experience indicates that the probability that a «new» organism will become established in a new environment is small (Williamson 1992). Historical examples of biological invasions of pests or of classical biological control agents demonstrate the lack of predictability, the low level of successful establishment, the importance of scale, specificity, and the speed of evolution (Ehler 1990). Transgenic arthropods could pose somewhat increased risks because they will be released in large numbers. Williamson (1992) also speculated that the greater the genetic novelty, the greater the possibility of surprising results, and recommended using molecular markers to begin to understand dispersal and the interactions between species in natural communities.

Discussions of risk probably will include questions about survival, reproduction, and dispersal of transgenic populations and their effects on other species in the community. Questions also will be asked about the inserted DNA, its stability, and its possible effect on other species should the genetic material be transferred (Table 3). In the USA, both state and federal regulatory agencies, including state departments of agriculture and USDA-APHIS, will have to be consulted for permission to release transgenic arthropods. Questions about the impact of the transgenic arthropod on threatened and endangered species will be asked by state and federal agencies, including the US Department of Interior Fish and Wildlife Service.

Hadrys et al. (1992) point out that several molecular genetic techniques now are

available to analyze behavioral ecology and population biology. Thus, DNA fingerprinting and PCR-RFLP techniques can be used to determine taxonomic identity, assess kinship, analyze mixed genome samples, and create specific probes. The RAPD method of PCR is useful in situations in which limited amounts of DNA are available, for species with minimal genetic information, and because it is relatively efficient and inexpensive. The use of molecular techniques in ecological studies promises to provide powerful tools to help assess the risks of releasing transgenic arthropods. These techniques and others, such as population genetic models that incorporate information on dispersal rates and gene frequencies (Caprio et al. 1991), will provide methods for improving our knowledge of the ecology and behavior of both pest and beneficial arthropods in pest management programs, whether they have been genetically manipulated or not.

HOW TO DEPLOY TRANSGENIC ARTHROPOD NATURAL ENEMIES

Figure 1 is incomplete in several ways. A similar flow diagram, developed for natural enemies that were genetically manipulated using traditional genetic techniques, illustrated additional steps in the genetic manipulation project (Hoy 1990a). In that more complete flow diagram, the manipulated strains are evaluated in the laboratory, then in field cages and small field plots, and finally in large scale plots. After evaluation in large plots, implementation on a commercial scale can occur if the manipulated strains perform as expected. Once the genetically-manipulated strain has been employed in a pest management program, a cost : benefit analysis should be conducted (Headley & Hoy 1987).

A critically-important step not shown in Figure 1 is consideration of *how* to employ the genetically-manipulated strain in pest management programs. Ideally, the following questions should be considered when *initiating* the project, because genetic manipulation projects of beneficial or pest arthropods are neither rapid, inexpensive, nor simple.

1) Do you understand the biology, ecology, and behavior of the target species in its natural environment? Do you understand how it disperses, reproduces, and behaves under field conditions? What are its relationships with other organisms in the accessible environment?
2) Can the transgenic strain be mass reared easily and inexpensively?
3) Will the transgenic strain eventually be released into the environment for permanent establishment or will it be released periodically?
4) Will the transgenic strain be released into a geographic region where conspecific populations exist with which it can interbreed?
5) Will the transgenic strain be expected to replace the «native» population in order to achieve the desired effect?
6) Do you know how to ensure that the transgenic strain will replace the established native population, if that is crucial to the success of the project? Is it possible that the native population readily can develop «resistance» to the released strain?
7) Is improved strain likely to be stable?
8) Is the released population likely to become a pest or cause other significant harm in the environment? What could be done to mitigate any possible harm?

Answers to these questions were important to the success of pest management programs involving natural enemies improved by artificial selection. For example, the most successful genetic improvement programs with beneficial arthropods involved predatory mites (Phytoseiidae) selected for resistance to pesticides (Hoy 1990a, b). The programs were successful because a great deal is known about how to rear, release, and monitor phytoseiids. Because the manipulated species are effective biological control agents a key factor, resistance to pesticides, could be identified that would eliminate one of the critical constraints to their effectiveness in specific pest management programs.

Deployment of genetically-manipulated arthropods is complicated if some form of reproductive isolation or drive mechanism cannot be provided. One of the reasons genetically-modified predatory mites have been successfully employed in pest management programs may be because phytoseiids disperse relatively slowly. Releases of pesticide-resistant strains into pesticide-treated greenhouses, orchards, or vineyards has provided sufficient isolation that the genetically-manipulated strains have been able to establish without extensive competition from, or interbreeding with, susceptible «native» populations (Hoy 1991). Likewise, releases of a pesticide-resistant strain of the parasitoid *Aphytis melinus* into Israeli citrus groves did not involve competition or interbreeding with susceptible populations because this species was not present in Israel.

The population genetic issues were different when a pesticide-resistant strain of walnut aphid parasite, *Trioxys pallidus*, was released into pesticide-treated California walnut orchards for control of the walnut aphid. The outcome of these releases was more complicated. Because the resistant strain is expensive to rear in very large numbers, inoculative releases were made and the released parasites were expected to establish (Hoy et al. 1990, Caprio et al. 1991). Because susceptible populations of parasites were abundant in nearby orchards, the released population could interbreed with them and resistance could be lost unless strong selection was maintained.

Predicting whether, and how, genetically-modified arthropod natural enemies will establish is difficult. There are at least two models that could be employed in the establishment of a genetically-modified strain in situations in which a «native» population exists: 1) The released strain displaces the «native» population and replaces it (replacement model). This model assumes relatively little interbreeding occurs between the released and native populations. 2) Alternatively, the released strain interbreeds with the native population and a hybrid population is produced. By appropriate strong selection, often with pesticide applications, the desired trait is selected for and the resultant population contains the desired gene (introgression model).

Until recently, it has been difficult to determine what was happening when genetically-modified arthropod natural enemies became established. The only method for determining whether the released population was present was to conduct bioassays for resistance, which are unable to resolve whether replacement or introgression occurred. However, RAPD-PCR of DNA markers allowed monitoring of establishment and dispersal of pesticide-resistant strains of *Trioxys pallidus* in several California walnut orchards. The results suggest that introgression has occurred in at least two release sites (Edwards & Hoy, unpubl.). Thus, molecular genetic techniques may help resolve the population genetics of released populations, as well as providing new tools for genetic manipulation of pest and beneficial arthropods.

RESEARCH NEEDS

One factor hindering progress is the lack of a «universal» transformation system. Also, we lack an example that demonstrates that recombinant DNA technology can yield an effective beneficial arthropod.

Because the potential risks of releasing transgenic arthropods into the environment have been discussed only in a preliminary manner, it may be appropriate to release a relatively risk-free example first. This might involve the release of a transgenic beneficial arthropod that is carrying either a noncoding segment of exogenous DNA or a marker gene such as β-galactosidase. The transgenic strain should not contain an active transposable element vector (Hoy 1992a).

One early candidate for release might be a transgenic strain of the phytoseiid predator *Metaseiulus occidentalis* with a *lacZ* construct. *M. occidentalis* is an obligatory predator, has a low dispersal rate, and is unlikely to become a pest (Hoy 1992). Ideally, the transgenic strain of *M. occidentalis* could be released into a site where it is unlikely to become permanently established or be able to interbreed with native populations. Such a releases would allow a relatively risk-free evaluation of release, monitoring, and mitigation procedures. Later releases could evaluate strains with more useful characters.

Once risk assessment issues and safety have been demonstrated with such a beneficial species, releases of transgenic pest arthropods might be more readily assessed. Releases in the USA will be evaluated as a two-step process. Initial releases will be experimental and on a small scale. No guidelines are available for evaluating the risks associated with permanent releases of transgenic arthropods into the environment. Permission for long term and large scale releases may require five to ten years of evaluating small scale releases. Thus, risk assessment of transgenic arthropods, as it has with transgenic crops and microorganisms, will add a significant cost in both time and resources to pest management projects. It has taken about ten years for the first transgenic crop to become commercially available and may take as long for transgenic arthropods to be released permanently into the environment.

Significant, exciting, and unpredictable advances are being achieved in molecular biology and genetics. As a result of rapid advances in molecular genetic techniques and knowledge of basic developmental mechanisms, it is very difficult to anticipate the opportunities that might arise over the next few years for genetically manipulating arthropod natural enemies. However, additional research is required if we are to gain an understanding of the attributes other than resistance to pesticides that we might manipulate. Furthermore, getting a transgenic arthropod into a pest management program will be an awesome challenge, requiring risk assessments, detailed knowledge of the population genetics, biology, and behavior of the target species, and coordinated efforts between molecular and population geneticists, ecologists, regulatory agencies, and pest management specialists.

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How can Parasitoids Regulate the Population Densities of their Hosts?

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The ways in which parasitoid insects can regulate the population densities of their hosts are reviewed. In interactions between hosts and specialist parasitoids, the most important stabilising factors are those that reduce parasitoid efficiency at high parasitoid density (interference and pseudo-interference). However, recent work with spatially explicit models reveal other stabilising processes that are not observed in models with complete population mixing. The paper also reviews age-structured models of host-parasitoid interactions in continuous time and asks how the population dynamic study of individual host-parasitoid systems can be extended to host-parasitoid communities.

Keywords: age-structure, hosts, parasitoids, population dynamics, spatial processes

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The fact that herbivores rather seldom defoliate their host plants suggests that some agency is regulating their numbers below their carrying capacity. Prime candidates as regulating agents for many herbivores are parasitoid wasps and flies. The two types of evidence supporting this role are the high mortality that many herbivores suffer from parasitoids, and the many successes of parasitoids in controlling pests in biological control programmes. But exactly how can a parasitoid regulate the numbers of its host? This paper reviews the major explanations for host regulation and asks what are the most promising avenues of future exploration.

THE NULL MODELS

The question of how parasitoids regulate their hosts was first posed in the 1920's and 1930's by entomologists such as A.J. Nicholson and W.R. Thompson. Thompson (1924) considered the population dynamics of egg-limited parasitoids (see Varley, Gradwell & Hassell, 1973) while Nicholson (1933) placed more emphasis on parasitoids limited by their ability to locate hosts. In 1935, Nicholson & Bailey constructed a mathematical model of a parasitoid-host system with discrete generations and random parasitoid search, and observed that it displayed divergent oscillations in population density. This immensely influential paper established that what might be called a null model of a specialist host-parasitoid interaction fails to predict host regulation. Although parasitoids show a density-dependent response to

increased host numbers, the response is delayed by one generation, and the delayed feedback causes overcompensation and the consequent divergent oscillations. Much of the research in parasitoid population dynamics in the sixty years since the Nicholson-Bailey model was formulated has involved relaxing the assumptions of this null model and investigating how this affects host regulation.

Nicholson, perhaps because he worked in a temperate region, framed his mathematical model of a host-parasitoid interaction as a difference equation and assumed that the host and parasitoid had synchronised and non-overlapping generations. Until recently, most parasitoid models have followed this lead. It might be thought that the famous Lotka-Volterra predator-prey model would provide a null host-parasitoid model in continuous time; like the Nicholson-Bailey model it assumes that encounters between predators/parasitoids and prey/hosts are random, and no predator/parasitoid satiation is included. The model predicts neutral population oscillations which are unlikely to be found in nature, but such a model is much easier to stabilise than the decidedly unstable Nicholson Bailey. However, in moving from the Nicholson-Bailey to the Lotka-Volterra model, an important aspect of the biology of host-parasitoid systems has been jettisoned. All host-parasitoid systems have developmental lags which are incorporated implicitly in the discrete generation framework of the Nicholson-Bailey model, but are omitted in the Lotka-Volterra model which assumes instantaneous recruitment. It is this added unrealism that makes the Lotka-Volterra model comparatively more stable. It is only relatively recently, beginning with Murdoch et al. (1987), that developmental lags have been placed in Lotka-Volterra models and the models applied to host-parasitoid systems. When the host and parasitoid have similar generation lags – the assumption of the Nicholson-Bailey model – the dynamics of the two models are very similar (Godfray & Hassell 1989): overlapping generations per se do not influence the regulating potential of parasitoids. However, if the host and parasitoid developmental lags differ, more interesting conclusions are possible. Even retaining the assumptions of random search, host regulation can occur when adult hosts live for a long period of time relative to the system's developmental lags (Murdoch et al. 1987). The reason why this is stabilising is that the pool of long-lived adult hosts, immune from parasitism, acts as refuge for the host population when parasitoid densities rise. The destabilising delayed density dependence is tempered by the «storage» of hosts in a refuge from parasitism. The host-parasitoid systems which most closely approximate these assumptions are tropical and semitropical homopteran-parasitoid interactions, particularly those involving scale insects and mealy bugs. It is perhaps significant that biological control has been particularly successful with this category of pest. In contrast to this type of system, if we assume that adult hosts are very short lived, but still retain random search, host-parasitoid generation cycles are possible (Briggs & Godfray 1994), a subject we shall return to below.

HANDLING TIME AND INTERFERENCE

The first attempts to develop the Nicholson-Bailey framework and to construct a more realistic model of host-parasitoid interactions involved studies of handling time and egg limitation, and of interference between searching parasitoids. The Nicholson-Bailey model implicitly assumes that a parasitoid encounters hosts at random and

instantaneously attacks them. If hosts are very abundant, then the model can assume that a parasitoid lays a very large number of eggs in a very short time period of time which is obviously unrealistic. This problem can be cured by assuming that host attack takes a finite amount of time that is normally referred to as the handling time (Holling 1959a,b). Time spent handling hosts is time that cannot be used searching for new hosts, and so the incorporation of handling time effectively places an upper limit on the number of hosts that can be attacked by an individual parasitoid, whatever the abundance of hosts in the environment. Although motivated by a different aspect of parasitoid biology, egg depletion enters population dynamic models in a similar way to handling time and has the same consequences: a cap on the number of hosts a single parasitoid can attack.

The incorporation of handling time or egg depletion reduces the ability of the parasitoid to act as a density-dependent break on the growth of the host population and so decreases the likelihood of host regulation (Hassell & May 1973, Hassell 1978). Whether handling time or egg depletion is important depends on the balance between the intrinsic rate of host increase and the length of the handling time (or severity of the egg limitation). While the Nicholson-Bailey model always predicts divergent oscillations, a different although still unstable outcome is now possible: host fecundity can be large enough that the host outstrips its natural enemy so that its population increases exponentially.

The motivation for the incorporation of interference in host-parasitoid models stemmed from behavioural observations of parasitoids searching for hosts in the laboratory. At high densities, individual searching adults would meet each other frequently and, as a result, waste potential searching time. Often, the outcome of the interaction would be the dispersal of one of the insects. The process was termed interference and results in a decrease in the searching efficiency of the parasitoid with increasing parasitoid density. A similar effect can occur without two parasitoids directly encountering each other. Many, perhaps the majority, of parasitoids can detect the presence of parasitoids in an area by chemical markers deposited in the environment or on the host. These chemicals may increase the likelihood of parasitoid dispersal.

Interference can be a strongly stabilising factor because it reduces the strength of the negative feedback inherent in the Nicholson-Bailey model but does not destroy the stabilising potential of the density dependence (Hassell 1971, Hassell & Varley 1969). Another way of describing the consequences of interference is to say that it introduces a density dependent reduction in the efficiency of the parasitoids: as parasitoid density rises, the efficiency with which an individual parasitoid locates hosts declines because of the increased rate of encounters with other parasitoids.

Although the stabilising potential of interference is clear, it is generally thought to be of limited importance in stabilising host-parasitoid interactions in the field. The problem is that the rate at which parasitoids encounter each other in the field is unlikely to be high enough for a sufficient reduction in parasitoid efficiency to occur. There are, however, other factors that can act in a similar way to interference that may be much more important in the field.

PHYSICAL, PHENOLOGICAL AND PHYSIOLOGICAL REFUGES

An important assumption of the Nicholson-Bailey model is that all hosts are equally susceptible to attack by the parasitoids. There are number of biological reasons why one may want to relax this assumption. First, the natural history of the host may suggest that not all individuals are equally susceptible to attack (Bailey et al. 1962). For example, Weis (1983) showed that the probability that a gall midge was parasitised by a torymid wasp depended on the number of midges in the gall which varied between one and four. Refuges are stabilising for the same reason that a long adult stage is stabilising in the continuous time model of Murdoch et al. (1987) discussed above; they provide a means for part of the population to be protected from the worst excesses of delayed density dependence via parasitism. However, too big a refuge, and the parasitoid will be unable to locate sufficient hosts to regulate its population. Hassell (1978) discusses some of the details of the dynamics of host-parasitoid systems with refuges. Whether a fixed proportion or a fixed number of hosts lie in a refuge affects the predicted population dynamics. For some parameter values, the host-parasitoid system is predicted to display sustained limit cycles. Recently, Hochberg & Hawkins (1992) have argued for a central role for refuges in determining parasitoid community structure; we shall return to this point in the last section of the paper.

Some hosts may escape parasitism because they emerge before or after the period in which parasitoids are active searching for hosts. Like physical refuges, the temporal refuges that are caused by this type of phenological asynchrony can stabilise, or contribute towards the stability, of a host-parasitoid interaction (Münster-Swendsen & Nachman 1978, Godfray et al. 1994)

Hosts are not inanimate resource items for parasitoid reproduction but can fight back. This is especially true of koinobiont parasitoids which temporarily recover from parasitism and continue to feed and grow. Although they normally succumb to the parasitoid in the end, they have a breathing space within which they can attempt to destroy the developing larva, typically through the cellular immune response termed encapsulation. There has been some speculation that hosts vary in their ability to encapsulate parasitoids and that a class of well defended individuals might form a refuge that contributes towards the stability of the interaction (Hassell & Anderson 1984, Godfray & Hassell 1990). Very recently, a dramatic example of genetic variance in the ability of a population of aphids to withstand parasitoid attack, and of the same parasitoid to survive on the aphid, has been demonstrated by Henter & Via (1994) and Henter (1994). Within-population variance in encapsulation ability has also been demonstrated recently in *Drosophila* parasitoids (Kraaijeveld 1994). These empirical findings justify greater attention being paid to the population dynamic role of genetic heterogeneity in host defences. They also raise several new questions concerning how the genetic heterogeneity is maintained, the answers to which will probably require an approach that simultaneously considers population and genetic dynamics.

Physical, phenological and physiological refuges all share some dynamic features with interference. If the logarithm of the fraction of hosts surviving parasitism is plotted against parasitoid density, then with random search and the other assumptions of the Nicholson-Bailey model, a straight line results (Figure 1, curve a). If interference occurs, then the reduction in survival with parasitoid density is sub-

linear (e.g. Figure 1, curve b) which contributes towards stability. Similarly, the plot of the logarithm of survival against parasitoid density in the cases of refuges and partial immunity is sub-linear (Figure 1, curve c) and this has led to this stabilising mechanism being termed pseudointerference (although the only real justification for this term is historical: at the time these new mechanisms were being investigated interference was considered the most important stabilising factor). We now turn to another stabilising factor that can also be classed as a type of pseudointerference.

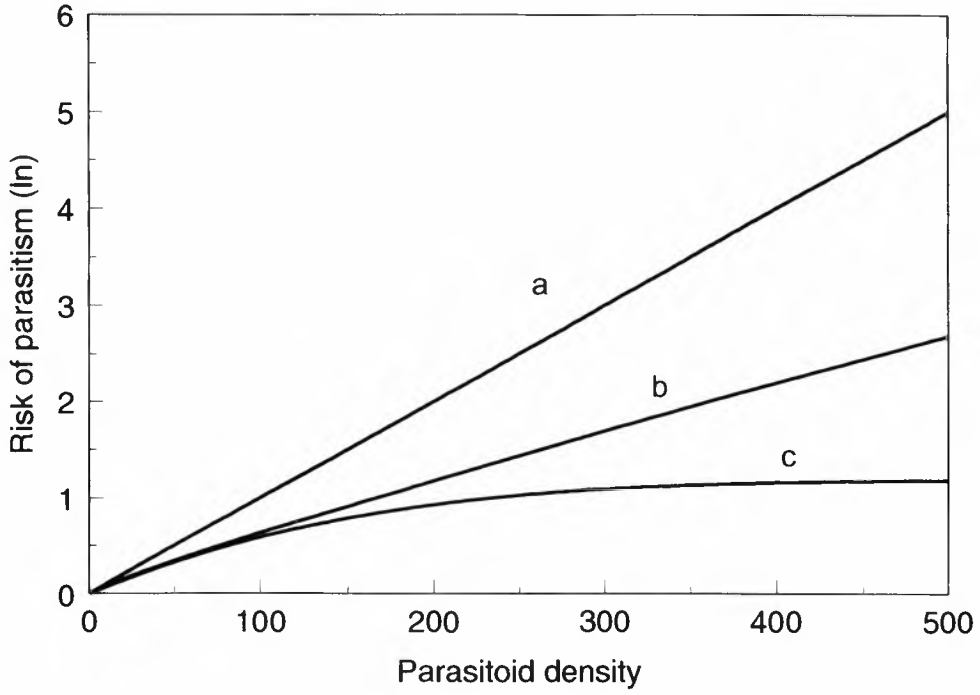

Figure 1. The relationship between the risk of parasitism and parasitoid density for a, the Nicholson Bailey model; b, the interference model (Hassell & Varley 1969); and c, a proportional refuge model (Hassell 1978). (The graph shows a plot of Log_e (Initial Hosts/Surviving Hosts) against parasitoid density. The searching efficiency parameter throughout is 0.01; in (b) the interference parameter is 0.3; in (c) the proportional refuge parameter is 0.3.)

HOST PATCHINESS AND PARASITOID AGGREGATION

The hosts of all parasitoids are distributed non-randomly across the environment and most are probably found in discrete patches. Parasitoids search in the environment and locate patches using an impressive variety of cues (normally chemical ones). For the Nicholson-Bailey model to apply to hosts and parasitoids in a patchy environment, every host must suffer the same risk of parasitism. A simple way by which this might be achieved is for the parasitoids to move very quickly between patches; thus having

hosts in patches has no influence on dynamics. However, what is more likely to happen is that parasitoids distribute themselves in other ways across patches. Two important cases can be distinguished which will be discussed in turn. First, parasitoids may aggregate in patches of high density; and second, parasitoids may enter certain patches irrespective of host density. The two types of parasitoid aggregation are conveniently referred as host density dependent (HDD) and host density independent (HDI) (Pacala et. al. 1990). Before continuing, a word should be said about the term aggregation which has been used in two separate senses in the parasitoid literature. First, in can be used to refer to the behaviours of the parasitoid that result in certain patches containing more individuals than others. If parasitoids detect the kairomones associated with their hosts, then aggregation in patches of high host density may result (HDD). This is the sense in which we shall use the word. Alternatively, aggregation can be used in a statistical sense to describe the variance and covariance of hosts and parasitoids across patches. The two uses of the word tend to converge in thinking about parasitoids with non-overlapping generations. However, confusion can arise when generations overlap and the degree of statistical aggregation changes, not through parasitoid movement, but simply through the process of host depletion as parasitoids search within a patch.

The stabilising power of HDD aggregation was demonstrated by Hassell & May (1973, 1974, 1988), Hassell (1978), Hassell & Pacala (1990), Hassell et al. (1991), Pacala et al. (1990) and Pacala & Hassell (1991). The reason why HDD aggregation is stabilising is that although parasitoids initially congregate in patches of high density, they deplete the patch so that an initial positive correlation between parasitoids and unparasitised hosts at the beginning of the season becomes a negative correlation by the end of the season (Hassell & Pacala 1990). An important assumption of these models, which we return to in a moment, is that parasitoids are distributed across host patches at the beginning of the season and remain in the patch throughout the season. Stability occurs because hosts in patches of low host density are in a probabilistic refuge and so the interaction is protected from the divergent oscillations that characterise the simple Nicholson-Bailey model. The refuge means that the decline in host survival with increasing parasitoid density is sub-linear and hence this is another type of pseudointerference. Proof that the stabilising mechanism involves refuges is provided by the initially counterintuitive observation that inverse density dependent aggregation can also stabilise an interaction (Hassell 1984). However, the range of parameter values for which inverse density dependence is stabilising is restricted because there is a great danger that hosts are able to evade regulation completely.

In 1989, Murdoch & Stewart-Oaten published a host-parasitoid model phrased in continuous time that came to a very different conclusions about the stabilising role of HDD aggregation. The model was based on the Lotka-Volterra model (without time lags) and hence, as described in the first section of this paper, one would expect it to predict a stable interaction more readily than a model from the Nicholson-Bailey stable. In fact they concluded that aggregation to patches of high host density was typically destabilising, the opposite conclusion from the discrete-time models.

Modelling parasitoid aggregation in continuous time is very much harder than modelling aggregation in discrete time with parasitoids remaining for the whole generation in one patch. Murdoch & Stewart-Oaten's (1989) model, while ingenious, was open to a number of criticisms, some semantic (what exactly was meant by

aggregation), and some involving the biological assumptions implicit in the model. However, the consensus of the debate prompted by this work (Godfray & Pacala 1992, Ives 1992, Murdoch et al. 1992) was that the different results from the two approaches were caused by the assumption of the absence of within-generation movement in the traditional models, and the very much faster rates of movement between patches implicit in Murdoch & Stewart-Oaten's models. To bridge this gap, Rohani et al. (1994) recently constructed a hybrid model of a host-parasitoid interaction with discrete, synchronised generations (as in the Nicholson-Bailey model) but with continuous within-generation dynamics (as in the Lotka-Volterra model with time lags). The model was sufficiently complicated that most results had to be obtained numerically, but the stability of the system could be examined as the rate of parasitoid movement among patches changed. The results were mixed but overall favoured Murdoch & Stewart-Oaten's conclusion. In the face of within-generation movement, HDD aggregation could still make some contribution to stability, but even relatively modest amounts of movement severely curtailed its stabilising power. That it can actually be destabilizing, as Murdoch & Oaten also found, depends on having within-generation redistribution of the susceptible host stages, which is unlikely for most host-parasitoid systems.

Another approach that has been taken to studying patch use in parasitoids is to ask how a population of parasitoids should distribute themselves across patches if natural selection acts on every individual to maximise its fitness (i.e. the number of hosts it locates). The answer is that the parasitoids should adopt a temporally varying ideal free distribution (Cook & Hubbard 1977, Hubbard & Cook 1978, Comins & Hassell 1979, Lessells 1985, Godfray 1994). With an ideal free distribution, no parasitoid can increase its fitness by moving to another patch. In a temporally varying ideal free distribution, the parasitoids continuously adjust their distribution so that at any one time all individuals are achieving the same rate of host encounter. In the simplest case (with no interference and zero handling time), parasitoids initially congregate in the best patch until it is reduced to the same profitability as the second best patch when the group of parasitoids distributes itself equally between the two best patches. This process continues with patches of decreasing quality being added to the exploited set.

It might be thought that if optimal searching maximises the fitness of individual parasitoids, then it would be strongly destabilising as it would result in the discovery and exploitation of any host refuge. However, Comins & Hassell (1979) discovered that for at least some parameter values, a host-parasitoid system with optimally searching parasitoids can be stable. Although the precise stabilising mechanism requires further exploration, there again appears to be a refuge for hosts in patches of low host density. Dynamic models incorporating optimal searching have to date been relatively simple and have made rather unrealistic assumptions about parasitoid behaviour, for example assuming that parasitoids are omniscient and that travel time is not important. However, Bernstein et al. (1988, 1991) have conducted simulations of populations of searching parasitoids in patchy environments with more realistic descriptions of learning and travel costs. They conclude that as long as patch depletion does not proceed too fast, it is not unrealistic to expect populations of parasitoids using relatively simple behavioural rules to approximate the ideal free distribution.

We now turn to aggregation of parasitoids in patches independent of host

density (HDI). Discrete generation models based on the Nicholson-Bailey model again predict that this is a potent stabilising influence (Hassell et al. 1991, Pacala et al. 1990, Pacala & Hassell 1991). Yet again, a pseudointerference mechanism is responsible: hosts in those patches that are not visited by parasitoids are in a probabilistic refuge and as a result there is a density-dependent reduction in the per capita efficiency of the parasitoid. A useful way of studying this effect is through the negative binomial model introduced by May (1978). The hosts escaping parasitism in a Nicholson-Bailey model can be thought of as the zero class of a Poisson distribution, the mean of which is the average number of times a host is encountered. May proposed that the hosts escaping parasitism could be though of as the zero class of a negative binomial distribution, a distribution that can be characterised by the average number of host encounters and a second parameter that is conventionally called k. When k is infinity, the logarithm of host survival decreases linearly with increasing parasitoid density as in the Nicholson-Bailey model (in fact the two models are identical as $k \to \infty$). For smaller k, the decline in log survival is sub-linear, and for $k<1$, the sub-linearity is enough to stabilise the system. Once more, we have stabilisation through a probabilistic refuge and pseudointerference. The negative binomial model was originally introduced as a description of HDD aggregation but Chesson & Murdoch (1986) showed that it was a more natural representation of HDI aggregation.

Murdoch & Stewart-Oaten (1989) also considered HDI aggregation in their analysis of aggregation with overlapping generations. They concluded that the dynamics of the system were uninfluenced by this form of aggregation. However, Godfray & Pacala (1992) argued that this was a structurally unstable conclusion that depended on a statistical definition of aggregation, rather than the more standard behavioural definition. In their hybrid approach discussed above, Rohani et al. (1994) concluded that the results from the discrete generation models without redistribution were robust to the addition of within-generation redistribution.

Throughout this section and the last, attention has been drawn to the way in which many of the effects of physical, physiological and probabilistic refuges can be interpreted within the same framework of pseudointerference. All these mechanisms also share the property that they increase the variance in the risk of parasitism across hosts. This suggests that a common criteria for stability may be derived, based on the distribution of risk. Hassell & Pacala (1990) and Hassell et al. (1991) show that an approximate but robust criterion is that the coefficient of variation in the risk of parasitism across hosts should be greater than one. In studies of parasitoids in patchy environments, the contribution of HDI and HDD to the coefficient of variation in risk and hence to stability can be partitioned. In a survey of 65 species, Hassell & Pacala (1990) and Pacala & Hassell (1991) found that the most important stabilising force was HDI aggregation rather than HDD aggregation. In interpreting these results, caution is required as the underlying model assumes no parasitoid redistribution within the season, something that is now known to be important in modulating the stabilising power of HDD aggregation. However, it is interesting that HDI aggregation is so important, and that the stabilising potential of this form of aggregation is not affected by within-generation redistribution.

The reason that parasitoids aggregate in some patches irrespective of host density is puzzling. Why does natural selection not lead to more efficient parasitoid behaviour? Possibly there are constraints operating, such as some patches exposing

the parasitoid to greater risks of predation or abiotic mortality. Alternatively, the variability may be operating on larger spatial scales with concentrations of parasitoids shifting in position from generation to generation. If the latter process is occurring, then we need to move from the type of models discussed so far with no explicit representation of space, to models in which the densities of hosts and parasitoids have spatial as well temporal coordinates.

SPACE: THE FINAL FRONTIER

The discrete generation models of host-parasitoid interaction, based on the Nicholson-Bailey model, all assume complete mixing of host and parasitoid populations between generations. Spatially explicit models relax this assumption and track the spatial position of host and parasitoid populations from one generation to the next. A halfway house is provided by a metapopulation model of a host-parasitoid interaction in continuous time developed by Murdoch et al. (1992). Although space was not represented explicitly in the model, populations of hosts and parasitoids in discrete patches existed for many generations and could send out and receive migrants from other patches. Most importantly, populations in the two patches could fluctuate out of synchrony, and this allows the ensemble of populations (or metapopulation) to persist, even when the dynamics in an isolated patch are unstable. The ensemble persists because a patch doomed to extinction can recover through immigration from another patch which, because its fluctuations are out of phase, is not currently in the same perilous position. This mechanism leading to metapopulation stability is termed a rescue effect and has been invoked in models of many different types of metapopulation (Taylor 1988).

A series of explicitly spatial host-parasitoid models have been developed by Hassell et al. (1991) and Comins et al. (1992). They used two types of model, both based on a square array of populations connected by dispersal to adjacent or local sites. In one set of models, the dynamics within each component population were described by the Nicholson-Bailey model, while the second set of models were constructed in the form of a rule-based cellular automata. Although, local dynamics were unstable, the ensemble of populations persisted. Moreover, interesting spatial population patterns emerged. The two most biologically relevant types of pattern were spirals and spatial chaos. In the first case, a spiral of areas of high host density radiated out from a focus with a spiral area of high parasitoid density along the trailing edge of the expanding host spiral. In effect, the parasitoid population chases the host population through space, destroying it at the trailing edge of the spiral, and moving into the new areas of high host density caused by host dispersal along the leading edge. The second pattern observed was called spatial chaos and also consisted of local high density regions of hosts which were chased through space by high density regions of parasitoids, but here the spatially ordered spiral patterns had broken down and regions of high insect density moved through space in unpredictable ways, frequently merging and generating new shapes.

Spiral waves and spatial chaos are known from a variety of spatial extended dynamic systems, both in biology and physics (Murray 1989), but had not previously been found in host-parasitoid models. What of their possible occurrence in nature? The spatial patterns observed in the simulation require a minimum sized spatial arena

and so are best looked for in host-parasitoid systems occupying relatively extensive and fairly homogenous habitats. A time series of host and parasitoid densities from a single site would exhibit periods of low or zero insect densities, with episodic explosions in host density followed quickly by a period of very high parasitoid density. Time series from several sites will tend to be uncorrelated or show complex delayed correlation. If time series were available from sufficient sites, then it might be possible to identify the actual spatial pattern.

AGE STRUCTURE

If both hosts and parasitoids have discrete, synchronised generations, as in the Nicholson Bailey model, then there is little need for an explicit consideration of age structure. However, if generations overlap then an explicit consideration of age structure becomes more important. In the first section of this paper we noted that the introduction of developmental lags into a continuous-time parasitoid model derived from the Lotka-Volterra model had little effect on the observed dynamics, as long as the developmental lags of the host and parasitoid were the same (although a long-lived, invulnerable adult stage did promote stability). If developmental lags are allowed to vary, then some novel dynamic behaviour is predicted.

Godfray & Hassell (1987, 1989; see also Godfray & Chan 1990 and Briggs & Godfray 1994) studied age-structured continuous time models of host-parasitoid interactions, largely using the lumped age class technique of Nisbet & Gurney (1983) which had first been introduced into host-parasitoid studies by Murdoch et al. (1987). They assumed that an unspecified factor of the pseudointerference type was stabilising the interaction and concentrated on exploring the effects of age structure on the system's dynamics. When the extrinsic pseudointerference was weak, the system was always unstable (unless the adult stage was very long lived, see above), and if the extrinsic pseudointerference was very strong, the interaction was always stable. Now, define the developmental lag of the host as one time unit and consider what happens as the developmental lag of the parasitoid varies. When the lag is very short, the parasitoid acts as a direct density dependent factor and this enhances stability. When the parasitoid lag is the same as that of the host, we recover dynamics that are very similar to the Nicholson-Bailey model. However, when the lag is approximately 0.5 (or 1.5), we obtain a new type of population dynamic behaviour. For intermediate levels of pseudointerference, both hosts and parasitoids cycle with a period approximately equal to the host developmental lag.

The reason why different developmental rates can cause cycles can be understood by considering a host and parasitoid at equilibrium where the equilibrium may be stable or unstable. Now suppose that there is a temporary increase in host numbers. One host generation later, the progeny of this temporary increase will cause a secondary increase. The parasitoid population will be able to capitalise on the first temporary increase of hosts and in consequence their numbers will go up one parasitoid generation later. If the host and parasitoid have similar developmental lags, the secondary increases of hosts and parasitoids will coincide, and this will tend to be stabilising. If the developmental lags are not the same, the two secondary increases will fail to coincide and this will tend to cause oscillations. Cycles with period approximately equal to the host developmental lag have been noted in many tropical

insect pests and Godfray & Hassell (1989) argued that the age-structured interaction between the host and the parasitoid may be responsible.

TOWARDS A PARASITOID COMMUNITY ECOLOGY

All the models discussed so far have concerned specialist parasitoids attacking one species of host. However, most herbivores are attacked by more than one species of parasitoid which may be a specialist, oligophage or generalist (polyphage). In the context of population dynamics, these three terms can be given a special meaning: the dynamics of a specialist parasitoid are uniquely driven by its host; the dynamics of a generalist can be considered as decoupled from that of any particular host, while the dynamics of an oligophage are intermediate – determined by their interactions with several hosts.

It is no surprise that the two-parasitoid equivalent of the Nicholson-Bailey model is unstable. May & Hassell (1981) took May's (1979) negative binomial model and adapted it for the case of two specialist parasitoids attacking a single host. They assumed that the two parasitoids attacked different host stages (and that the parasitised individuals of the first species were immune to the second) or that one species was invariably the superior competitor – the two assumptions give an identical model. They found coexistence was possible for certain combinations of host fecundity and parasitoid searching, as long as the pseudointerference terms were strong enough to provide some stability.

For coexistence to occur, both species must be able to invade when rare. Because pseudointerference in these models is purely intraspecific, the rare species is at an advantage in comparison with the common species, especially when pseudointerference is strong. The form of pseudointerference is thus critical in determining coexistence. Kakehashi et al. (1984) relaxed this assumption by replacing the negative binomial distribution with the negative multinomial distribution, and also by exploring a niche overlap model where the host has refuges from each parasitoid which may or may not overlap. Coexistence is less likely in the negative multinomial model because the pseudointerference term of the second parasitoid is influenced by the density of the first. In the niche partition model, coexistence is more likely when niches do not overlap. The latter is a standard result in population biology and occurs because in these circumstances intraspecific competition is more powerful than interspecific. Some related models in continuous time that incorporate the effects of age-structure have been analysed by Briggs (1993) and Briggs et al. (1993).

If one parasitoid attacks two hosts, then the most likely outcome is that one species of host will be driven to extinction (Holt & Lawton 1993). For each host species, there is a threshold density of parasitoids in the environment above which the species can no longer replace itself. The species that wins out is that which can support and survive the highest density of parasitoids. Holt & Lawton (1993) refer to the outcome of this process as dynamic monophagy. Coexistence of the two hosts may be possible in the presence of refuges from parasitism, or if the parasitoid preferentially switches to the more common host.

Moving from specialists and oligophages to generalists, Hassell & May (1986) considered a host attacked by a specialist and a generalist where the dynamics of the specialist were determined by a negative binomial pseudointerference term, and the

generalist were assumed to have decoupled dynamics, but to increase in importance as host densities rose (switching). One outcome that can occur is for the generalist to drive the host population to a sufficiently low level that the specialist can no longer persist. This is especially likely to occur if the generalist attacks the host prior to the specialist. However, coexistence is also possible if the generalist shows switching behaviour (Murdoch 1969), thereby inflicting a density dependent mortality with no time lags. For some parameter values, the different non-linearities in the model interact to produce complex cyclic and chaotic dynamics.

The stabilising power of generalist (or possibly oligophagous) parasitoids has recently been demonstrated in a remarkable experiment by Gould et al. (1990) on the gypsy moth (*Lymantra dispar*). The gypsy moth exists over much of North America in an endemic state but occasionally increases in numbers to cause a major outbreak. The outbreak is normally controlled by viruses. Gould et al. attempted to engineer an outbreak by releasing up to 34 million gypsy moth larvae at a single site. They failed, largely because generalist and oligophagous parasitoids killed the larvae. It appears that the parasitoid wasps and flies switched to the temporarily abundant host. Gypsy moth is also heavily predated by small mammals in the winter. Gould et al. speculate that outbreaks are caused by a decline in the small mammal populations over large geographic areas which results in so many larvae over so large an area that the generalist parasitoids are saturated and the host population escapes.

The studies discussed so far in this section have generally considered three species of host and parasitoid. The only work to have gone beyond this is Hochberg & Hawkins (1992) who modelled one host species attacked by a large number of parasitoid species. The parasitoids were a mixture of specialists (with dynamics described by the negative binomial model) and generalists with the type of switching behaviour described earlier in this section. Furthermore, the model included a host carrying capacity, and a variable refuge (from all parasitoids). The aim of the exercise was to see whether there was a relationship between the size of the host refuge and the number of parasitoid species maintained by the host (see Hochberg & Hawkins (1992) for the relationship between this work and Hawkins' (1994) surveys of the number of species of parasitoids attacking different types of hosts; a slightly different view is given in the commentary by Godfray 1994). The model is started initially with 50 species each of generalist and specialist and iterated numerically until a stable number of parasitoids are observed. They found the largest parasitoid community when the refuge was of intermediate strength.

To understand these results, consider the number of hosts available for parasitism (i.e. outside the refuge). When the refuge is large, few hosts are available, even though the host population density is near carrying capacity. When the refuge is small, the host population density is very low and largely determined by the specialist parasitoid or parasitoids (several can coexist because of the assumption of intraspecific interference). The population of hosts available for parasitism is thus highest for intermediate refuges and it is here that the greatest numbers of generalists, which respond in a simple manner to available host density, are recorded from the host. It is the number of these generalists that largely determine the observed pattern of parasitoid community size.

It is easy to criticise the assumptions of this model and to construct alternatives that are likely to give different results. However, Hochberg & Hawkins' work is important in that it is the first attempt to take the simple models discussed in this paper and examine their behaviour at the community level.

CONCLUSIONS

This review has focused on the aspects of parasitoid biology that are likely to lead to the persistence of host-parasitoid interactions with the parasitoid regulating the host substantially below its carrying capacity. We have thus ignored a variety of issues, such as the interaction of parasitoids with other regulatory processes (for example direct host density dependence (Beddington et al. 1978, May et al. 1981) and pathogens (Hochberg et al. 1990)); the role of hyperparasitoids (Beddington & Hammond 1977, May & Hassell 1981); and the controversy over the wisdom of multiple and single introductions during biological control (May & Hassell 1981, Kakehashi et al. 1984, Godfray & Waage 1991, Briggs 1993). We have also, for reasons of space, concentrated on theoretical rather than experimental issues.

The simplest models of host-parasitoid systems are not intrinsically stable and explaining how host regulation occurs in nature has been a major challenge to population biologists. The answers are not yet clear, although great progress in understanding how different processes mesh together has been made. The most likely mechanisms stabilising interactions between specialist parasitoids and their hosts are forms of pseudointerference associated with heterogeneity in the host risk of parasitism. Density dependent aggregation by parasitoids in patches of high host density is probably not as important as has been thought until recently, but heterogeneity in host defences and aggregation irrespective of host density may be more important. Comprehending these processes in an explicitly spatial setting continues to challenge theorists.

Another major challenge is to develop a population-based community ecology of parasitoids. Understanding parasitoid communities is likely to involve an understanding of the dynamics of oligophagous parasitoids: species whose dynamics can neither be decoupled from their host, nor understood by considering one or two hosts in isolation. To do this will be difficult, but the simple and often strong trophic link between parasitoids and their hosts suggests that this research programme has a better chance of succeeding than equivalent programmes revolving around any other type of animal trophic relationship.

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The reliability of published host-parasitoid records: a taxonomist's view

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The unreliability of published host-parasitoid lists is illustrated with several examples from the literature. Possible reasons for erroneous records are discussed and recommendations are made which could reduce the number of dubious records published in the future.

Keywords: causes, Host-parasitoid lists, remedies, unreliability

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Insect parasitoids play a major role in the regulation of numbers of many insect species (LaSalle & Gauld, 1993). Therefore, understanding the relationship between insects and their parasitoids can be crucial to improving pest control methods, and to the study of biodiversity or the evolution of insect parasitism.

Investigations of insect parasitism frequently include a search through published catalogues that list natural enemies recorded for various insect species. Perhaps the best known of these is the series of publications prepared under the direction of W.R. Thompson (1943–1958), generally known as «Thompson's Parasite Catalogue» and continued by Herting (1971–1982). Other catalogues frequently used for this purpose are those of De Santis (1967, 1979, 1980, 1983, 1989: Chalcidoidea of South America), Krombein et al. (1979: Hymenoptera of North America), Mackauer (1968: Braconidae, Aphidiinae), Shenefelt (1969–1980: Braconidae), Shenefelt & Marsh (1976: Braconidae), and Peck (1963: Chalcidoidea of North America).

This approach provides a preliminary list of natural enemies recorded in association with a given insect species as well as providing an entry point into the literature. But how reliable is this information, and can it be taken at face value? The purpose of this paper is to demonstrate that published host-parasitoid lists of «well-known» species can be extremely inaccurate and that the same may also be true of papers dealing with even a single parasitoid species and its host. Indeed, Shaw (1994) put the view that host-parasitoid lists in the literature can be so misleading as to be generally useless and suggested that we should reject the notion that literature is an adequate source of reliable data and focus our attention on data held in taxonomic collections.

The possible reasons for these inaccuracies are discussed below and some recommendations are made which, if followed, may improve the accuracy of host-parasitoid lists published in the future.

1. EXAMPLES OF UNRELIABLE HOST-PARASITOID LISTS

Of the very many host-parasitoid lists that can be compiled from catalogues, the following examples illustrate the level at which inaccuracies can occur.

1.1 Host lists of a parasitoid species

a) *Aleiodes* spp. (Braconidae). Shenefelt (1975) records 34 host species for *Aleiodes alternator (=geniculator)* and 45 host species for *A. gastritor* (=*testaceus* sensu Shenefelt). Shaw (1994) has worked extensively with species of this genus and has made many hundreds of rearings, studied oviposition habits in the laboratory and examined extensive material in collections with preserved hosts. He is of the opinion that *alternator* only parasitises caterpillars of Lasiocampidae, Arctiidae and Lymantriidae on low plants and, of the host species recorded in Shenefelt, not more than 9 or 10 host records can be considered reliable. *A. gastritor* appears to parasitise caterpillars of Geometridae and Drepanidae and only 14 of the species listed as hosts for *gastritor* belong to these families.

b) *Cotesia glomerata* (Braconidae). The list of published hosts for this species is very extensive (Table 1) and illustrates the problem very well. The recorded hosts belong to a very wide range of Lepidoptera. There is no possibility of determining what proportion of these records are reliable, but it is very unlikely that a single species of endoparasitoid would attack such a wide variety of hosts in the larval stage because it would have to overcome the immune system of every species. Laboratory studies have shown that immature stages of *Cotesia glomerata* are encapsulated by the caterpillars of at least 14 species of lepidopterans (see Laing & Levin 1982), although encapsulation rates may vary with geographical distribution. Even so, it is certain that *C. glomerata* could not have been reared from eggs of Pentatomidae! On the other hand, some species of ectoparasitoids are known to parasitise a wide range of hosts in certain situations, eg. *Colastes braconius* will attack suitably-sized lepidoptera, dipteran, coleopteran and hymenopteran leaf-miners (see Shaw & Huddleston 1991: 94).

c) *Trichogramma evanescens* (Trichogrammatidae). This is perhaps the most frequently cited species of chalcidoid in the entomological literature. Being an egg parasitoid it does not have to deal with an advanced host immune system and the list of published hosts is very extensive with nearly 200 host species recorded from at least 33 families in 5 insect orders (Thompson 1958, Herting 1973–1982; *Review of Applied Entomology,* 1980–1992). However, the taxonomy of this genus is in an extremely confused state, not least the recognition of *evanescens* which is the type species of *Trichogramma.* The identification of species of *Trichogramma* is based almost entirely on male characters whilst *evanescens* was described by Westwood (1833) from a single female collected in London, England. Even though the type specimen (now poorly mounted in balsam on a microscope slide) is one of the most borrowed specimens of the Oxford University Museum (C. O'Toole, pers comm.) the identity of the species remains a problem with at least two conflicting interpretations of the species: that of Russian authors and of French authors (see Kostadinov & Pintureau 1991). Until the identity of the species can be established with certainty, all records, other than that of Westwood (1833), must be treated with caution.

1.2 Parasitoid lists for one host species

Table 2 provides a summary of the parasitoid species recorded for the diamond-back moth, *Plutella xylostella*. It is far from complete but must represent one of the longest lists of parasitoids recorded from a single host species. The parasitoids of this moth pest are presently the subject of investigation (see Fitton & Walker 1992, Fitton et al. 1991). It seems likely that only about one third of the recorded species of parasitoids is correct (M.G. Fitton, A.K. Walker, J. LaSalle, Z. Boucek, pers. comm.), the remaining two-thirds require confirmation or are incorrect.

2. POSSIBLE REASONS FOR ERRONEOUS HOST-PARASITOID RECORDS

2.1 Mixed series of hosts

This is common problem and may be the cause of a very high proportion of erroneous host records. This can be illustrated by the list of hosts given in Peck (1963:27) for *Alaptus eriococci* Girault (Chalcidoidea: Mymaridae). Here the recorded hosts are given as one species of Psocoptera, two species of Diaspididae (Homoptera) and one species of Eriococcidae (Homoptera). Where their biology is known, all species of Mymaridae are parasitoids of the eggs of other insects and species of *Alaptus* have been recorded reliably only from the eggs of Psocoptera (see Huber 1986). It is probable that in such cases psocopteran eggs are mixed up with diaspidids or eriococcids on a twig, or some other substrate, thus leading to the mymarid being erroneously recorded from the coccoids instead of from the unseen psocopteran eggs.

2.2 Mixed series of parasitoids and/or hyperparasitoids

This is also a common problem which can lead to erroneous records in the literature. Material sent for identification to taxonomists, and thought to be of single species by the non-specialist, frequently consists of more than one species which may even belong to more than one family. If, for one reason or another, the whole series is not examined by the taxonomist, incorrect identifications may result. A very good example of this is from the biocontrol literature where a species of *Prochiloneurus* (Chalcidoidea: Encyrtidae) has been attributed with the successful control of *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae) in Egypt (Kamal 1951, as *Achrysopophagus*). Where their biology is known, species of *Prochiloneurus* are hyperparasitoids mostly of mealybugs, via encyrtid primary parasitoids (Noyes & Hayat 1984). No species are known that act as primary parasitoids of mealybugs and it is extremely unlikely that this will ever be found to be the case. Even for the non-specialist, it is difficult to confuse a species of *Prochiloneurus* with any species of primary parasitoid of mealybugs. The likely cause of this error is that a primary parasitoid species, probably of the genus *Anagyrus,* was introduced into Egypt but the material used for identification by a specialist consisted of the hyperparasitoid alone.

2.3 Misidentification of host and/or parasitoid due to inadequate taxonomy

Misidentifications may occur as a result of insufficient knowledge of a group. There are many good examples, especially in biological control, where a pest must be identified correctly to determine its natural geographical range in order to locate natural enemies. This can be critical to the successful outcome of the project. An example of this was the cassava mealybug, *Phenacoccus manihoti*, a serious pest of

cassava throughout tropical Africa. This mealybug species was initially thought to be a native of Central America or northern South America, but parasites recovered from mealybugs in these areas would not parasitise the species from Africa. Later it was discovered that the African species was different and originated from central South America. Almost immediately natural enemies were found in Paraguay, Bolivia and southern Brazil that would attack the cassava mealybug pest in Africa. One of these, *Apoanagyrus lopezi* (Chalcidoidea: Encyrtidae), has since proven to be extremely successful in controlling the cassava mealybug (see Herren & Neuenschwander 1991).

2.4 Misidentification of host/parasitoid due to poor taxonomy

This is a very serious and common problem, especially where experienced taxonomists have not been consulted with regards to specimen identification. A recent example of this is with work published on the biology of a pest of a *Eucalytpus* in the Philippines where *Ooencyrtus erionotae* (Chalcidoidea: Encyrtidae) was recorded as a parasitoid of the eggs of *Agrilus sexsignatus* (Coleoptera: Buprestidae) (Braza 1988, 1989). No species of *Ooencyrtus* have ever been recorded reliably as parasitoids of coleopteran eggs and further to this, *Ooencyrtus erionotae* is well known as a parasite of *Erionota thrax* (Lepidoptera: Hesperiidae) a serious pest of banana in S.E. Asia. The parasitoid of the buprestid does not even remotely resemble a species of *Ooencyrtus* and was later described as a new genus and species of Encyrtidae (*Orianos brazai* Noyes, 1990).

2.5 Author disregarding opinion of a taxonomist

Perhaps the most common instance of this occurrence is when a species has been provisionally identified e.g. *Pteromalus puparum*, or as a «sp. near», e.g. *Pteromalus* sp. nr *puparum*. There is often a tendency for the name to be published subsequently without the query or «sp. near» prefix.

2.6 Misspelt parasitoid or host names

This is a fairly frequent occurrence, but perhaps the least serious in that it is the most easily detected. These errors may be introduced by printers and are not always attributable directly to the author. The problem can illustrated by the list of parasitoids recorded by Lim (1986:163, Table 1) for *Plutella xylostella* in Malaysia. Of the 55 species recorded here, the names of four parasitoids are misspelt, viz: *Brachymeria phyta* (Walker) for *Brachymeria phya*, *Compoletis* sp. for *Campoletis* sp., *Euptromalus viridescens* (Walsh) for *Eupteromalus viridescens* (Walsh) and *Celis tenellus* (Say) for *Gelis tenellus* (Say).

2.7 Misidentification resulting from inadequate material

The Natural History Museum and International Institute of Entomology, London, UK, and no doubt other identification services, frequently receive material that has been poorly prepared or has been damaged in transit because of inadequate packaging. Sometimes specimens are mounted in so much glue that important taxonomic characters are obscured, or they may have been mechanically damaged by bad

handling. On occasions, material is covered in fungus having been left in a jar, polythene bag or similar receptacle for several days in a hot, humid climate. Serious problems can result from material being posted with insufficient shock-absorbent packaging or with poorly fixed specimens that work their way loose. These problems may result in the material being incorrectly identified, although it is unlikely that good taxonomists would attempt to identify material that is unsuitable for the purpose.

2.8 Misidentification resulting from inadequate information

This may be a problem when a specialist is identifying material from a wide area. For instance, an unlocalised sample may be sent for identification from a laboratory in a country where only a single species of a particular genus is known. The material may actually have been collected in another country where several other, similar species occur. Some taxonomists may incorrectly assume that the material being identified belongs to the single species known from the first country and identify it as such. Adequate information may also allow taxonomists to comment on unlikely or erroneous host records.

3. RECOMMENDATIONS

Given that much of the information available in the literature relating to parasitoids and their hosts is unreliable, the value of *any* information stored in host-parasitoid lists must be questionable. To attempt to overcome this, two basic requirements are suggested below.

3.1 The reliability, and thus the value, of individual records can be indicated in published catalogues which provide host-parasitoid lists. This would probably be best done by specialist taxonomists who have a wide knowledge of the groups of insect parasitoids that they study. Their expertise can be incorporated into host-parasitoid lists by scoring the probability of a given record being correct. This can be applied easily to electronic databases. The computerised database of world Chalcidoidea being developed at The Natural History Museum, London (see elsewhere in these proceedings) has this feature inbuilt and allows for any record to be scored, on a dual system, from 1-4. A score of 1 indicates that the identification is certainly correct, 2 that it needs confirmation, 3 that it is probably incorrect and 4 that it is certainly incorrect. The same scoring applies for both parasitoid and its host. Whilst it would be a gargantuan task to systematically score this for all records, over a period of time scores can be added by specialists when the need arises.

3.2 Secondly, it should be possible to improve the accuracy of published host-parasitoid lists by following the recommendations given below.

– Do not repeat published host-parasitoid lists in subsequent publications unless they have been checked by specialists in both parasitoid and host taxonomy.

– Consult appropriate taxonomists **before** starting a research project, or even before starting to prepare a grant application.

A complaint of specialists, working for identification services or other organisations, is that they frequently receive collections of insects for identification at

the end of a project and without any previous consultation. Consulting a taxonomist early may ensure goodwill but it will also indicate the likely systematic problems that may arise and how long identification may take, or indeed whether reliable identification is possible. Preparation of material is also very important and will vary from group to group. This may be absolutely crucial to the correct identification of a species, particularly so with small chalcidoids and other microhymenoptera. The cost of identification necessary for a project can also be included in any grant proposal.

– Isolate hosts and preserve their remains with the parasitoids that emerge.

It cannot be stressed strongly enough how important it is to try to isolate parasitised hosts and to preserve their remains with any parasites that emerge. In many cases where the actual host has been misidentified initially it is possible to reidentify the host's remains. It may also be important in reassessing cases where the host/parasitoid association seems improbable such as a leaf miner parasitoid being reared from a syrphid larva.

– Include at least locality and host information with any material to be identified.

This is beneficial in two ways: a) it may prevent the specialist from making incorrect assumptions about the area or host from which a parasitoid has originated, or b) it may allow the specialist to make helpful comments about the material that has been identified.

– Deposit voucher material of parasitoids and their host remains in a well-maintained, major collection.

– State in any publication who identified the host and/or parasitoids and state where the material has been deposited.

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Table 1. Recorded parasitoids of Plutella xylostella. Information obtained mainly from Shenefelt (1969–1980), Thompson (1943–1958), Herting (1971–1982), and Review of Applied Entomology (A) (1980–1992).

Reliably recorded species

Aphanogmus fijiensis
Spilochalcis albifrons
Spilochalcis fulvovariegatus
Spilochalcis side
Brachymeria excarinata
Brachymeria phya
= Brachymeria plutellophaga
Elasmus sp.
Oomyzus sokolowskii
Tetrastichus howardi
= Tetrastichus ayyari
= Tetrastichus israeli
Eurytoma sp. indet.
Trichogramma chilonis
Cotesia marginiventris
Cotesia plutellae
Apanteles ippeus
Microplitis plutellae
Protomicroplitis claritibia
Diadegma fenestrale
Diadegma insulare
= Diadegma polynesialis
= Diadegma hellulae
= Diadegma plutellae
= Diadegma pygmaeus
= Diadegma congregator
Diadegma leontiniae
Diadegma rapi
Diadegma semiclausum
= Diadegma eucerophaga
Diadegma varuna
Diadegma xylostellae
Diadromus collaris
= Diadromus brischkei
Diadromus subtilicornis
= Diadromus plutellae
= Diadromus ustulatus
Phaeogenes plutellae
Mesochorus facialis
Stictopisthus sp.
Macromalon orientale
Triclistus xylostellae
Diatora prodeniae
Nepiera moldavica
Apanteles halfordi

Confirmation required

Calliceras sp.
Hockeria sp.
Antrocephalus sp.
Spilochalcis chapadae
Spilochalcis flavopicata
= Spilochalcis delira
Spilochalcis hirtifemora
Brachymeria sidnica
Brachymeria femorata
Dibrachys cavus
Habrocytus phycitidis
Megadicyclus dubius
Mokrzeckia indica
Trichomalopsis apanteloctena
= Trichomalopsis parnarae
Trichomalopsis oryzae
Trichomalopsis shirakii
Trichomalopsis viridescens
Trichogramma brasiliensis
Trichogramma evanescens
Trichogramma minutum
Trichogramma ostriniae
Trichogramma pretiosum
Trichogrammatoidea armigera
Apanteles aciculatus
Apanteles albipennis
Cotesia rubecula
Dolichogenidea sicarius
Macrobracon hebetor
Bracon gelechiae
Bracon greeni
Meteorus pulchricornis
Apanteles glomeratus
Microplitis pseudomediana
Apanteles ruficornis
Apanteles sodalis
Apanteles viminetorum
Apanteles wilkinsoni
Campoletis sp.
Diadegma neocerophaga
Diadromus erythrostomus
Dicaelotus parvulus
Phaeogenes ischiomelinus
Gelis tenellus
Itoplectis alternans
= Itoplectis spectabilis
Itoplectis maculator
Itoplectis naranyae
Itoplectis viduata
Itoplectis tenetanus
Pimpla nipponica
Voria rurlalis
Apanteles alexanderi
Apanteles emarginatus
Apanteles eriophyes
Apanteles fuliginosus
Apanteles lacteipennis
Apanteles limbatus
Apanteles litae
Apanteles piceotrichosus
Apanteles ruficrus
Apanteles vestalis

Species erroneously recorded as parasitoids associated with *Plutella xylostella*

Pediobius bruchicida
Cotesia vestalis
Dolichogenidea sicarius (New Zealand)
Chelonus ?versatalis
Chelonus ritchiei
Chirotica sp.
Diadegma armillata
Diadegma cerophaga
Diadegma chrysosticta
Diadegma gibula
Diadegma holopyga
Diadegma gracilis
Diadegma interrupta
Diadegma lateralis
Diadegma monospila
Diadegma tibialis
Diadegma trochanterata
Diadegma vestigialis
Hyposoter ebeninus
Lienella sp. indet.

Table 2. Recorded hosts of *Cotesia glomerata.* List obtained mainly from Shenefelt (1969–1980), Thompson (1943–1958), Herting (1971–1982), and Review of Applied Entomology (A) (1980–1992). (Names as they appear as cited in these publications and not checked for current status.)

Pieris brassicae
Pieris monustae
Pieris oler acea
Pieris prodice
Pieris rapae
Pieris daplidace
Aporia crataegi
Pieris napi
Leptidea synapis
Rhodocera rhamni
Colias lesbia
Tatochile autodice
Thais polyxena
Melitaea aurinia
Vanessa cardui
Vanessa urticae
Nymphalis polychloros
Nymphalis antiopa
Naranga aenescens
Archips xylosteanus
Ascia monuste
Orgyia antiqua
Panolis piniperda
Cirphis unipunctata
Callophrys rubi
Lampides boeticus
Thyris fenestrella
Bombyx mori
Cymatophora acicularis
Chelonia caja
Sphenarches caffer
Phylactaenia ferrugalis
Geometra papilionaris
Abraxas grossulariata
Geometra padella
Dicranura vinula
Phigalia pilosaria
Phigalia pedaria
Deilinia pusaria
Acronycta bidens
Lycaena phlaes
Polia psi
Acronicta tridens
Triphaena fimbria
Amathes xanthographa
Amphira pyramidea
Meganephria oxyacanthae
Dichonia areola
Lithophane ornithopus
Brachyonicha sphinx
Catocala nupta
Autographa gamma
Phytometra brassicae
Agrotis triangulum
Notodonta ziczac
Pygaera pigra
Pygaera anachoreta
Cheimatobia brumata
Anthophila pariana
Gracillaria syringella
Isophrictis tanacetella
Larentia vernaria
Lozotaenia foersterana
Lysandra coridon
Phalonia posterana
Platyptilia rhododactyla
Macroglossum stellatarum
Amorpha populi
Cochlidion limacodes
Notodonta dromedarius
Zygaena peucedani
Zygaena ephialtes
Bembicia hylaeiformis
Actornis chrysorrhoea
Liparis auriflua
Lymantria monacha
Porthetria dispar
Euproctis similis
Dendrolimus pini
Malacosoma neustria
Chesias spartiata
Larentia viridaria
Cidaria galiata
Colotois pennaria
Selenia bilunaria
Epiblemma immundana
Hypogymna morio
Phragmatobia fuliginosa
Botys forficalis
Phlyctaena rubigalis
Zygaena trifolii
Mamestra brassicae
Trichoplusia ni
Limentis camilla
Polia pisi
Plutella maculipennis
Pentatoma (eggs)

Host families for
Cotesia glomerata

Arctiidae
Gelechiidae
Geometridae
Glyphipterygidae
Gracillariidae
Lasiocampidae
Limacodidae
Lycaenidae
Lymantriidae
Noctuidae
Notodontidae
Nymphalidae
Pieridae
Papilionidae
Pterophoridae
Pyralidae
Saturniidae
Sessiidae
Sphingidae
Thyatiridae
Tortricidae
Yponomeutidae
Zygaenidae

Pentatomidae

Computerised Database of World Chalcidoidea: an introduction

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Noyes, J. S. 1994. Computerised Database of World Chalcidoidea: an introduction. Norwegian Journal of Agricultural Sciences. Supplement 16. 71-75. ISSN 0802-1600.

Information is provided on a computerised database of the taxonomy, biology and distribution of Chalcidoidea being developed currently at The Natural History Museum, London.

Keywords: biology, Chalcidoidea, computerised database, distribution, taxonomy.

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INTRODUCTION

Of all parasitic insects used in biological control programmes, the most successful group is probably the Chalcidoidea. Two recent independent surveys (Greathead 1986, Noyes, 1985) have shown that chalcidoids are important in at least half of all biological control programmes and have been used in at least twice as many programmes as the Ichneumonoidea. The success of any biological control programme ultimately depends upon the correct recognition of the pest, discovering its point of origin and identifying its natural enemies. This process can be enhanced given rapid access to a comprehensive and reliable source of information concerning the pest and its natural enemies. At present, such information is extremely difficult to obtain because it is scattered through the literature in many thousands of separate publications. This situation is further complicated by the dynamic state of zoological nomenclature, many species appearing in the literature under several quite different names. To add to this, a recent survey (see Noyes, elsewhere in these proceedings) of some well known pest (eg. *Plutella xylostella*) and parasitoid (eg. *Cotesia glomerata*) species has shown that as many as two thirds of all published host-parasitoid records may be incorrect because of misidentifications or wrong host associations.

The taxonomic catalogue and bibliography of World Chalcidoidea being maintained at The Natural History Museum, London is the most complete and up-to-date on this group in the World. The transfer of records from 5X3" library record cards was started in August 1991 and since then the total bibliography of some 27,000 references has been computerised, as well as the transfer of taxonomic data for the 27.000 or so described taxa of Chalcidoidea. Current work includes the entry of information on hosts, biology and distribution from the literature as well as checking reference citations for accuracy and keyword content.

The Natural History Museum, London is the ideal place to undertake this project. The library facilities are the most comprehensive in the World, with at least 90% of all entomological literature easily accessible within the museum. Other literature is available in the nearby libraries of the Royal Entomological Society and CAB International. Further to this, five of the top chalcid taxonomists are on site or are frequent visitors, i.e. Z. Boucek, M.W.R. de V. Graham, J. LaSalle, J.S. Noyes and A. Polaszek. Other specialists are frequent visitors. Additionally, taxonomists of other insect groups, which may be important pest species and act as hosts for chalcids, also work in the museum. Their expertise is available for comment on host identifications, etc.

THE DATABASE

The software used for the the database is Borland's «Paradox 4.0». Paradox was chosen in preference to other database systems because it is extremely user friendly and very flexible with regards to even the most specialised search.

The database allows for rapid access to information that might be important to workers in any aspect of entomology, but especially biocontrol. To date, the main priority has been to complete the entry of taxonomic data and currently the database is very large, at about 60mb. However, it is envisaged that its size can be reduced eventually to about 20mb, without losing any information, by improving the structure of its user interface. This should also improve its performance.

The database is relational and consists of two parts.

The first part is a functional taxonomic database which presently consists of seven tables, six of which deal with taxonomy, hosts, key words and distribution. The seventh («Master») table provides a link between the cited taxonomic name and the currently accepted valid name and is linked to the other six tables via the cited taxonomic name (family level, generic level or species level). Each of the six subsidiary tables is also directly linked to the separate bibliographic database. This part of the database is designed to be updated regularly and easily. Information remains linked to the cited parasitoid name, even if that name is currently being treated as invalid due to synonymy, homonymy, etc. This is an important aspect in the design which is based on a 1-to-many relationship and which allows for the dynamic properties of classification and nomenclature. Thus if a taxon is synonymised and later removed from synonymy for any reason, information attached to that name does not become irretrievably lost as a result of the initial synonymy. A summary of the main fields associated with each table and the relationship between the tables can be found below.

The second part is the bibliography which contains two linked tables. The smaller table allows for «overflows» from certain fields in the larger table and also includes four unique fields which summarise information on publishers, editors, available English translations (where necessary).

A) *Summary of main fields in each of the taxonomic database tables*

A. a) MASTER TABLE

11 fields including:
Valid genus; valid species; author (of valid genus or species); cited genus; cited species; author (of cited genus or species); cited subgenus; cited subspecific name

A. b) FAMILY GROUP NAMES
– Linked to MASTER

7 fields including:
Reference to original citation or subsequent emendations;
original spelling

A. c) GENUS GROUP NAMES
– Linked to MASTER

8 fields including:
Reference to original citation; type species designation

A. d) SPECIES GROUP NAMES
– Linked to MASTER

16 fields including:
Reference to original citation; primary type designation; depository of primary type; sexes described; locality data for primary type

A. e) TAXONOMIC STATUS/CHANGES
– Linked to MASTER

16 fields including:
Taxonomic status (new synonymy; new combination; misidentification, etc.); reference to original paper where taxonomic change was proposed; reference to paper where misidentification was noted; key words

A. e) BIOLOGY/HOSTS/KEYWORDS
– Linked to MASTER

24 fields including:
Reference to cited paper; parasitoid type (internal, external, phytophagous, hyperparasitoid, etc.); primary host; secondary host; associate animal species (eg. for parasitoids reared from a host in a gall other than the primary gall maker); host families; associated plant species; country of record; statement of reliability of record as scored by experts (various levels from totally reliable to totally unreliable).

A. f) DISTRIBUTION
– Linked to MASTER
9 fields including:
Reference to cited paper; zoogeographic region; country; state; statement of reliability of record.

B) *Summary of fields used in Bibliography database tables*

B. a) REFERENCE
– Linked as a look-up table to all taxonomic tables except MASTER

20 fields including:
Author(s); year; actual date of publication; title of article; journal/meeting/book/etc. title; volume; pages; language; keywords; source of summary

B. b) REFERENCE EXTENSION
– Linked to REFERENCE

7 fields including:
overflows for author, title and journal fields from Reference table; publishers; notes (to be changed to Paradox «memo» field); available English translation

C) *Look-up tables*

C. a) KEYWORDS
– Linked to REFERENCES, TAXONOMIC STATUS, BIOLOGY/HOSTS/KEYWORDS

A maximum of 110 predefined keywords, including:
Classical biological control; Inundative biological control; Natural biological control; Adult morphology; Behaviour; Bibliography; Biological control (all types); Biology; Catalogue; Chalcidoid phytophagy; Chalcidoidea as pests; Cited in key; Classification; Climate; Competition; Courtship; Cytology; Diapause; Dispersal/movement of parasitoid; Distribution; Diversity/species richness; Ecology; Economics (Biocontrol); Effect of temperature on parasite; Egg morphology; Egg production; Embryology; Enzymes; Evolution; Fecundity; Fossil; Genetics; Greenhouse; Hibernation/overwintering; Host development; Host effect on p. morphology/size; Host feeding; Host immunity; Host locating; Host reaction to parasitism; Host selection; Hosts; Humidity, effect of; Hybridization; Hyperparasitism; Integrated pest management; Intraspecific variation; Kairomones; Key to genera; Key to species; Key to subfamilies/tribes; Laboratory experiments/studies; Laboratory rearing; Larval morphology; Life history; Mass rearing; Mathematical modelling; Mating; Micro organisms; Mimicry; Molecular genetics; Morphology of immature stages; Nutrition; Oviposition; Parasitism; Parasitoid development; Parasitoid longevity; Pathogens; Percentage parasitism; Pesticide, effect of; Photoperiodism; Phylogenetics; Physiology; Pollination; Pupal morphology; Races/strains, parasitoid; Release methods for BC agents; Reproduction; Review; Revision; Sampling; Semiochemicals; Sex pheromone; Sex ratio; Stored products; Swarming; Taxonomy; Ultrastructure; Venoms; Viruses; Zoogeography

C. b) LANGUAGES
– Linked to REFERENCES

Language of original publication

C. c) COUNTRY
– Linked to SPECIES, BIOLOGY/HOSTS/KEYWORDS and DISTRIBUTION

All the World's countries listed and each assigned to zoogeographic region. Larger countries such as P.R. China, Russia, USA, India, Brazil subdivided into states or administrative regions.

C. d) HOST FAMILIES
– Linked to BIOLOGY/HOSTS/KEYWORDS and HOST MISIDENTIFICATIONS

All known host families, including junior synonyms, are listed and assigned to order and where possible superfamily.

C. e) PARASITOID TYPE
– Linked to BIOLOGY/HOSTS/KEYWORDS

The main parasitoid lifeways are listed.

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Variations in diapause among populations of Eubazus semirugosus (Nees) (Hym.: Braconidae), a parasitoid of *Pissodes* spp. (Col.: Curculionidae)

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Kenis, M. 1994. Variations in diapause among populations of Eubazus semirugosus (Nees) (Hym.: Braconidae), a parasitoid of *Pissodes* spp. (Col.: Curculionidae). Norwegian Journal of Agricultural Sciences. Supplement 16. 77-82. ISSN 0802-1600.

Different populations of *Pissodes* spp. and their parasitoid *Eubazus semirugosus* were collected at various altitudes and reared in the laboratory for comparative studies on their phenology. When reared on non-diapausing *P. castaneus*, *E. semirugosus* from *P. pini* and *P. castaneus* from high altitudes showed a tendency to enter into an obligatory diapause in their host while the lowland populations developed without diapause. Mountain populations of *P. pini* also tended to enter into diapause in laboratory rearing while lowland populations were reared continuously. These variations in parasitoid development are regarded as an adaptation to the cycle of their hosts in natural environments. The mountain biotype of *E. semirugosus* is considered to be a better candidate for the biological control of *P. strobi* in North America than the lowland biotypes because it is more likely to synchronize with the seasonal occurrence of the target host.

Keywords: Biological control, diapause, *Eubazus semirugosus*, intraspecific variations, *Pissodes*.

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The braconid *Eubazus semirugosus* (Nees) is a common parasitoid of *Pissodes castaneus* DeGeer (= *notatus* F.), *P. pini* (L.) and *P. piniphilus* (Hbst.), three European weevils whose larvae feed in the inner bark of pine trunks (Kenis and Mills in press). *E. semirugosus* oviposits into host eggs and the hatching first instar larva remains quiescent until the host is in its last instar. The parasitoid larva then develops quickly and emerges to complete its development externally (Alauzet, 1987). In temperate regions, the weevils are usually univoltine, but any stage of development can be found at any time of the year, which allows *E. semirugosus* to develop two generations per year. In cooler climates, their development can be biennial (Kangas 1938, Kudela 1974, Alauzet 1982, 1987).

Recently, *E. semirugosus* has been chosen by Kenis and Mills (in press) as a possible candidate for the biological control of *Pissodes strobi* (Peck), a serious pest of pines and spruces in North America. In order to select the strain of *E. semirugosus* which would be the best adapted to the life cycle of the target host, different host and geographic biotypes were compared in their phenology. Preliminary observations showed important differences with respect to diapause between these ecotypes. These observations are summarized in this paper.

MATERIAL AND METHODS

Pine logs containing last instar larvae of *Pissodes* spp. parasitized by *E. semirugosus* were collected at various sites in central Europe between 1990 and 1993. *Pissodes castaneus* was collected at Beaumotte (France, Haute-Saône; 6°09'E, 47°26'N; 300m alt.) and Derborence (Switzerland, Valais; 7°14'E, 46°17'N; 1300m alt.). *P. pini* was found at Delémont (Switzerland, Jura; 7°19'E, 47°27'N; 500m alt.), S-Charl (Switzerland, Graubünden; 10°19'E, 46°43'N; 1600m alt.), Zernez (Switzerland, Graubünden; 10°09'E, 46°41'N; 1900m alt.) and Brusson (Italy, Valle d'Aosta; 7°43'E, 45°45'N; 1500m alt.). Finally, a single population of *P. piniphilus* was collected at Vicques (Switzerland, Jura; 7°26'E, 47°22'N; 600m alt.). All the host trees were Scots pine (*Pinus sylvestris* L.) except at the sites S-Charl and Zernez where mountain pine (*P. uncinata* Miller) was collected. Logs were incubated in the laboratory for host and parasitoid emergence. Parasitoid adults were reared in gauze covered wooden cages (50x30x30cm) and fed with honey. Water was provided as moistened cellulose paper. *Pissodes* weevils were reared in larger cages (100x50x50cm) with fresh Scots pine branches immersed in water as food source. Branches were renewed every fourth day. Freshly cut Scots pine logs (4–10 cm diameter, 20–40 cm length) sealed at the end with paraffin wax were offered for oviposition.

For comparative studies on the development of *E. semirugosus* biotypes, parasitoids were reared in the laboratory under standard conditions on non-diapausing *P. castaneus* as standard host. Pine logs were exposed to *P. castaneus* for oviposition during four days before being offered to the parasitoids for two days. Logs were then placed singly in cages for parasitoid emergence. Four months after parasitoid oviposition, the logs were debarked to count remaining host larvae which were dissected for parasitism. Temperature, humidity and light in the laboratory were 23±0.5°C, 70±10% RH and 16h daylength, respectively.

When sufficient numbers of *Pissodes* spp. were obtained from field collected logs, these were reared in the laboratory. Scots pine logs were exposed for four days to the weevils and then kept singly in cages for weevil emergence. Four months after oviposition, the logs were debarked to count *Pissodes* larvae in diapause.

In order to observe whether *E. semirugosus* enters into diapause also in the field on its natural host, logs containing parasitized *P. pini* were collected in the lowlands at Delémont and at high altitude at Zernez at two different times of the year. Larvae that had overwintered were collected in spring while, later in the season, another collection was made of a generation that had not yet overwintered. Logs were brought to the laboratory for *Pissodes* and *Eubazus* emergence. One month after the last emergence, the logs were debarked to count hosts and parasitoids in diapause.

RESULTS

When *E. semirugosus* from different hosts and altitudes was reared on *P. castaneus* from lowland, there were important differences in the development of the parasitoid strains. Parasitoids reared from the three *Pissodes* species collected at low altitudes emerged without diapause (Fig. 1) 4 to 7 weeks after oviposition. By contrast, the majority of *E. semirugosus* reared from *P. castaneus* and *P. pini* collected at the four

sites at high altitude did not complete their development on *P. castaneus*. Four months after oviposition, between 70% and 100% of the parasitized larvae were found as young larvae in living *Pissodes* larvae while all the unparasitized weevils had already emerged, suggesting that parasitoids had arrested host development and had entered into an obligatory diapause.

The rearing of *Pissodes* weevils gave similar results. Weevils of the three species collected at low altitudes developed without diapause, but two mountain populations of *P. pini* partly entered into a diapause.

P. pini and *Eubazus* from high altitudes in their natural environment also have a partial obligatory diapause. When hosts and parasitoids that had not yet overwintered were collected at Zernez in October, 41.7% of the *Eubazus* (n=84) and 16.2% of the hosts (n=37) emerged, while the logs collected in May after overwintering gave 92.7% emergence of *Eubazus* (n=55) and 96.7% of *P. pini* (n=124). Logs collected at Delémont always gave 100% emergence of both hosts and parasitoids, no matter whether the insects had already overwintered or not.

DISCUSSION

Populations of *E. semirugosus* in Central Europe can be separated into two groups with respect to their diapause behaviour and development. A first group reared from *P. castaneus*, *P. pini* and *P. piniphilus* collected at low altitudes (600m or lower)

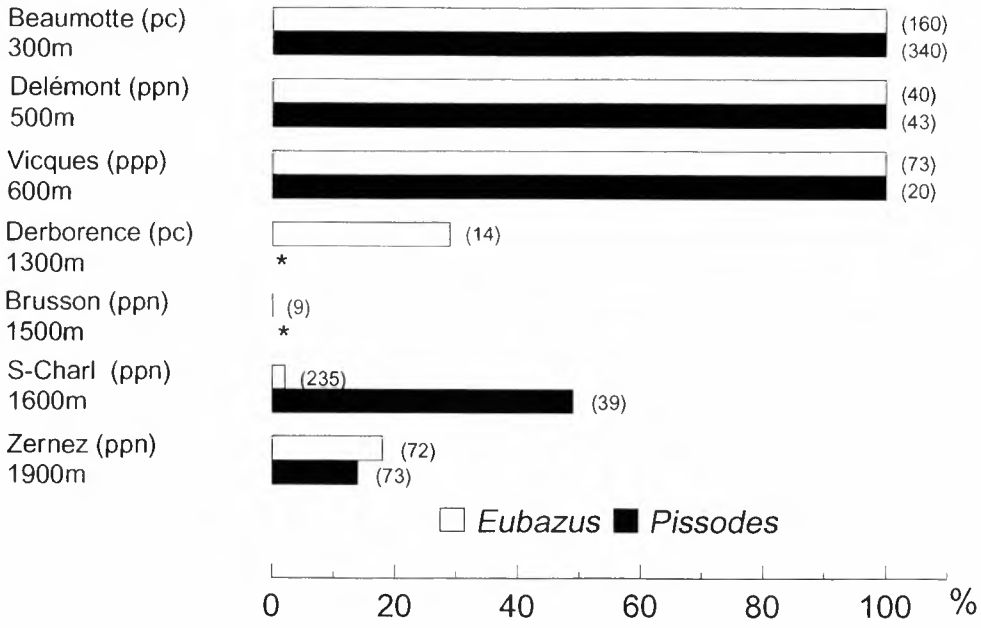

Fig. 1. Percentage of emergence without diapause of *Pissodes castaneus* (pc), *P. pini* (ppn) and *P. piniphilus* (ppp) and their *Eubazus semirugosus* from various sites reared in the laboratory. *E. semirugosus* were reared on a non-diapausing strain of *P. castaneus*. (n): number of insects emerged + in diapause.
*: *Pissodes* not tested

develops on *P. castaneus* in the laboratory without a diapause. A second group comprises populations from high altitudes (1300m or higher) from *P. castaneus* and *P. pini*. When these are reared on non-diapausing *P. castaneus* in the laboratory, the majority of them block the development of their host and enter into an obligatory diapause in the living *Pissodes* larva. Investigations are currently under way to determine the factors that induce and break diapause. Temperature is probably a key factor as in the field these *Eubazus* need to overwinter before they emerge, as was shown for the strain from *P. pini* at Zernez. However, *P. pini* from high altitudes also has a partial diapause, regardless of whether it is reared in the laboratory or in the field.

These variations in development between lowland and mountain parasitoid populations suggest genotypic differences which are probably a result of an adaptation to the cycle of their hosts in natural environments. At high altitudes, as observed at Zernez (Kenis, unpublished data), *P. pini*, and consequently *E. semirugosus*, only oviposit during the warmest months of the year, from June to August, and the weevil eggs laid in spring will only give rise to adult emergence the following year. Hence, *Eubazus* does not emerge before the winter when no *Pissodes* eggs are present but instead stays in the host larva until the following year when *Pissodes* adults oviposit. By contrast, in the lowland where development of *Pissodes* spp. is faster, eggs laid in spring will produce summer emergence. As the average temperature is higher, the oviposition period lasts much longer and every developmental stage can be found at any time (Alauzet 1982, Kenis unpublished data). Therefore, there is no reason for *Eubazus* not to emerge during the year of oviposition and, consequently, these *Eubazus* do not have an obligatory diapause.

Hymenopteran parasitoids often show important variations between strains when reared under the same conditions (see Hopper et al. 1993 and Ruberson et al. 1989 for review). Differences were found e.g. in acceptance of hosts, courtship, development time, fecundity, host range, insecticide resistance, morphology, mortality, pheromones, search, sex ratio, host suitability and temperature tolerance. However, very few observations were made on variations in diapause induction in living hosts. A notable exception is the study of Maslennikova (in Danilevskii 1965) on two geographic races of *Apanteles glomeratus* (L.) (Braconidae), a parasitoid of the cabbage white butterfly, *Pieris brassicae* (L.) She showed that diapause in these two races was induced by two different photoperiods and that diapause induction occurred when the parasitoid is still in the host.

In this example, the parasitoid kills its host before it enters into diapause. No case was found in the literature of an endoparasitoid which is able to enter into diapause in its host while this latter is still alive and has no diapause itself. But there are some examples of endoparasitoids that are able to delay their emergence from their host to allow synchronization with the latter. Carl (1976) reports the case of *Synomelix* sp. (Ichneumonidae), a parasitoid of the pear-slug, *Caliroa cerasi* (Tenthredinidae). This parasitoid overwinters as first instar larva in its host. While the latter is bivoltine, the parasitoid is strictly univoltine. *Synomelix* blocks the development of its host to emerge in autumn in order to synchronize with the second generation of the pear-slug. *Eubazus robustus* (Ratz.), a parasitoid of the pine cone weevil *P. validirostris* (Annila 1975, Roques 1975), is a braconid closely related to *E. semirugosus* for which it has often been mistaken (Kenis and Mills in press). The weevil and its parasitoid oviposit in pine cones in May and June. *P. validirostris* emerges from the cone in late summer,

overwinters in the litter and attacks new cones in the following spring. By contrast, only a small percentage of *E. robustus* emerges in summer, the rest overwinter in the host and emerge in spring to be synchronized with host oviposition. When the overwintering parasitized larvae are incubated in the laboratory in autumn, all the parasitoid adults emerge suggesting that these are not in true diapause but in simple quiescence (Roques 1975).

There is a great need to intensify studies on the biosystematics of natural enemies in biological control (Caltagirone 1985). Identification and characterization of biotypes should be part of each biological control programme before the release of natural enemies. Variations in developmental responses, temperature tolerance, pesticide resistance and encapsulation resistance are most likely to affect establishment or control of natural enemies (Ruberson et al. 1989, Hopper et al. 1993). Choosing a biotype which ensures seasonal synchronization with its host is particularly important for biological control programmes against native pests for which the control agents are selected from hosts which might have a different phenology from that of the target host. Most of the European *Pissodes* species overwinter at least partly as larvae while *P. strobi* typically overwinters as an adult. It lays eggs in spruce and pine leaders in spring and emerges between July and October. If *E. semirugosus* is selected for release against *P. strobi*, it would be of high importance to choose a strain which consistently induces larval overwintering in *P. strobi*. It is probable that the strains from the lowland would develop quickly and emerge in the year of oviposition, as they do on European *Pissodes* species. On the other hand, the mountain strains are more likely to block the development of *P. strobi* to overwinter in their host larva and thus, would ensure the synchronization of its phenology with that of the target host.

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Life history parameters of parasitoids attacking cereal aphids

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A comparison of parasitoid life histories was used to investigate the effects of parasitoids on population dynamics of cereal aphids. Data from the literature on developmental time, longevity, and fecundity of seven common parasitoid species under laboratory conditions were compared. As a single measure, age-specific fecundity accounts for variation in all three life history parameters. Therefore, we fitted a function to age-specific fecundity data from the literature. Data were found for only three of the parasitoid species. Some tentative conclusions on the effects of variation in age-specific fecundity on the population dynamics of cereal aphids are presented.

Keywords: Aphelinidae, Aphidiidae, cereal aphids, development, fecundity, life tables, longevity.

INTRODUCTION

To investigate the population dynamics of cereal aphids and their parasitoids, we compared the life histories of the parasitoids. Accurate estimates of life history parameters are an essential part of the development of realistic population simulation models (Gutierrez & Baumgärtner 1984) which are the core of an ongoing Danish research project on cereal aphids. Here, the literature on laboratory life tables of the dominant cereal aphid parasitoids is reviewed, and the essential life history parameters are extracted and compared.

Parasitoids attacking cereal aphids belong mainly to the hymenopterous families Aphidiidae (Ichneumonoidea) and Aphelinidae (Chalcidoidea). The general life histories of both families are similar, but only aphelinids feed on their hosts (Hagen & van den Bosch 1968, Stary 1988). Development, longevity, and fecundity vary between the species of parasitoids, and these life history traits are investigated here. For the sake of simplicity, effects of body size (Kouame & Mackauer 1992) and host-feeding by aphelinids (Jervis & Kidd 1986) were ignored. On a physiological time scale (i.e., degree-days (°D) above the developmental threshold), development and longevity are comparable among species. Fecundity is frequently reported as a lifetime cumulative quantity (Hofsvang 1991), but the use of age-specific fecundity rates appears more appropriate for studies on population dynamics, because the fecundity early in life has the greatest effect on the reproductive capacity. Due to their interdependence, development and longevity are expected to correlate with age-specific fecundity. Thus, age-specific fecundity may account for variation in parasitoid development, longevity and fecundity as a single measure. Fitting a function to existing data allows us to compare differences in age-specific fecundities between species. The fecundity function proposed by Bieri et al. (1983) has two important properties: the parameters can be interpreted biologically, and it has been used successfully for a range of insects (e.g., Graf et al. 1990).

Complete life tables of parasitoids attacking cereal aphids are sparse, and some assumptions were necessary to make the comparisons. The spectrum of parasitoid life histories range from those with a fast development, a short life, and a high initial fecundity to those at the other extreme with a slow development, a long life, and a low fecundity.

MATERIALS AND METHODS

The literature on six common (Kroeber & Carl 1991) aphidiid species, i.e., *Aphidius ervi* Hal., *A. picipes* (Nees), *A. rhopalosiphi* De Stefani-Perez, *A. uzbekistanicus* Luzhetski, *Ephedrus plagiator* (Nees), and *P. volucre* (Hal.) and the less common (Hoeller et al. 1993) aphelinid *Aphelinus varipes* (Foerster) was reviewed. These parasitoids have been recorded from all of the three main cereal aphid species, *Sitobion avenae* (F.), *Metopolophium dirhodum* (Wlk.), and *Rhopalosiphum padi* (L.) (Kroeber & Carl 1991).

The electronic databases *Agricola* (1970-June 1993), *CAB Abstracts* (1984-1992), and *Life Sciences* (January-November 1993) were searched for data on (1) the developmental threshold, (2) developmental time, (3) longevity, and (4) fecundity of the selected parasitoid species.

When estimates of the lower developmental threshold could not be determined from the data, a value of 6°C was assumed. The accuracy of the threshold estimate is not crucial when ambient temperatures are well above the threshold (Campbell et al. 1974), and 6°C is close to the thresholds reported for other northern temperate aphidiids (Campbell et al. 1974; Kambhampati & Mackauer 1989). The developmental time of pupae and average adult longevity were expressed in physiological time above the respective thresholds. Fecundity was determined as the number of eggs deposited, the number of larvae developing, or the number of mummies formed.

Bieri et al. (1983) proposed a function (1) that is easily fit to age-specific fecundity rates.

$$y = \frac{a * (x+c)}{b^{(x+c)}}$$

Parameters *a* and *b* were originally used by Bieri et al. (1983), but here we add a third parameter *c* which shifts the function to the left rather than forcing it through the origin. This modification allows for parasitoids to deposit fully developed eggs immediately after emergence. Parameters *a* and *b* affect the maximum and the shape of the curve with parameter *b* having the greater effect.

RESULTS

Life history data compiled from our literature research were not complete (Table 1).

In general, data on temperature dependency of parasitoid development were sparse with the developmental thresholds for only three of the species available. The assumed threshold of 6°C was approximately equal to the average of the estimated thresholds (6.12°C).

Table 1. Life history parameters of parasitoids attacking cereal aphids.

parasitoids	hosts	Θ_L [1]	T [2]	developmental time [3]		adult longevity		total fecundity	age-specific fecundity parameters			References
				days	°D	days	°D		a	b	c	
Aphidiidae												
A. picipes	*S. avenae*	6[5]	21[5]	3.5[4]	52.5	4.3[4]	64.5					Han 1983
Aphidius ervi	*S. avenae*	6[5]	21.5[4]	5	77.5							Feng et al. 1992
	M. dirhodum	6[5]	21.5[4]	5.5	85.3							Feng et al. 1992
	M. persicae	1.1[4]	21			15.4	258.72					Hofsvang & Hågvar 1975
	A. pisum	6	20	5.9	82.5	15.1	211.25	354	3.42	1.026	8.34	Digilio & Pennacchio 1989
	A. pisum	4.2		4.9[4]	76.7							Campbell et al. 1974
	A. pisum	6.7	23.6	4.1[4]	68.5			284				Kambhampati & Mackauer 1989
A. uzbekistanicus	*M. dirhodum*	7.2	20	5.4[4]	69.2			123				Dransfield 1979
A. rhopalosiphi	*S. avenae*	6[5]	18			11.25	135	240	1.46	1.022	6.63	Shirota et al. 1983
	S. avenae	6[5]	20	8.5	119							Powell 1986
Ephedrus plagiator	*S. avenae*	6.6	21	8.8	127.1	23	332.12					Jackson et al. 1974
	R. padi	7.5	21	8	108	15	202.5					Jackson et al. 1974
	S. graminum	6[5]	23.9	7.5	134.3							Dureseau et al. 1972
Praon volucre	*S. avenae*	6[5]	20	10.7	171.2	21	294	253	1.05	1.016	24.63	Ruggle (unpubl.)
Aphelinidae												
Aphelinus varipes	*S. avenae*	6[5]	21.5[4]	10.1	156.6							Feng et al. 1992
	D. noxia	9.65	25.7	13.9	223.1							Lajeunesse & Johnson 1992
	S. avenae	6[5]	20	13.3	186.2							Ruggle (unpubl.)

[1]) lower developmental treshold (°C)
[2]) temperature of the experiments (°C)
[3]) from mummy formation to adult emergence
[4]) estimated from data
[5]) assumed

Data on the developmental period was the most complete. The species of the genus *Aphidius* have the fastest development, with the order *A. picipes*, *A. ervi*, and *A. uzbekistanicus* and *A. rhopalosiphi*. *Ephedrus plagiator* and *Praon volucre* develop more slowly than any of the *Aphidius* species, and the chalcidoid *Aphelinus varipes* exhibits the slowest development.

Data on longevity tend to correlate with development: *A. picipes* shows the shortest, *A. ervi* an intermediate, and *E. plagiator*, and *P. volucre* the longest life span. Longevity estimates may be less reliable, because they strongly depend on food availability to the adult parasitoid (Digilio & Pennacchio 1989).

Comparable fecundity data were rare, and age-specific fecundity rates were found only for *Aphidius ervi* and *A. rhopalosiphi*. (Fig. 1). Although the peak fecundity of *Aphidius ervi* is twice that of *A. rhopalosiphi*, the total fecundity is only 50% higher. The parameters *a* and *b* (Table 1) were lower for *A. rhopalosiphi*. For *P. volucre* lower parameters *a* and *b* were estimated from preliminary data. *Aphelinus varipes* feeds on its hosts and lays few eggs (personal observations) for a very long time like other aphelinids (Hagen & van den Bosch 1968; Stary 1988). This may be related to the host-feeding behavior.

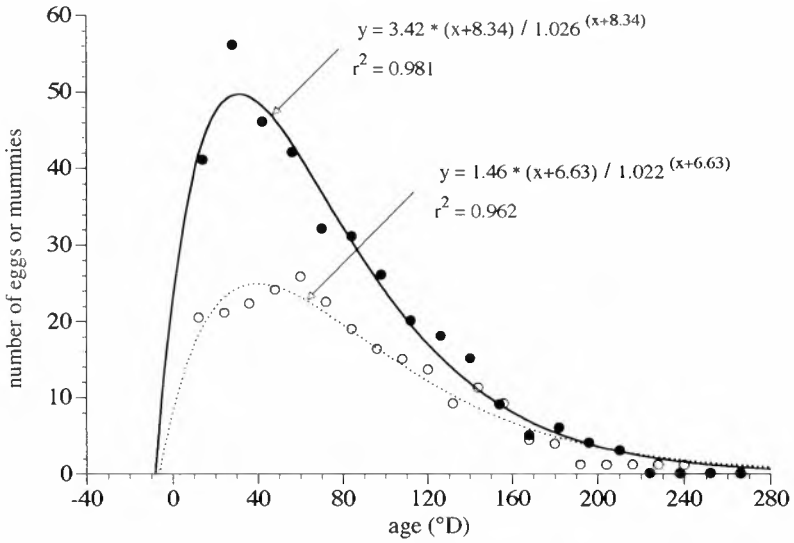

Fig. 1. Mean age.specific fecundity of *Aphidius ervi* (dots) and *Aphidius rhopalosiphi* (circles) (data from Shirota et al. 1983 and Digilio & Pennacchio 1986).

DISCUSSION

If age-specific fecundities of the parasitoids investigated are correlated with the data on development and longevity, then we expect the fecundity function to yield decreasing values for parameters *a* and *b*, and increasing values for parameter *c* in the following order: *Aphidius spp.* -> *Ephedrus plagiator* -> *Praon volucre* -> *Aphelinus varipes*. Variation in age-specific fecundity is especially important for the dynamics of the cereal aphid-parasitoid system, because of the short season of about 1000 °D (Denmark). More age-specific fecundity data are necessary to determine the relationships between the different life history parameters of aphid parasitoids and to draw final conclusions about their role in population dynamics of cereal aphids. Force & Messenger (1964) used the innate capacity of increase (r_m) to assess the effectiveness of three parasitoids (two aphidiid and one aphelinid species) of the spotted alfalfa aphid in California alfalfa fields. They present three distinct patterns of age-specific fecundity similar to the cases presented here. An analysis of the cereal aphid-parasitoid system analogous to Force & Messenger (1964) could be performed, if complete age-specific life tables of the parasitoids became available. In addition to such an analysis, simulation modeling based on the results presented here can be used to investigate the population dynamics of parasitoids and cereal aphids.

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Differential impact of three *Sitobion avenae* parasitoids

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In order to evaluate their efficiency, the demographic parameters and the intrinsic rate of natural increase of *Aphidius rhopalosiphi*, *Aphidius ervi* and *Praon volucre*, three species of *Sitobion avenae* parasitoids, were compared at 23.7±1.2°C. The comparison of single demographic parameters couldn't give a clear conclusion: the short length of development of *A. rhopalosiphi* and *A. ervi* gave them an advantage but the greater fecundity of *P. volucre* counterbalanced it. However, the r_m of *P. volucre* (0.292 f/f/d) was greater than the r_m of the other species: 0.278 f/f/d for *A. rhopalosiphi* and 0.211f/f/d for *A. ervi* but not significantly so (Jackknife confident intervals). These r_m were equivalent to the r_m of *S. avenae* published by Acreman and Dixon in 1989 at 25°C, on the flag leaf. The three species have equivalent potentials as parasitoids of *S. avenae* under those experimental conditions.

Key words: aphid parasitoids, development, fecundity, intrinsic rate

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INTRODUCTION

Sitobion avenae (Fabricius) is, with *Metopolophium dirhodum* (Walker) and, to a lesser extent, *Rhopalosiphum padi* (Linnaeus) (Homoptera : Aphididae) one of the major insect pests of wheat in Europe (Dean & Luuring 1970, Latteur 1970). Entomophagous fungi (Latteur 1976) and Aphidiidae parasitoids (Jones 1972, Latteur 1976) are important control agents of those species. Field investigation have shown that *Aphidius rhopalosiphi* (De Stefani-Perez), *Aphidius ervi* (Haliday) and *Praon volucre* (Haliday) are major parasitoid species of *S. avenae* in Western Europe. The objective of this study was to compare the efficiency of those species to control *S. avenae* populations. We have established the life-tables of the three species and determined their intrinsic rate of natural increase (r_m) (Birch 1948).

The comparison of the r_m obtained for the three parasitoid species with the r_m of *S. avenae,* obtained in the similar conditions, (Dean 1974, Simon et al. 1991, Acreman & Dixon 1989) will indicate which parasitoid has the best fitness for *S. avenae* (Bhatt & Singh 1991, Mackauer 1983).

MATERIALS AND METHODS

Insect rearings

The culture of *S. avenae* was started in May 1993 with 10 green parthenogenetic females collected in the same field near Louvain-la-Neuve (Belgium) on winter wheat (*Triticum aestivum*), cv. Estica. These aphids were maintained on winter wheat seedlings, cv. Camp Remy, at 20±3°C, 60% r.h. and 16 hr light. The cultures of *A. rhopalosiphi* and *A. ervi* were initiated with individuals collected twenty days later from the same location. The culture of *P. volucre* was started one month before the experiment, in July, with females and males collected on *Acyrthosiphon pisum* (Harris) in a pea culture near Louvain-la-Neuve. Each parasitoid rearing was started with at least 3 females and 3 males and maintained on *S. avenae* on wheat seedling. All rearings were done in 0.4m^3 wooden cages in the same conditions as the aphids.

Standardisation of the aphids and of the females parasitoids for the experiment

Aphid parasitoids can parasitize all host instars but second and third instars are often preferred (Kirsten & Kfir 1991, Shu-sheng 1985b, Shirota et al. 1983). The female parasitoids were provided with larvae of 2nd and 3rd instars (48 to 96 hr old) of *S. avenae*. Larvae were obtained by placing between 50 to 70 adults of *S. avenae* on 15 seedlings, 10 cm high, of winter wheat, cv. Camp Remy, for 2 days. After that period, the adults were removed. The larvae were kept on the seedlings during two more days before the introduction of a female parasitoid.

Because size of the female parasitoids (Shirota et al. 1983), and then the size of the host influence the bionomics of aphid parasitoids, the parasitoids used for the experiment came only from 2nd and 3rd instar aphids.

Fecondity, longevity, length of development, sex ratio and rate of mummies emergence

Females, less than 24 hr old provided with a honey solution, and in contact with other female and male parasitoids, were used for this experiment. Nine females of *P. volucre*, eleven of *A. rhopalosiphi* and fourteen of *A. ervi* were followed during all their adult life. This experiment was performed at 23.7±1.2°C, 56±4% r.h. and 16 hr of light (±6000 lux of intensity). In order to establish the daily fecundity of the females during the first 9 days, each female was offered daily more than 100 *S. avenae* larvae (106±11, n=27) on 15 wheat seedlings in an experiment cage made with a clear plastic bottle, 26 cm high and 8 cm of diameter. The top of the bottle was covered with a 300 µm muslin maintained with a rubber band. During the first day, one male was also introduced in the cage. After, from the 10th day until it died, each female was provided with 100 aphid larvae but this time every two days. Female Aphidiidae oviposit most of their egg complement during the first week of their adult life (Cohen & Mackauer 1987, Shu-sheng 1985, Messenger 1964, Shirota et al. 1983), and therefore 100 larvae per two days are enough to avoid significant superparasitism after the first week of female life. The longevity of the females was also noted.

After 9–10 days, mummies were collected with a piece of leaf and placed by groups of 10 or less in a petri dish. They were incubated in the same conditions as the experiment and checked daily until the emergence of the adults parasitoids which were counted and sexed. From those data, the length of development, the sex ratio and the rate of mummies emergence were calculated. Only the results obtained with mated females were used in the analysis.

Larval survival rate and superparasitism rate

During its life, a female parasitoid was offered daily ca 100 aphids of L2–L3 instars. Fifty of these aphids were stored, 4 days later, in 95% alcohol until dissection to determine the number of eggs and L1 larvae they contained. The other aphids were kept on wheat until mummies formation. This experiment was replicated 3 times.

Superparasitism, which was calculated as the number of eggs found per host attacked (Cohen & Mackauer 1987), had to be included in life-table analysis because it is a major larval mortality factor for first instar parasitoid. In those species, elimination of supernumerary individuals occurs by physical attack between first instar larvae (Chow & Sullivan 1984). The mortality of the other larval instars was estimated as the difference between the parasitism rate of the aphids found by dissection and those found by counting the mummies formed among the non-dissected aphids attacked by the same female and from the same cohort.

Data analysis

The demographical parameters were analyzed separately and then used to prepare a life-table. The intrinsic rate of increase (r_m) was obtained by resolving the Birch's approximation of Lotka's equation (Lotka 1956) :

$$e^{-r_m \cdot x} \cdot lx \cdot mx = 1$$

with
x = age
lx = age-specific survival
mx = age-specific fecundity

The solution is obtained by recurrence. To estimate the variance of r_m, the Jackknife method was used (Meyer et al. 1986). All these analysis were performed with the SAS software (SAS Institute 1985).

RESULTS

Length of development and females longevity

The length of development of the three species of parasitoids on *S. avenae* were highly significantly different (GT2 method (Sokal & Rohlf 1981)) between species and also between sexes of the same species (Table 1). The females of *P. volucre* with 13.6 days, took one more day longer to develop than *A. ervi* and two days more than *A. rhopalosiphi*. The males emerged sooner than the females.

The longevity of the females (Figure 1) was also very different between the three species. After seven days, 100% of *A. ervi* females died, while 45% of *A. rhopalosiphi* and 89% of *P. volucre* females were still alive. The last female of *A. rhopalosiphi* died after 13 days whereas 55% of the *P. volucre* specimens continued to reproduce. The last female of *P. volucre* died after 22 days.

These two parameters disadvantage *P. volucre* from a demographical point of view. In fact, the longer duration of development and longevity of *P. volucre* extend considerably its generation time and population doubling time.

Table 1. Length of development of three aphid parasitoids. Means with the same letter are not different at the 0.01 level – GT2 method (Sokal & Rohlf 1981).

spcies	sex	N	mean ± SD
P. volucre	m	721	13.1 ± 0.9 a
	f	858	13.6 ± 0.9 b
A.rhopalosiphi	m	421	11.1 ± 0.9 c
	f	815	11.6 ± 1.0 d
A.ervi	m	726	11.7 ± 0.9 d
	f	419	12.3 ± 1.0 e

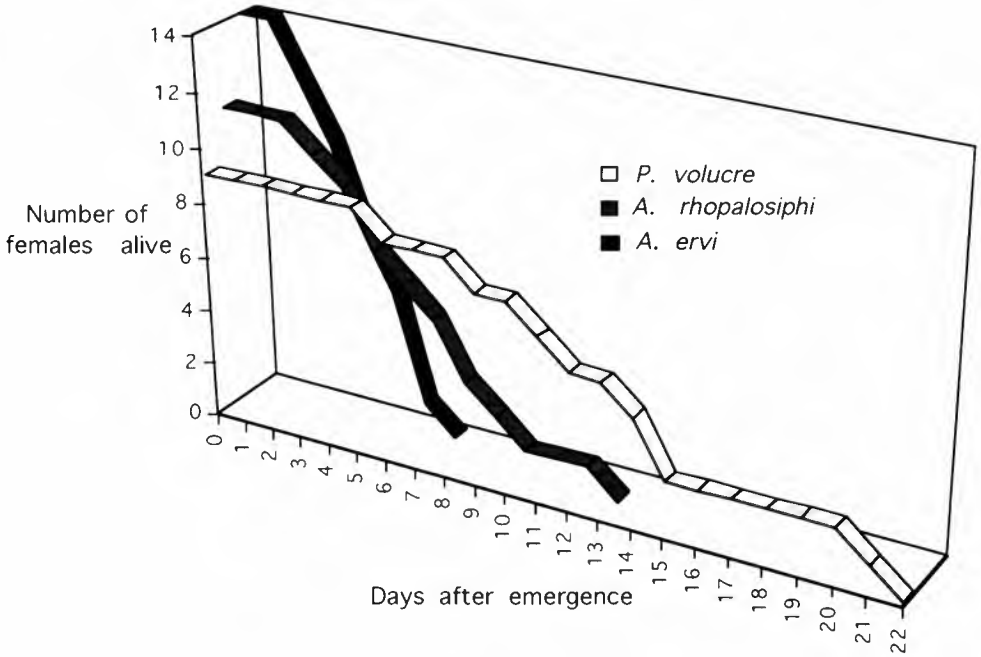

Fig. 1: Survivorship of the females of *A. rhopalosiphi, A. ervi* and *P. volucre* at 23.7 °C.

Pre-adult mortality

The pre-adult mortality of *A. ervi* (46%) was larger and probably occurred before the mummies formation (Table 2). Moreover, a large proportion of *A. ervi* mummies (17%) never emerged at this temperature. *A. ervi* mummies (n=24) maintained at this temperature were then dissected after three weeks: 46% were diapausing larvae (L4 alive), 42% were dead L4, 8% were mature alive and 4% dead adults. For the two other species, 24-25% of the specimens died before adult emergence. This mortality was mostly due to superparasitism for *A. rhopalosiphi* and to death before the formation of the mummies for *P. volucre*.

Table 2. Pre-adult mortality for the three parasitoids species.

	A. rhopalosiphi	*A. ervi*	*P. volucre*
Superparasitism (mortality between instar L1 and L2) [1])	11.00%	6.00%	7.00%
Other larval instars mortality [2])	6.65%	17.00%	13.39%
Mummies died or in diapause [3])	8.00%	18.00%	7.50%
Total pre-adult mortality	24.00%	46.00%	25.00%

[1]) (number of eggs and L1 found by dissection – number of parasitize aphids obtained by dissection) / number of eggs and L1 found by dissection.
[2]) ((number of parasitize aphids obtain by dissection – number of mummies formed among the second half of the same cohort) / number of parasitize aphids obtain by dissection)
[3]) ((number of mummies-number of adults) / number of mummies

Sex-ratio

Except for *P. volucre*, the sex-ratio was significantly different from 1:1. *A. rhopalosiphi* produced more females than males (1:0.5) and *A. ervi* significantly more males than females (0.6:1) under those experimental conditions. The proportion of females among the progeny was greater at the beginning of the reproductive period.

Number of mummies by female

There was no significant difference between the number of mummies obtained for *A. ervi* and *A. rhopalosiphi*. One of the nine females of *P. volucre* gave no mummy at all. This female induced a large variance of the mean number of mummies by female *P. volucre* and, therefore no significant difference with the two other species was found. If this female is ignored, *P. volucre* produced significantly more mummies by females than the two *Aphidius* species. The same was true with the number of females by female (Table 3).

No clear difference in fitness resulted from those analysis. The length of development disadvantages *P. volucre* but advantages *A. rhopalosiphi*; the sex-ratio of *A. rhopalosiphi* is favorable but not the one of *A. ervi*; and the fecundity of *P. volucre* is interesting. The intrinsic rate of increase of the three species were calculated to better distinguish the species.

Life-table analysis

The product of the age-specific survival by the age-specific fecundity synthetizes the previous observations. The peak of *P. volucre* was higher than the two other species but it was reached three days after *A. ervi* and two days after *A. rhopalosiphi* (Figure 2). *A. rhopalosiphi*, which has a shorter length of development, took more time to

Table 3. Fecundity of the three parasitoid species.

	Mean number of mummies per female	IC95[1])	Mean number of females per female	IC95[1])
A. ervi	101.8	[45.5–124.6]	30.9	[10.8– 35.7]
A. rhopalosiphi	106.4	[58.6–136.3]	65.2	[32.7– 86.6]
P. volucre[2])	370.9	[43.5–988.5]	170.9	[25.2–398.5]
P. volucre[3])	417.3	[339.1–491.5]	192.3	[132.7–248.6]

[1]) Obtain after a log. tranformation of (x+1).
[2]) With the female which laid no eggs.
[3]) Without the female which laid no eggs.

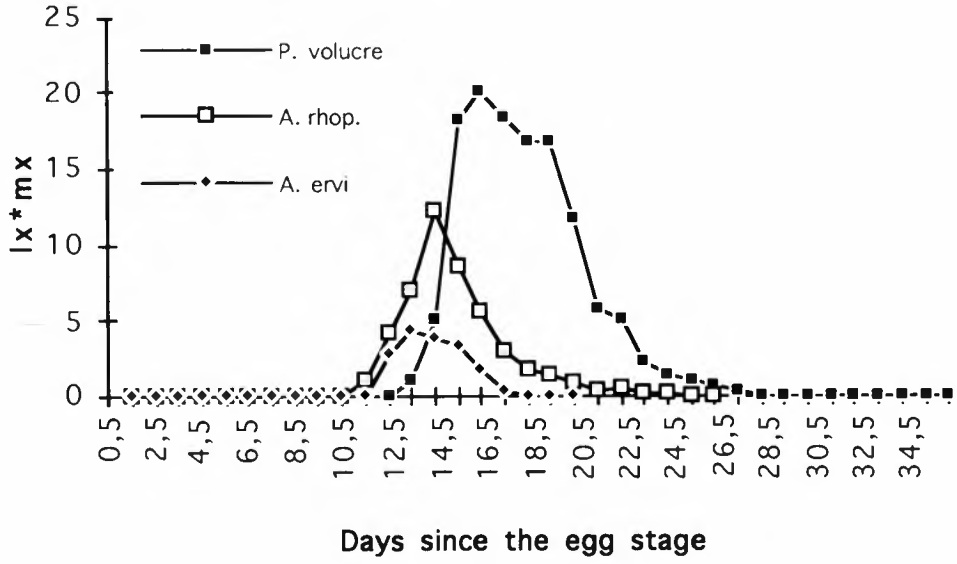

Fig. 2: Evolution of the lx∗mx of *A. rhopalosiphi, A. ervi* and *P. volucre* at 23.7°C.

reach its peak than *A. ervi*. Its peak was more clearly defined and narrower than for the two other species.

After the resolution of the Birch's approximation of the Lotka's equation, the r_m of our cohorts were determined. *P. volucre* had the greater r_m with 0.292 f/f/d, followed by *A. rhopalosiphi* with 0.278 f/f/d, and *A. ervi* with 0.211 f/f/d (Table 4). In spite of the differences, none of them was significantly different from the other by the Jackknife method (Figure 3).

A. ervi was the only species for which the Jackknife estimator of r_m was not close to the experimental value of the r_m. That seems to be due to the large *A. ervi*

larval mortality (Meyer et al. 1986) that caused the variance around this estimator to be twice as great as the estimator variance of the two other species.

In spite of large differences between their net reproduction rate, the finish rates of increase of *A. rhopalosiphi* and *P. volucre* are very similar (Table 4).

Table 4. Results of life-tables analysis of three parasitoid species.

	A. rhopalosiphi	*A. ervi*	*P. volucre*
Number of females followed	11	14	9
Females longevity (days)	7.1±2.9 a[1])	4.5±1.6 a	13.9±5.2 b
Sex-ratio (f:m)	1:0.5	0.6:1	1:1
Larval mortality (%)	24%	46%	25%
Intrinsic rate of natural increase r_m (f/f/d)	0.278	0.211	0.292
r_{est} found with the Jackknife method (f/f/d)	0.274	0.241	0.293
Standard Error (r_{est})	0.017	0.034	0.016
Confident Intervals (0.95)	(0.237–0.311)	(0.171–0.311)	(0.258–0.328)
λ, Finish Rate of Increase (f/f/d)	1.32	1.23	1.34
R_0, Net Reproduction Rate (f/f)	46.4	14.7	123.9
T, generation time (d)	13.8	12.7	17.7
t_2, doubling time (d)	2.49	2.88	2.37
G.R.R., Growth Reproduction Rate (f/f)	95.4	38.0	174.1

1) Number with the same letter are not significantly different (GT-2 test)
f: females – m: males – d: days

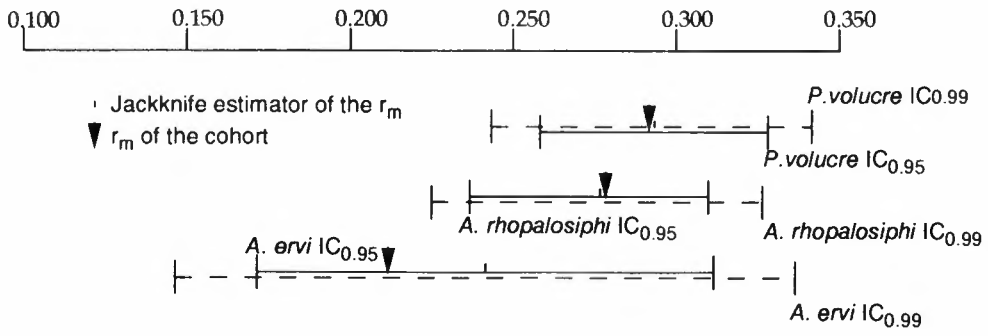

Fig. 3: Confident intervals of the r_m estimator.

DISCUSSION

Its longer developmental time and longevity disadvantages *P. volucre* because the factors involving timing in the life cycle influence more the r_m than the fecundity during the adult life (Messenger 1964).

In the literature, the pre-adult mortality of aphid parasitoids has often been considered as nul: either the number of eggs was estimated from the number of mummies formed (Bhatt & Singh 1991), or the number of mummies was deducted from the number of eggs laid (Cohen & Mackauer 1987). The r_m were then overestimated, in the first case by a surestimation of the lx and in the second case by a surestimation of the lx and the mx. This pre-adult mortality can be divided into host death before mummies formation (the nymphal mortality of *S. avenae* at 25°C is between 8.5 and 12.1% (Acreman & Dixon 1989)), mortality due to superparasitism, parasitoid's larval mortality and parasitoid's pupal mortality. Diapause was considered here as a mortality factor, and host mortality was incorporated in the parasitoid's larval mortality.

The results obtained here showed that the immature stage mortality was more important than expected and reached 25%. The 46% mortality obtained for *A. ervi* was mostly due to the diapause of the L4 parasitoids under those conditions.

The sex-ratio of *A. ervi* (0.6:1) disadvantages this species while the sex-ratio of *A. rhopalosiphi* (1:0.5) advantaged it. The fecundity of *P. volucre* was greater than the two other species resulting in three times more mummies.

It is difficult to describe the evolution of natural populations of host-parasitoids using only the r_m of the two species because these intrinsic rates of increase are subjected to two contraints. The experimental conditions are kept constant, and the r_m is of value only under these conditions. A stable age distribution is also necessary to permit the r_m calculation (Lotka 1956). Stable age distribution is not reached during the colonisation of a field by a pest (Hance 1988, 1990), however it is during this phase that the parasitoid must be present and control its host. The intrinsic rate of natural increase remains however of great value to compare demographical performances between species and between different strains and morphs of the same species in a given condition (Acreman & Dixon 1989, Dean 1974, Kambhampati & Mackauer 1989, Reed et al. 1992, Simon et al. 1991).

The simple demographical variables observed permitted no clear conclusion. The r_m of the three parasitoid species integrate all these variables and is thus a good index of a parasitoid on a given host (Kambhampati & Mackauer 1989, Messenger 1964).

The r_m of *P. volucre* (0.292 f/f/d) was larger than *A. rhopalosiphi* (0.278 f/f/d) and *A. ervi* (0.211 f/f/d) one's but never significantly so. The Jackknife method gave a less precise estimate of *A. ervi* r_m probably because of a larger larval mortality for this species (Meyer et al. 1986). However, when the r_m is calculated with a nul larval mortality and a sex-ratio of 1:1, *A. ervi* (0.340 f/f/d) had the greatest r_m, followed by *P. volucre* (0.326 f/f/d) and *A. rhopalosiphi* (0.307 f/f/d)), although these differences are not significant. The larger Gross Reproduction Rate of *P. volucre* is conterbalanced and surpassed by the earlier emergence (2-3 days) of the two *Aphidius* species.

Under those conditions, the r_m of *S. avenae* is around 0.275 aphid/aphid/day, on the flag leaf, and 0.375 aphid/aphid/day, on the ear (Acreman & Dixon 1989). The r_m

of *A. rhopalosiphi* and *P. volucre* are equivalent on the flag leaf, but on the ears the pest can easily compensate for the attack of the parasitoid.

In conlusions, the three parasitoid species seem to have a similar fitness for *S. avenae* at 23.7°C. The three species have an intrinsic rate of increase near the one of their host when *S. avenae* is on the flag leaf, a situation occuring at the beginning of the season.

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Behavioral ecology of host feeding in *Aphytis* parasitoids

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We studied host feeding strategies of *Aphytis* parasitoids using laboratory and field studies as well as dynamic state variable modeling. Further laboratory studies showed that host feeding can increase fecundity, but not longevity of *A. melinus* and *A. lingnanensis*. Dynamic modeling predicted host feeding to be favored when (i) parasitoids are young, (ii) egg loads are low, (iii) nutritional reserves are low, and (iv) host availability is high. A laboratory study showed that *A. melinus* are more likely to host feed at low egg loads. *A. melinus* fed pure sucrose were also more likely to host feed than *A. melinus* fed sucrose + yeast. Younger *A. melinus*, however, were not more likely to host feed than older *A. melinus*. In the field, *A. aonidiae* with lower egg loads were more likely to host feed than *A. aonidiae* with higher egg loads.

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The adult females of many species of parasitoid Hymenoptera feed upon the hemolymph or tissues of their host, a practice known as «host feeding». The importance of host feeding has been recognized for some time by applied entomologists (e.g. DeBach 1943; Flanders 1953; Bartlett 1964), and a recent exhaustive review of the topic has brought host feeding to the attention of behavioral ecologists as well (Jervis & Kidd 1986). The review by Jervis & Kidd has sparked a number of theoretical treatments of host feeding (e.g. Houston et al. 1992; Chan & Godfray 1993; Collier et al. 1994) as well as empirical tests of theory (e.g. Bai & Mackauer 1990; Sahragard et al. 1991; Rosenheim & Rosen 1992; Collier et al. 1994; Heimpel & Rosenheim unpubl.).

The main goal of these studies has been an increased understanding of the conditions under which host feeding is favored over oviposition. Factors investigated have included conditions associated with the host (e.g. host quality and availability) as well as states of the parasitoid (e.g. parasitoid egg load and nutritional reserves). Specific theoretical predictions vary with assumptions about the biology of host feeding, and are discussed elsewhere (see refs. cited above). Generally speaking, however, host feeding is increasingly favored as parasitoids become more egg-limited, and oviposition is favored under conditions of time limitation.

In this paper, we discuss our empirical and theoretical investigations of host feeding strategies in *Aphytis* parasitoids (Hymenoptera: Aphelinidae). We have reviewed host feeding behavior in *Aphytis* spp. elsewhere (Rosenheim & Heimpel 1994). The main goal of this paper is to give an overview of our work with three species of *Aphytis*: *A. aonidiae* (Mercet), *A. lingnanensis* Compere, *and A. melinus*

DeBach. The research involved four phases. First, we investigated the influence of host feeding on longevity and fecundity of *Aphytis*. Second, we constructed a dynamic state variable model to generate formal predictions for the effect of egg load on host feeding strategies. Third, we conducted a laboratory test of the model. Lastly, we tested the predictions of the model in a field setting.

EXPERIMENTS AND A MODEL

I. Influence of host feeding on longevity and fecundity

We used the following protocol to determine whether host feeding alone can substantially increase the longevity of *A. melinus* and *A. lingnanensis*. One-day old adult females were placed in an arena with excess hosts, and the arenas were checked three days later for the presence of live or dead parasitoids. As a control treatment, parasitoids were placed in an arena containing hosts and a streak of honey. Studies by other workers have shown that longevity of *Aphytis* in the presence of hosts and honey greatly exceeds 3 days (Quednau 1963; Abdelrahman 1974; Gulmuhamad & DeBach 1978; Rosenheim & Hoy 1988). For the experiment with *A. melinus*, the hosts used were third instar oleander scale, *Aspidiotus nerii* Bouché. For *A. lingnanensis*, hosts were first instar California red scale, *Aonidiella aurantii* (Maskell). In the experiment involving *A. lingnanensis*, an additional control treatment was included: a group of parasitoids was placed in arenas containing neither hosts nor honey.

The results of these experiments are presented in Table 1. In both cases, most *Aphytis* held with only hosts died within three days. As was expected, almost all *Aphytis* held with hosts and honey lived for at least three days. Inspections of hosts in arenas after the parasitoids were removed showed that host feeding had taken place in all arenas containing hosts. None of the *A. lingnanensis* held in arenas with neither hosts nor honey survived for three days. This absence of survivors in arenas with no hosts was significantly different from the survivorship in arenas containing only hosts (Likelihood ratio $\chi^2 = 4.7$, $P < 0.05$), indicating that host feeding was making a slight contribution to longevity.

Table 1. Proportion of *Aphytis* adults surviving for three days in arenas containing hosts only, or hosts and honey.

	PROPORTION OF PARASITOIDS SURVIVING 3 DAYS (n)	
Contents of Arena	*A. melinus* with oleander scale (3rd instar)[a]	*A. lingnanensis* with California red scale (1st instar)
Hosts only	0.10 (20)	0.22 (18)
Hosts and honey	0.92 (13)	1.00 (14)
No hosts or honey	–	0.00 (13)
Likelihood ratio χ^{2b}	24.9	24.8
P	<0.0001	<0.0001

[a] Results from this experiment are also reported in Rosenheim & Heimpel (1994)
[b] The chi-square test excluded arenas wih no hosts

Next, we determined the value of one host-feeding meal for egg production by *A. lingnanensis* on virgin female third instar California red scale. Two-day old female parasitoids that had been held individually in vials containing honey were observed continuously as they oviposited on a series of hosts until they began to host feed. At this point, they were randomly allocated to either of two treatment groups: oviposit only, or oviposit and host feed. Individuals in the oviposit only group were removed from the host, just as host feeding started, and individuals in the oviposit and host feed group were allowed to feed at will on one host. Parasitoids from both groups were then returned to vials containing honey and held for two additional days. After these two days, the then four day-old parasitoids were dissected and the number of mature eggs present in the ovaries (the egg load) was determined. The length of both hind tibiae of each parasitoid were measured as an index of size. Also, to determine the relationship between parasitoid size and egg load for two-day old parasitoids, a separate group of *A. lingnanensis* was dissected for egg load, and hind tibia lengths were measured. All parasitoids were held at 26.7±1° C and 75±10% R.H. A more detailed description of the arenas and dissection protocol will be given by Heimpel & Rosenheim (unpubl.).

Egg loads at four days of age for parasitoids from the oviposit and host feed group were not significantly different from those of the oviposit only group (Fig 1a; $t = 1.0$, $P > 0.3$). However, because parasitoids varied in initial egg load and the number of eggs laid (from 1-8), the mean egg load at four days is not a sensitive measure of egg maturation. A more sensitive comparison would take into account (i) egg loads of the parasitoids at the age of two days, when the oviposition and host feeding took place, and (ii) the number of eggs laid by each parasitoid. We knew how many eggs were laid, but could not know the exact egg load at two days, since parasitoids were held until the fourth day of life for dissection. However, dissections from a separate group of 2-day old *A. lingnanensis* showed that there was a very strong positive relationship between hind tibia length and egg load (Fig. 2; $r^2 = 0.91$, $F = 191.7$, $n = 20$, $P < 0.0001$). The linear regression equation for egg load at two days (EL_2) is:

$$EL_2 = 1.53(\text{hind tibia length}) - 19.34 \qquad (1).$$

With this equation, an estimated change in egg load during the two days following the assay (Δ_{el}) can be easily calculated if the egg load at day four (EL_4) and the number of eggs laid during the assay is known:

$$\Delta_{el} = EL_4 - (EL_2 - \text{eggs laid}) \qquad (2),$$

where (EL_2 – eggs laid) represents the estimated egg load after oviposition. By applying this equation, we found that the estimated increase in egg load between days two and four was significantly greater for parasitoids that had oviposited and host fed than for parasitoids that had oviposited only (Fig. 1b; $t = 3.0$, $P < 0.01$). The group ovipositing matured an estimated 0.9 eggs in the two day period, while the group ovipositing and host feeding matured an estimated 3.2 eggs (Fig. 1b). In summary, we have shown that host feeding alone does not substantially increase longevity in *A. melinus* and *A. lingnanensis*, and that host feeding does promote egg production in *A. lingnanensis*. We have also shown that, for *A. lingnanensis* feeding on 3rd instar

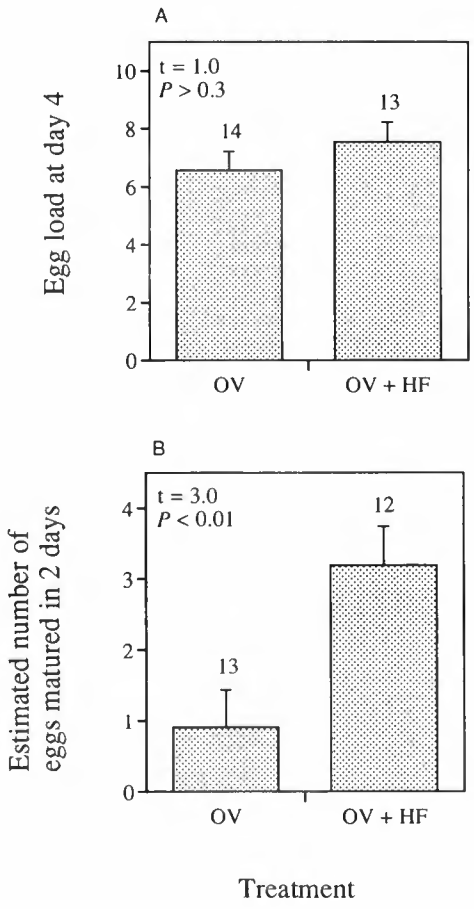

Figure 1. (A), Egg load at day four, and (B), estimated number of eggs matured in two days (Δel from eq. 2) for *A. lingnanensis* individuals that were prevented from host feeding after ovipositing (OV) and individuals that were allowed to host feed after ovipositing (OV + HF). Numbers above columns are sample sizes.

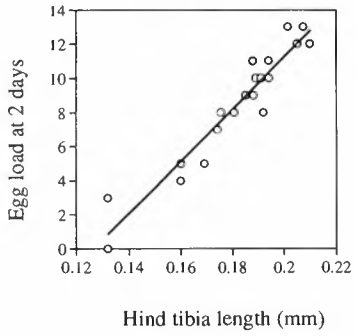

Figure 2. Relationship between mean hind tibia length and egg load at the age of two days for *A. lingnanensis*. The linear regression equation is: egg load = 1.53 (hind tibia length) – 19.34 (see also equation 1 in text); r2 = 0.914, F = 191.7, P < 0.0001.

California red scale, one host feeding meal translated into the production of about two eggs two days later in life. Our next objective was to use this information in constructing a predictive model of host feeding behavior.

II. A dynamic state variable host-feeding model

Dynamic state-variable modeling is an appropriate method for generating predictions for the outcome of the oviposit vs. host feeding «decision». Its main strength lies in its sensitivity to parasitoid life expectancy and «states», such as egg load and nutritional reserves (Mangel & Clark 1988). Dynamic modeling has been used to investigate host acceptance and clutch size decisions in parasitoids (e.g. Mangel 1987; 1989; Mangel et al. 1994), as well as the decision of whether to forage for hosts or (non-host) food sources (Mangel 1987). Recently, a number of dynamic state-variable models have been constructed for host feeding behavior as well (Houston et al. 1992, Chan & Godfray 1993, Collier et al. 1994). Here, we construct a model that is similar to the ones presented by Chan & Godfray (1993) and Collier et al. (1994). It does differ from these other models in some respects, however. One difference is that concurrent oviposition and host feeding on one host is allowed in our model but not in those of Chan & Godfray or Collier et al. Our rationale for including concurrent host feeding and oviposition was that (i) we have evidence that *A. melinus* and *A. lingnanensis* sometimes engage in concurrent oviposition and host feeding (Heimpel & Rosenheim unpubl.), and (ii) concurrent oviposition and host feeding is not uncommon in parasitoids in general (Jervis & Kidd 1986). We also included an explicit physiological cost of reproduction in our model. Chan & Godfray (1993) included cost of reproduction («state dependent mortality») in one of their models, but it is otherwise absent from host feeding models. We have no direct evidence for a physiological cost of reproduction in *Aphytis*, but we know that egg resorption takes place in *A. melinus* and *A. lingnanensis* (Heimpel & Rosenheim in press). If we assume that egg resorption serves some beneficial function, then oviposition must in part deprive the parasitoid of that function. Following this reasoning, we believe it is likely that *Aphytis* incur a physiological cost of reproduction.

In the model, four decisions are available to a parasitoid upon encountering a host: rejection of the host, host feeding only, concurrent host feeding and oviposition, and oviposition only. Assumptions include (i) host feeding contributes to fecundity, but not longevity, (ii) there is one host type only, and (iii) only one egg can be laid per host. The model uses the technique of backward iteration over time periods (Mangel & Clark 1988) to find the decision maximizing lifetime reproductive success. This optimal decision is calculated for each of a series of time periods. Within each time period, the optimal decision is calculated for each level of a state variable, which in this case is egg load. A second state variable, nutritional reserves, is evaluated within each egg load class as well. Host feeding contributes to the nutritional reserves, and material from the reserves is turned into eggs. There is therefore a time delay of one time unit between host feeding and egg maturation. This time delay is probably unrealistically short. Models constructed by Chan (1991) and Collier et al. (1994) have more realistic time delays, but such delays are beyond the scope of the current study. Longer time delays for egg maturation add realism, but the qualitative results of models with shorter time delays are similar. It is important, however, to include a time delay. Models lacking any time delay can give qualitatively different results from models that include even a slight delay.

The terms used in the model are listed in Table 2. To calculate which decisions maximize lifetime reproductive success, we formulated the following dynamic programming equation:

$$F(x, y, t, T) = (1-\lambda)p(x)\, F(x + r - \delta, y - r, t + 1, T) +$$

$$\max_d\ \lambda[Wf_d + p(x)\, F(x - c_d + r - \delta, y + hf_d - r, t + 1, T)] \qquad (3),$$

with the following constraints: (i) egg load, x, cannot exceed the egg capacity, which was set at 10 eggs, (ii) the nutritional reserves, y, cannot exceed an upper limit set at five egg units, (iii) the maximum number of eggs matured per unit time, r, is set at one, but if the nutritional reserves are depleted (y=0), or if the egg load is at capacity (x=10) then no eggs can be matured. Table 3 shows the host feeding values (hf_d), and immediate fitness (Wf_d) gained for each of the four decisions. By host feeding only, no immediate fitness is gained, but enough nutrients are acquired to mature two eggs. Ovipositing and host feeding on the same host reduces the quality of the host for offspring development. This lower quality can result either in a decreased probability of successful offspring development, or in lower quality (e.g. smaller) offspring; the model does not differentiate between these two scenarios. Also, when parasitoids host feed and oviposit concurrently, less of the host resource can be used for host feeding itself, since some must be left for the offspring. By ovipositing only, the parasitoid gains one unit of fitness immediately, but acquires no nutrients for future egg maturation.

As t approaches T, oviposition is increasingly favored over host feeding (Fig. 3). Fig. 3 also shows that oviposition is favored at higher egg loads and host feeding at lower egg loads. Note that concurrent host feeding and oviposition can be optimal under a relatively wide range of conditions. In our current formulation, by ovipositing and host feeding concurrently, the parasitoid obtains 75% of the host feeding value derived from host feeding only. To match this, we let the quality of the host as an oviposition site decline by 75% as well (Table 3). Obviously, different formulations of this trade-off will yield different optimal decisions. Another result of the model is that host feeding is favored at relatively high host availability, and oviposition is favored as host availability declines (compare Figs. 3a, 3b, 3c). In Figure 4, we consider the effect of egg load and nutritional reserves on optimal decisions at one time interval (t=7) and host availability level (λ = 0.9). Host feeding is favored at lower levels of reserves and, again, at lower egg loads (Fig. 4).

In summary, this dynamic state variable model has furnished us with a number of predictions:

P1: Host feeding is favored when parasitoids are young.

P2: Host feeding is favored when parasitoids have low egg loads.

P3: Host feeding is favored when nutritional reserves are low.

P4: Host feeding is favored at higher host availabilities.

We now describe a laboratory test of the first three of these predictions as well a field test of the second prediction.

III. A laboratory test of the model

We used direct observations of behavior to test predictions 1, 2 and 3 for *A. melinus* attacking second instar oleander scale. A detailed account of this experiment will be

Table 2. Terms used in the dynamic state variable model.

Term	Explanation
Decision variable:	
d	reject, host feed, host feed and oviposit, or oviposit
Independent variables:	
t	time period; last t is T, the time horizon; T set at 15
x	eggs; maximum egg capacity set at 10
y	nutritional reserves (in egg units); maximum reserves set at 5
Dependent variables:	
F(x,y,t,T)	fitness function: eggs deposited between t and T when X(t) = x and Y(t) = y
λ	host availability ($0 \leq \lambda \leq 1$)
p(x)	probability of survival = $(x/\text{ egg capacity})^{0.1}$; at x = 0, p(x) = 0.75
r	number of eggs potentially matured per t; set at 1
δ	number of eggs resorbed per t ; set at 0.2
Wf_d	immediate fitness gain, through oviposition (in egg units); see tab. 3
c_d	clutch (0 or 1 eggs); see table 3
hf_d	value of host feeding (in egg units); see table 3
z_d	change in host quality due to concurrent host feeding and ovipositing; see table 3

Table 3. Value of host feeding and immediate fitness gain through oviposition, with its derivations for the four decisions available to parasitoids in the dynamic state variable model.

Decision, d	Host feeding value, hf_d	Clutch size, c_d	Host quality change due to concurrent host feeding and oviposition, z_d	Immediate fitness gain, $Wf_d = c_d \times z_d$
Reject	0	0	1	0
Host feed only	2	0	1	0
Host feed and oviposit	1.5	1	0.25	0.25
Oviposit only	0	1	1	1

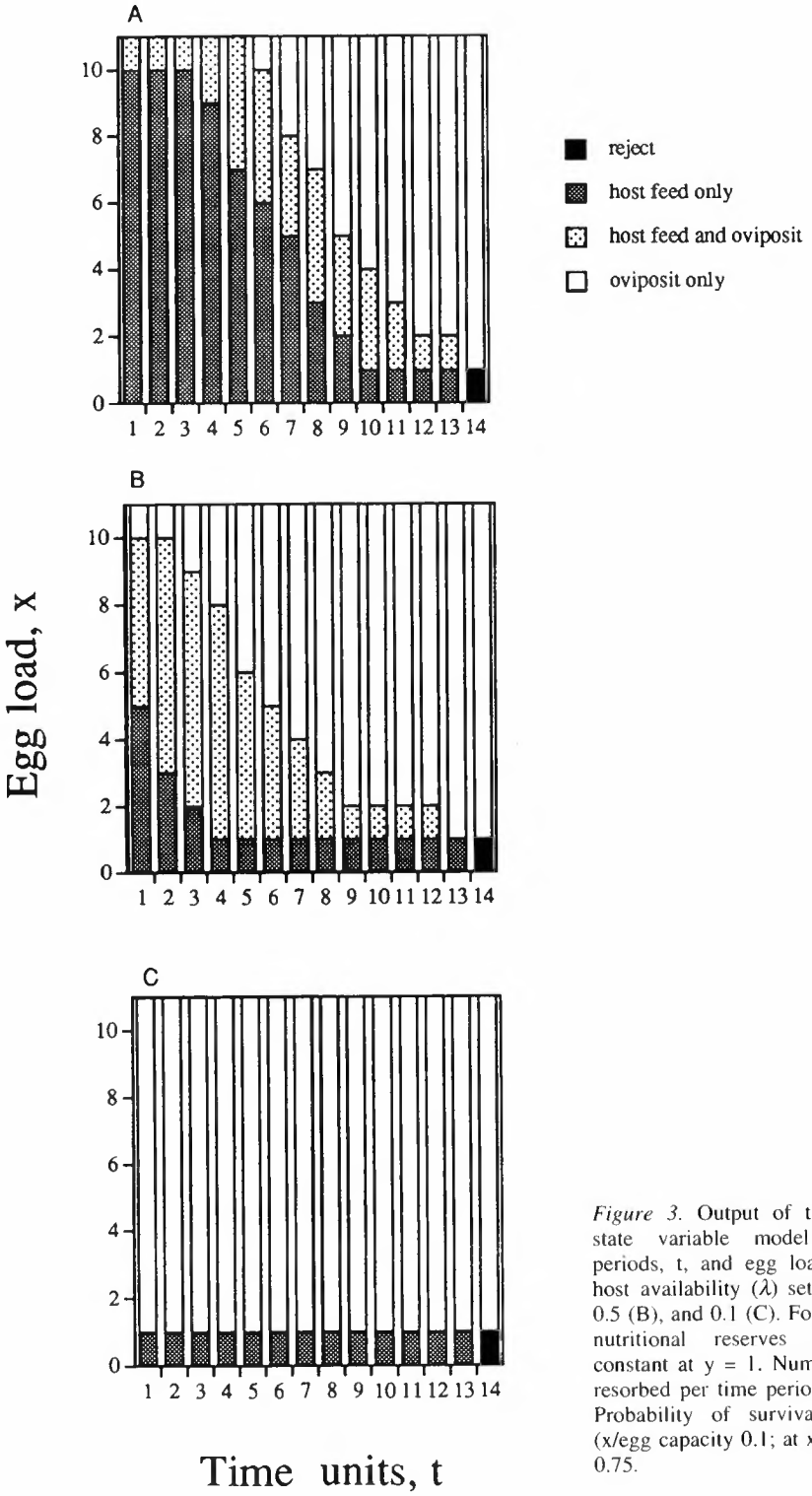

Figure 3. Output of the dynamic state variable model for time periods, t, and egg loads, x, with host availability (λ) set at 0.9 (A), 0.5 (B), and 0.1 (C). For these runs, nutritional reserves were held constant at y = 1. Number of eggs resorbed per time period (δ) is 0.2. Probability of survival (p(x)) is (x/egg capacity 0.1; at x = 0, p(x) = 0.75.

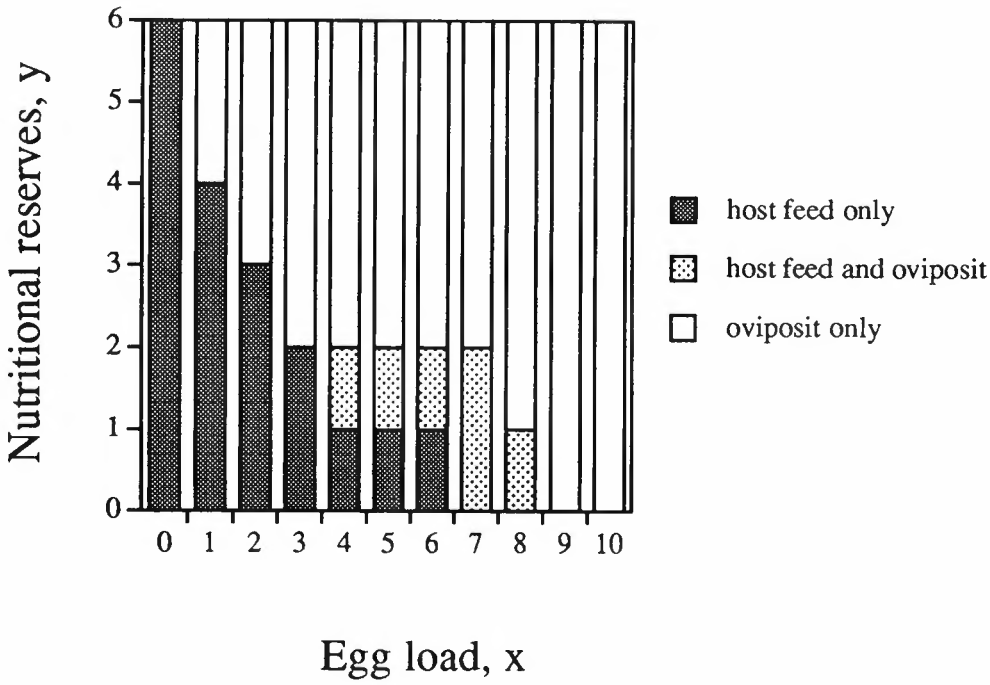

Figure 4. Output of the dynamic state variable model for egg load and nutritional reserves during one time period (t=7). Parameters as in Fig. 3.

given by Heimpel & Rosenheim (unpubl.). Here, we briefly review the methods and major results. Nutritional status was manipulated by feeding adult parasitoids either pure sucrose, or a solution of sucrose + yeast. Parasitoids were held in vials with only these foods and water until the observations were conducted. To manipulate parasitoid age, parasitoids were held for either 2, 5, or 15 days before observations took place. Egg resorption occurred by the 5th and 15th days in the wasps fed the pure sucrose diet, but parasitoids fed the sucrose + yeast diet resorbed very few eggs (Fig. 5). Egg load was therefore manipulated through the diet and age treatments: older wasps fed the sugar diet had lower egg loads than younger wasps fed the yeast diet. Also, larger parasitoids from all groups had, on average, higher egg loads than did smaller parasitoids (Fig. 5). Polychotomous stepwise logistic regression, a statistical modeling technique that allowed us to evaluate simultaneously a series of potentially confounded independent variables, was used to analyze the results.

The observations were performed as follows: individual parasitoids were placed into arenas containing one host at a time and observed continuously to determine whether they rejected the host, host fed only, host fed and oviposited concurrently, or oviposited only. After the observation, parasitoids were dissected for egg load and the length of a hind tibia was measured. Two host encounters were observed, but for this presentation only behavior on the first host is discussed.

Two of the three predictions being tested were supported. First, parasitoids with lower egg loads were more likely to host feed than parasitoids with higher egg loads

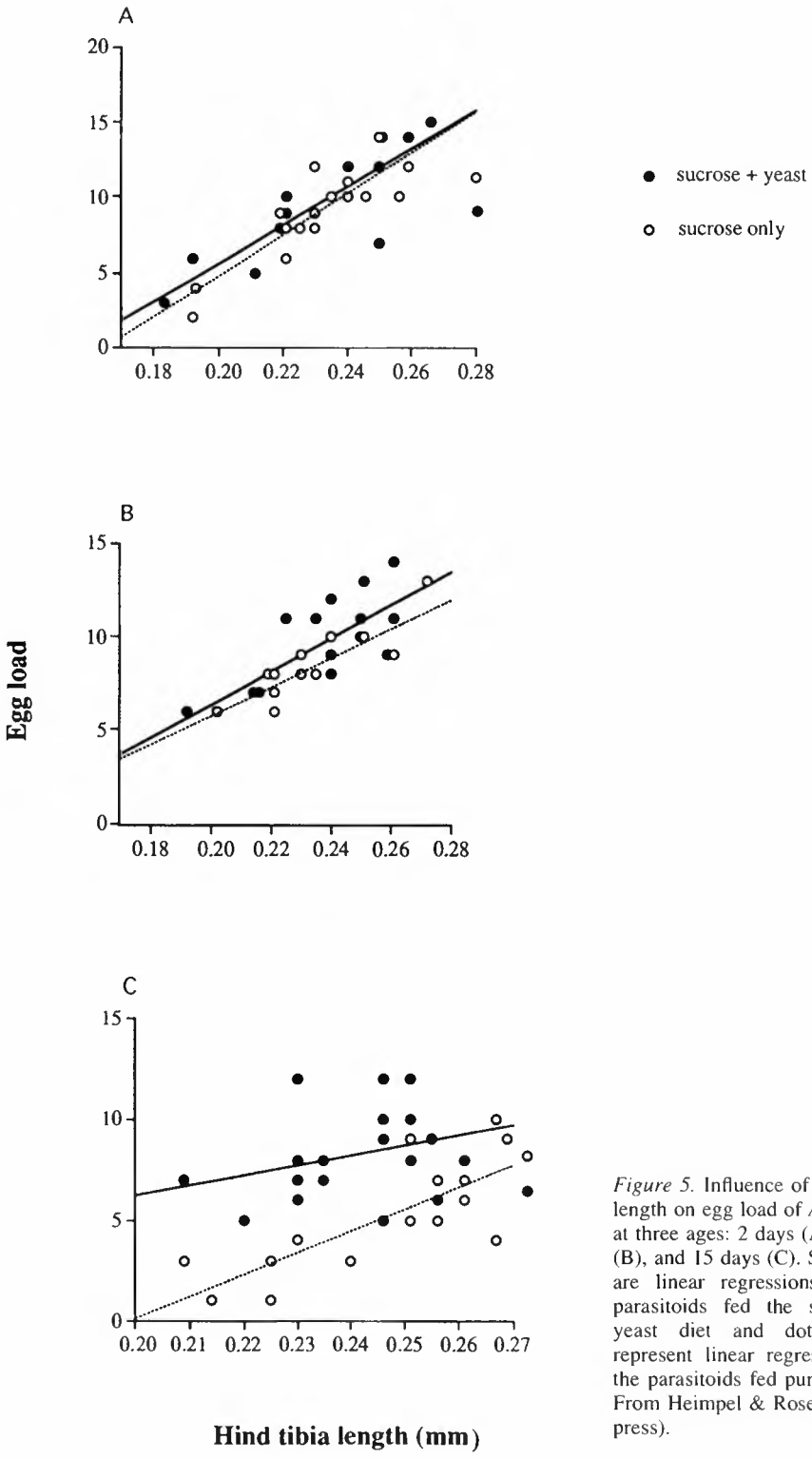

Figure 5. Influence of hind tibia length on egg load of *A. melinus* at three ages: 2 days (A), 5 days (B), and 15 days (C). Solid lines are linear regressions for the parasitoids fed the sucrose + yeast diet and dotted lines represent linear regressions for the parasitoids fed pure sucrose. From Heimpel & Rosenheim (in press).

(Fig. 6; stepwise logistic regression, $\chi 2 = 18.8$, $P < 0.001$). Second, parasitoids fed the pure sucrose diet were more likely to host feed than parasitoids fed the sucrose + yeast diet (Fig. 7; stepwise logistic regression, $\chi 2 = 13.4$, $P < 0.01$). Note that this difference was evident even in the youngest parasitoids, i.e. before there was an effect of diet on egg load (Fig. 7). However, younger parasitoids were not more likely to host feed than were older parasitoids (Fig. 7; stepwise logistic regression $\chi 2 = 3.4$, $P > 0.5$). Another result that was inconsistent with theory was that about 20% of the parasitoids host fed and oviposited on the same host, even though the eggs deposited during concurrent oviposition and host feeding never developed successfully to adulthood (Heimpel & Rosenheim unpubl.).

In summary, our laboratory studies showed that the behavior of *A. melinus* attacking second instar oleander scale is consistent with theory in some respects but not in others. Predictions for egg load and nutritional status were supported, but predictions for age were not. Our final objective was to test the theory in the field.

IV. A field test of the model

We conducted observations in the field of *A. aonidiae* attacking San Jose scale, *Quadraspidiotus perniciosus* (Comstock), growing on almond, *Prunus dulcis* in Sutter County, northern California in 1992 and 1993 (a detailed account of this study will appear later, Heimpel & Rosenheim in preparation). The natural population of *A. aonidiae* was providing moderate natural biological control of the San Jose scale. *A. aonidiae* were observed foraging on the bark, twigs and leaves of a tree until a host

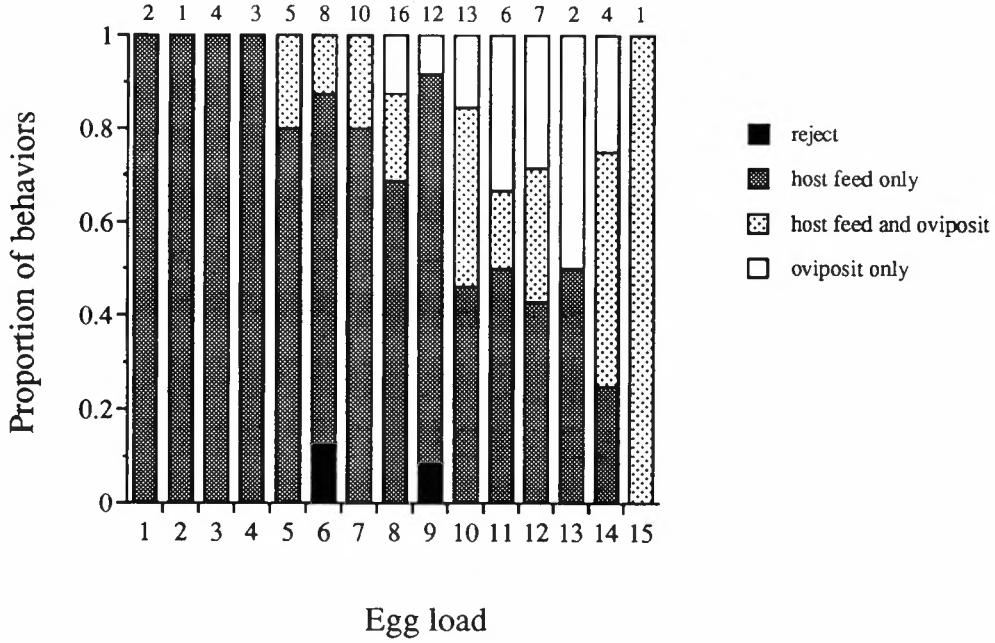

Figure 6. Influence of egg load on behaviors performed by *A. melinus* when offered one secondinstar oleander scale insect. Numbers above columns are sample sizes. From Heimpel & Rosenheim (in press).

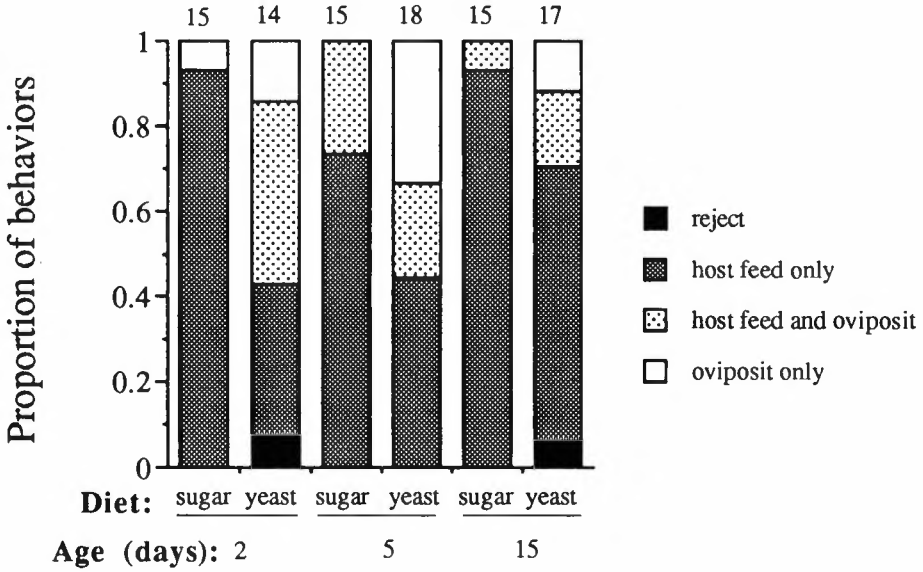

Figure 7. Influence of diet and age on behaviors performed by *A. melinus* when offered one secondinstar oleander scale insect. Numbers above columns are sample sizes. From Heimpel & Rosenheim (in press).

was encountered. The host encounter was then also observed. Host feeding events were easily recognized by the prostrate position of the parasitoid, but oviposition was difficult to distinguish from host rejection, so subsequent inspections of the hosts were used to distinguish these behaviors. After the host encounter was completed, the parasitoid was aspirated and put on ice and the host was removed from the tree and also put on ice. Both parasitoid and host were then returned to the laboratory where the parasitoid was dissected for egg load, and the host was inspected for signs of parasitism.

Eighteen *A. aonidiae* were observed host feeding ovipositing, and 28 were observed ovipositing. Parasitoids host feeding had significantly lower egg loads than parasitoids ovipositing (Fig. 8a; $t = 2.5$; $P < 0.05$). This result supports prediction #2, above, namely that host feeding should be favored at lower egg loads. It is also consistent with the results of the lab studies using *A. melinus* described above.

DISCUSSION

Our aim in this paper was to give an overview of our investigations into the behavioral ecology of host feeding in *Aphytis* parasitoids. We feel that, in general, the four research areas that we have discussed are key to forming an understanding of host feeding strategies. Before formulating hypotheses about optimal host feeding strategies, it is important to know what the parasitoid gains and does not gain by host feeding. We discovered that host feeding alone does not substantially increase *Aphytis*

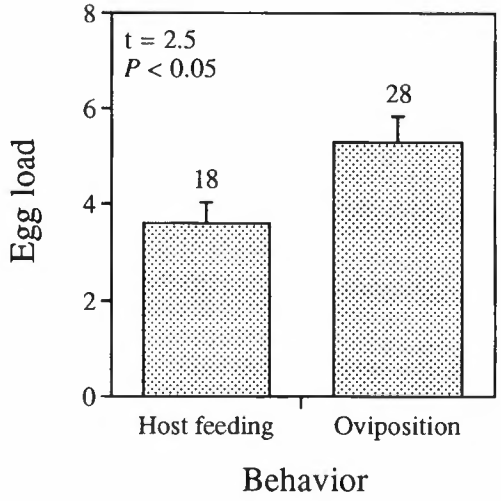

Figure 8. Egg loads of *A. aonidiae* host feeding or ovipositing on San Jose scale growing on almond. Numbers above columns are sample sizes.

longevity, but that it does increase fecundity. We also found that each host feeding meal translates into approximately two eggs. We used this information to construct a dynamic state-variable model to predict optimal outcomes of the host-feed vs. oviposit decision under various conditions. This model was not entirely novel (see Chan & Godfray 1993; Collier et al. 1994), but it was well-suited to our purposes and furnished us with four unambiguous predictions. First, that host feeding should be favored for younger, rather than older individuals; second, that host feeding should be favored when parasitoids have low egg loads; third, that host feeding should be favored when nutritional reserves are low and fourth, that host feeding should be favored at higher host densities. In empirical studies, the egg load prediction was supported in the laboratory and field, and the nutrition prediction was supported in the lab. The age prediction, however, was not supported in the laboratory.

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Influence of parthenogenesis *Wolbachia* on host fitness

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Stouthamer, R. & S. Lükö & F. Mak 1994. Influence of parthenogenesis *Wolbachia* on host fitness. Norwegian Journal of Agricultural Sciences. Supplement 16. 117-122. ISSN 0802-1600.

The influence of symbionts associated with parthenogenetic reproduction (*Wolbachia*) was studied in two species of parthenogenetic wasps: *Encarsia formosa* and *Muscidifurax uniraptor*. The experiments consisted of «curing» the wasps by feeding them the antibiotic rifampin in honey (10mg/ml), and comparing the offspring production of the cured wasps with that of honey fed individuals. In *E. formosa* the «cured» wasps produced significantly fewer offspring than the wasps with symbionts, whereas in *M. uniraptor* no significant difference could be found in offspring production. These results are discussed and improved experimental designs are suggested.

Keywords: *Encarsia formosa*, fitness, *Muscidifurax uniraptor*, parthenogenesis, thelytoky, *Wolbachia*,

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Rickettsialike bacteria of the genus *Wolbachia* have been implicated in a number of reproductive anomalies in insects. These bacteria are inherited from mother to offspring through the cytoplasm of the eggs, males are generally not able to transmit them to their offspring. This purely vertical transmission can explain some of the major effects of these bacteria, i.e. cytoplasmic incompatibility, sex conversion and parthenogenesis. The transmission of the symbiont in cytoplasmic incompatibility is favored by a mechanism in which eggs free of the symbiont fertilized by sperm from a male with symbionts will result in no offspring (most diploid-diploid insects) (O'Neill et al. 1991) or only in male offspring (in haplo-diploid insects) (Breeuwer & Werren 1990). The net result of this selective killing of eggs without symbionts is an increase in the frequency of the females with symbionts in the host population (Hurst 1993). Similarly the sex-conversion (i.e. genetic males become functional females) (Rousset et al. 1992) and parthenogenesis (virgin females produce only daughters) (Stouthamer et al. 1990a) also favor the transmission of the symbionts, because only females can pass on the symbionts of their offspring. It is easy to see how these mechanisms favor the transmission of the symbionts if there are no other effects of being infected (Hurst 1991). However even with some negative impact of the symbiont on the hosts offspring production the infection frequency within a population can increase (Stevens & Wade 1990). Similarly if parthenogenesis symbionts have a negative impact on the offspring production of their hosts, the infection rate can remain stable as long as the infected females produce the same number of daughters as females free of the symbionts (Stouthamer & Luck 1993).

The evolutionary fate of symbionts purely transmitted through the maternal line is closely linked to that of their host. A reduction of the negative impact of the symbiont on its host can be expected to take place over time once the hosts genome and the symbionts genome co-evolve (Ewald 1987). The extent of the co-evolution between the hosts and symbionts genome will be a function of the time that they have been associated with each other. The rate of this co-evolution may be expected to increase once the association between the hosts and the symbiont has become absolute, i.e. the hosts and the symbionts genome are always associated with each other. This means that the population carrying the symbiont is completely infected and no more gene flow takes place between infected and uninfected individuals.

Little information exists in the literature over the effect of the symbionts on the hosts reproduction. Stevens & Wade (1990) find that in populations of the flour beetle *Tribolium* the incompatibility symbionts have a substantial negative impact on the offspring production of the infected individuals. In this species the infection with *Wolbachia* has not gone to fixation and consequently one may expect that an evolution toward a reduction in negative impact of the symbiont on its host may not yet have taken place. Similar large negative impacts are found when the symbiont of *Aedes albopictus* is established in *Drosophila simulans* (Braig et al. 1994) . The effect of the parthenogenesis symbionts on their *Trichogramma* hosts also appears to be severe (Stouthamer & Luck 1993). And here also the association between host- and symbiont- genome is not complete because females infected with the parthenogenesis *Wolbachia* are not genetically isolated from the sexual part of the population in which they are found (Stouthamer & Kazmer 1994).

In this paper we study the effect of parthenogenesis symbionts on their hosts when the association between the hosts genome and that of the symbiont must have existed for a substantial time period. The effect of the symbionts on their hosts is studied in the parasitic wasp species *Muscidifurax uniraptor* and *Encarsia formosa*. Of both species only infected populations are known. Further evidence that the association between the parthenogenesis symbiont and their hosts is old stems from the observation that males of these species, created by antibiotic treatment of mothers, are not capable of successfully completing an insemination of females of their species (Zchori-Fein et al. 1992, Van den Assem & Povel 1973). This failure in their mating is an indication that mutations have accumulated in that part of the genome coding for mating behavior, sperm production etc. Such mutations are expected to accumulate when these traits are not selected for anymore during many generations of purely parthenogenetic reproduction when only virgin females are exposed to selection (Ikeda & Carson 1973, Stouthamer et al. 1990b).

MATERIAL AND METHODS

To test the effect of the symbionts on the hosts reproduction we compared the offspring production of females «cured» by a honey-antibiotic treatment with females that had received only honey. The antibiotic may itself have a side effect on the wasps. The best way to check for that effect is to simultaneously treat a closely related sexual form with the antibiotic and test for its effect on the offspring production. We did this experiment in case of *Muscidifurax*, unfortunately we did not have access to a sexual species that is closely related to *E. formosa*.

E. formosa

Encarsia formosa is an aphelinid parasitoid of the greenhouse whitefly (*Trialeurodes vaporariorum*). General information on its lifehistory is described in van Lenteren et al. (1976) *E. formosa* used in these experiments was supplied by a commercial insectary. Upon emergence the wasps were randomly assigned to either the treatment group or the control group. Both groups were kept for 24 hrs in the presence of either honey (control group) or honey mixed with the antibiotic rifampin (10mg/ml) (treatment group). Subsequently the wasps were allowed to parasitize greenhouse whitefly larvae. The whiteflies were reared on tobacco plants. Leaf disks containing large numbers of whitefly larvae of the L3–L4 stage were excised from plants and placed with the whitefly larvae on the top in petri-dish (55 mm diameter and 18 mm high) containing water agar (1% agar, 5 ml/l Nipagine in water). The leaf discs adhered to the agar and remained in good condition for about 10 days. The lid of the petridishes contained a hole (40mm diameter) covered with fine gauze to ensure ventilation. Per petridish one *E. formosa* individual was allowed to parasitize hosts for two days. After this time the wasps were transferred to a new petridish with whiteflies. The number of parasitized whiteflies were counted ten days after the wasps had been removed from the leaf disk and their gender was determined. All experiments were done in a climate chamber at 25°C and L:D=18:6.

M. uniraptor

Muscidifurax uniraptor and *M. raptor* are pteromalid parasitoids of pupae of synantropic flies. Wasps used in these experiments were originally collected by Legner (1988). *M. uniraptor* originated from Puerto Rico (Legner 1985) and *M. raptor* from California, USA. General information on their lifehistory can be found in Legner (1988). In these experiments we compared the influence of the antibiotic both on *M. uniraptor* a species infected with the parthenogenesis symbionts and in its close relative the arrhenotokous *M. raptor* that is free of symbionts (Stouthamer et al. 1993). Upon emergence in a petridish the wasps of each species were randomly assigned to either the treatment group or the control group, each consisting of ten females. The treatment groups were allowed to feed on the antibiotic rifampin in honey (10mg/ml) for 24 hrs, while the control group was fed honey during this time. The next day individual females placed in glass vials (7cm high, 1 cm diameter) covered with a cotton plug received 20 host pupae each. These pupae were replaced daily with fresh pupae. The parasitized pupae were kept in separate vials for emergence. All experiments were done in a temperature cabinet at 25°C, 24 hrs light. After the offspring had emerged they were counted and sexed.

Statistical analysis

Total number of offspring is compared between the control and the treatment group using the Wilcoxon-Mann-Whitney-Test (Siegel & Castellan 1988)

RESULTS

No significant differences were found in longevity or fecundity between the antibiotic treated and the control groups, with the exception of a significant difference in the total number of progeny produced by *E. formosa* (Table 1). This difference may

indicate that the antibiotic concentration had a negative influence on the number of offspring produced by these females.

Table 1. Influence of treatment with the antibiotic rifampin in honey (10 mg/ml) on total life time offspring production and longevity of the parthenogenetic species E. formosa, *M. uniraptor* and the sexual species *M. raptor.*

sample size	treatment	mean (S.E.) of total progeny	mean (S.E.) of longevity in days
Encarsia formosa			
24	control	193 * (16.11)	16.2 (1.30)
20	antibiotic	148.9* (8.83)	14.5 (0.92)
Muscidifurax uniraptor			
9	control	127.6 (22.2)	25.3 (3.43)
10	antibiotic	143.8 (31.9)	22.1 (3.45)
M. raptor			
10	control	203 (11.5)	25.9 (1.70)
9	antibiotic	180.2 (15.4)	26.8 (2.53)

* These values differ significantly from each other $p<0.05$, all other cases no significant differences between control and antibiotic treatment (i.e $p>0.05$)

DISCUSSION

A significant difference was found in the fecundity of the treated and control *E. formosa* groups, with the mean fecundity of the control group being higher. This may be indicative that the concentration of antibiotic used in this experiment was too high for these wasps. Not only did it result in the production of male offspring but it also affected the fecundity of the females. This result is in contrast with that reported by Zchori-Fein et al. (1992), who found an increase in offspring production by females treated with 50mg/ml tetracycline. They measured the offspring production of a group of wasps. It is difficult to compare these two data sets because the experimental setup differed substantially. The interpretation of the *Muscidifurax* data is relatively straight forward. There appears to be no significant influence of the antibiotic treatment on either life-history characters of the two *Muscidifurax* species. However there is a trend indicating that the antibiotic has a negative, but non-significant impact on the offspring production of the sexual species. Whereas the antibiotic-treated *Muscidifurax uniraptor* produced more offspring than the honey-fed control group. Unfortunately a heterogeneity in the variance of the offspring production in the *M. uniraptor* compared with the *M. raptor* group made a more detailed statistical analysis in which the interaction effect between species and treatment could be detected

impossible. However, we can conclude that if there is any increase in offspring production following the antibiotic treatment the magnitude of this increase is relatively limited. The *Muscidifurax* case is therefore not inconsistent with the hypothesis that the negative impact of the symbionts on this species is limited. Clearly additional data are needed to determine the extent of the impact of the symbionts on these lifehistory characters in these two parthenogenetic species.

There may however be a more fundamental problem with trying to establish the effect of the symbionts on their hosts. With these antibiotic treatments we are only able to «cure» the adult females of their infection, the negative impact of the symbionts on the fecundity of the adults may however already occur during the larval stage. In that case curing the adults of their symbionts will not alter the impact that these symbionts have exerted. The importance of this «larval» effect can be determined in experiments using species where sexual lines can be derived from parthenogenetic forms.

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Enhanced reproduction of an aphid in the presence of its hyperparasitoid

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Dendrocerus carpenteri is a common hyperparasitoid of the grain aphid *Sitobion avenae*. Reproductive behaviour of this aphid in the presence of its hyperparasitoid has been investigated. Using two-chamber clip cages, in which the insects could not get into contact with each other, we examined whether the number of larvae produced by a single aphid in 24 h and 96 h respectively changed in the presence of the hyperparasitoid. We found out that winged *S. avenae* increased their reproduction significantly in 24 h and 96 h when they were next to *D. carpenteri* females. Yet, they behave differently in the presence of *D. carpenteri* males. We suggest that this increased reproduction is effected by female volatiles.

Key words: aphid, *Dendrocerus carpenteri*, hyperparasitoid, reproduction, semiochemicals, *Sitobion avenae*

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INTRODUCTION

Aphids are hosts of hymenopterous primary parasitoids, which again are hosts of hymenopterous hyperparasitoids. Since hyperparasitoids kill larvae of primary parasitoids and thus raise the hosts pest equilibrium (Hagen & van den Bosch 1968, Sullivan 1988), obligatory hyperparasitism is often (at least to a certain extent) blamed for the poor effectiveness of primary parasitoids in the field, as far as biological control is concerned.

Furthermore, most recent field studies by Höller et al. (1993) suggest that primary parasitoid females leave the areas populated by hyperparasitoids. This seems to be another reason as to why the levels of aphid primary parasitism are often very low. This hypothesis, which is based on field data, could be supported by laboratory experiments with the primary parasitoid *Aphidius uzbekistanicus* and the hyperparasitoid *Alloxysta victrix* Westwood (Höller et al. 1994).

Yet, apart from these negative effects of the hyperparasitoids, a possible positive influence on the population of primary parasitoids and their host cannot be ruled out. By means of mathematical models, some authors (Luck et al. 1981, Beddington & Hammond 1977) proposed the hypothesis that the introduction of hyperparasitoids into a host-parasitoid system with extreme periodical fluctuations of population can lead to a stabilisation of this system. Doing research on the walnut-aphid-complex, van den Bosch et al. (1979) arrived at a similar conclusion: the immigrated hyperparasitoid had led to a significant decrease in the extreme fluctuations of populations.

Within the aphid-primary parasitoid-hyperparasitoid system the role of the hyperparasitoid is obviously very complex. While both the relation-ship between the latter and the primary parasitoid and the resulting consequences on the host population are discussed again and again, the direct relationship between the host aphid and the hyperparasitoid has hardly been investigated into. Hyperparasitoids nourish on the honeydew of aphids and develop on the parasitoid larvae. Aphids, however, benefit from the killing of their opponents by the hyperparasitoids. Thus we can assume that there are less primary parasitoids in areas with a high hyperparasitoid pressure. From the aphids' point of view it could therefore be of advantage to produce more larvae in these areas.

The question whether the presence of its hyperparasitoid can cause an increased reproduction of the aphid has been investigated into by means of laboratory experiments on the grain aphid *Sitobion avenae* F. and its hyperparasitoid *Dendrocerus carpenteri* Curtis. The design of the experiment ruled out any possible influences on the aphid by tactile, gustatoric and optical stimuli. The series of experiments focused on the effect of volatiles produced by hyperparasitoids.

MATERIAL AND METHODS

Sitobion avenae was maintained on oats (cv. Bovar) at 20°C in a light-dark circle of 16h/8h. Since 1989 they reproduce purely parthenogenetically. *Dendrocerus carpenteri* was reared on *Aphidius uzbekistanicus* (host aphid: *Sitobion avenae*) under the same climatic conditions in separate rooms.

Within the last 24 hours before the experiment started, the aphids had moulted to alatae and had not yet produced any larvae. All of them came from the same rearing unit and were of comparable size. When the experiment started, the hyperparasitoids were not older than 24 hours. Experimenting with oat plants (cv. Bojar; 8-10 days old), we used two chamber clip cages (3.5 cm diameter, each chamber being 1 cm high). The chambers were separated by a fine gauze net (mesh: 200 nm). In each chamber there was a hole (0.5 cm in diameter) which could be closed and through which the insects were let into the chamber (Figure 1). Two series of experiments were carried out: While series no. I took 24 hours, series no. II took 96 hours in order to have the opportunity to find out both the short-term and the long-term effects of the hyperparasitoid on the reproduction of *S. avenae*. While in series no. I the influence of males on the one hand and virgin and mated females on the other hand was tested, in series no. II it was only the effect of virgin and mated females. Throughout the experiment, in 50% of the number of cages there were two *D. carpen-teri* (treatment) while in the other 50% these upper chambers remained empty (control). In each of the lower chambers an aphid could suck at the tip of a leaf on an undamaged oat plant and produce larvae (22°C; light-dark circle of 16 h/8h).

24 and 96 hours respectively after letting the aphid into the clip cages, we registered the number of larvae per aphid which had been produced in the meantime. Seven replicates, each of them with 10 cages (parallels), were performed. All parallels in which the plants showed symptoms of wilting, the aphids were not situated on the leaves or had died in the meantime were ignored.

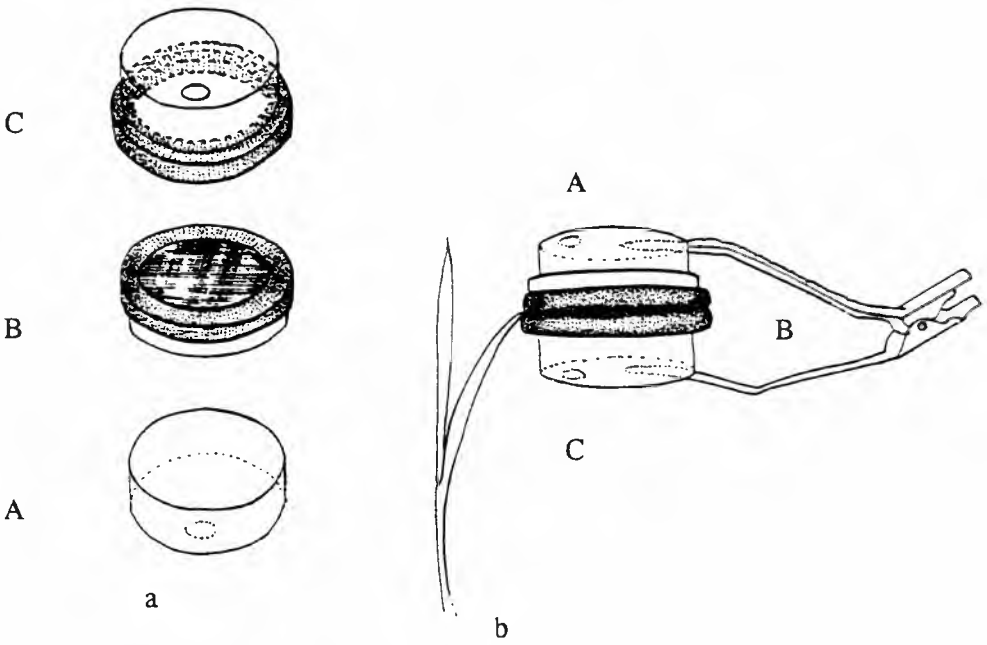

Figure 1: Two-chamber clip cage.
Hyperparasitoid chamber (A), partition gauze net (B), aphid/plant chamber (C), opening for insect introduction, sealed after introduction (a), oat plant (b).

RESULTS

24 hours

On avarage the *S. avenae* alates produced significantly more offspring when treated i.e. when the virgin females and mated females were in the upper chamber, than at the corresponding controls (Table 1). In comparison with the controls there were less test aphids without offspring with regard both to virgin and mated females, yet more with a relatively huge offspring. The differences in the distribution of treatment and control are highly significant (chisquare goodness of fit test). The presence of male hyperparasitoids, however, did not lead to significant differences at control and treatment. Therefore its 96 h version could be dispensed.

96 hours

Correspondingly at the four-day testing period the reproduction of the alatae was higher in the presence of hyperparasitoid females than at the controls (Table 2). In comparison with the 24-hour testing period, the differences of the avarage larvae production concerning control and treatment could not be increased significantly: 0.8 larvae per aphid with regard to mated and 1.08 larvae per aphid with regard to virgin hyperparasitoid females. Just the results of the second variant (virgin females) can be statistically ensured (Mediantest, $p<0.01$). Moreover, the basic totals in the distribution of the larvae do not differ significantly in the first variant but in the

Table 1: Reproduction of winged *Sitobion avenae* during 24 h (series no. I) in two-chamber clip cages. One aphid each was placed on an oat plant in the lower chamber at the presence of *D. carpenteri* virgin females, mated females or males (treatments) or no insects (control) in the upper chamber. The number of aphids producing 0, 1, 2 or more than 2 larvae were counted. The medians ($\bar{x}$) of control and treatments were compared statistically by the Mediantest and the basic totals of distribution were compared by the chi-square goodness of fit test.

	no. of *S. avenae* with 0/1/2/>2 larvae					
larvae per aphid	virgin females	control	mated females	control	males	control
0	8	21	20	29	24	20
1	15	8	5	7	15	17
2	25	27	21	13	7	9
>2	16	8	4	1	3	3
N	64	64	50	50	49	49
$\bar{X}$	1.92	1.41	1.20	0.72	0.80	0.90
Median-test[1])	*		*		n.s.	

[1]) n.s.: $p>0.05$; *: $p^3 \leq 0.05$; **: $p^3 \leq 0.01$; ***: $p^3 \leq 0.001$
chi^2 goodness of fit test: virgin females:***, mated females:**, males: n.s.

Table 2: Reproduction of winged *Sitobion avenae* during 96 h (series no. II) in two-chamber clip cages. Experimental conduct and calculation see table 1. The number of aphids producing 0-2, 3-5, or more than 5 larvae were counted.

	no. of *S. avenae* with 0–2/3–5/>5 larvae			
larvae per aphid	virgin females	control	mated females	control
0–2	1	5	8	13
3–5	14	26	18	20
>5	38	22	24	17
N	53	53	50	50
$\bar{X}$	6.21	5.13	5.52	4.72
Mediantest [1])	**		n.s.	

[1]) n.s.: $p>0.05$; *: $p^3 \leq 0.05$; **: $p^3 \leq 0.01$; ***: $p^3 \leq 0.001$
chi^2 goodness of fit test: virgin females:***, mated females: n.s.

second (chi-square goodness of fit; $p<0.001$). Yet at treatment in this test series, too, a small number of aphids tend to produce extremely few larvae (0-2) while a greater number tends to produce extremly many larvae (>8).

DISCUSSION

At the presence of females of the hyperparasitoid *D. carpenteri* in clip cages, the reproduction of the winged grain aphid *S. avenae* could be significantly increased in the 24 hours test phase although the hyperparasitoid could not get into direct contact with the aphid. Since this effect could only be observed when experimenting with virgin and mated females but not when experimenting with males, we suggest that it is due to sex-specific volatiles emitted by the female hyperparasitoid. This on average enhanced production of larvae could also be noticed in a test period of 96 hours when virgin hyperparasitoid females were present. Comparison with the 24 hours results suggest that the influence on reproduction of the *S. avenae* lasted only for the first 24 hours and that in the following period of time rates at control and at treatment equallized again. Producing more larvae under treatment conditions is very sensible since offspring near to hyperparasitoids is better protected against parasitism in comparison to offspring in hyperparasitoid free sites.

If the conditions are alike, aphids will possible start reproduction earlier and produce more larvae for a short time when a hyperparasitoid is present. In the whole lifetime of an aphid, however, the genetic potentials and the set enviromental conditions determine the number of larvae which can be produced at maximum. An evlier and more intensive reproduction will probably have consequences onto the energetical potential and the longevity of the aphid. Further experiments have to investigate these possibilities.

It is quite surprising that *S. avenae* is actually able to notice the presence of its hyperparasitoid, since in comparison with other insects aphids have relatively few receptor cells on their antennae. A great number of these cells serve perception of both intraspecific communication substances, such as alarm and sex pheromones, and scents produced by host plants (Klingauf 1988). The influence of volatile semiochemicals on the fecundity of aphids could repeatedly be shown (Brown et al. 1990, Hildebrand et al. 1993). Accordingly, Hildebrand et al. (1993) could prove that certain 6-carbon compounds reduce tabacco aphid fecundity. How these substances led to the observed modifications of behaviour has not been explained, yet. Apart from a perception through the antennae and the following reaction, it can theoretically also be possible that the chemicals which were taken in beforehand influence the metabolism of the herbivores.

By earlier reproduction increase of aphid population will be accelerated. Through this influence of the hyperparasitoid on the grain aphid, the efficiency of the primary parasitoids as biocontrol agent might be further reduced.

Carrying out laboratory experiments with *Dendrocerus carpenteri* and *Sitobion avenae* we have been able to find indications that the hyperparasitoid can not only influence the primary parasitoids with specific semiochemicals but also their hosts, as proved before (Höller et al. 1994). The aphid reproduction increased by hyperparasitoids could be an additional reason why the effectiveness of primary parasitoids employed for measures in the field is low. Further experiments are to be

carried out to identify the chemicals, which have caused the increased reproduction of *S. avenae*. Also it seems to be necessary to look for other influences of hyperparasitoids within the system aphid-primary parasitoid-hyperparasitoid.

SUMMARY

Grain aphids are hosts of primary parasitoids, which again are hosts of hyperparasitoids. The more female primary parasitoids are killed, the better the whole aphid population is protected against parasitism. The target of the investigations proposed here was to examine whether aphids which live in areas with a high hyperparasitoid population and thus at a lower risk of parasitism produced more larvae than those in areas without hyperparasitoids. In two-chamber clip cages the alatae of *Sitobion avenae* had significant more offspring in the presence of *Dendrocerus carpenteri* females than in the presence of hyperparasitoid males or at control (no hyperparasitoids present). The effect, however, could be observed for a period of 24 hours. During 96 hours the numerical difference of offspring between treatment and control only could be statistically ensured when virgin *D. carpenteri* females were present. But there was no significant difference in the number of produced larvae in the presence of mated females compared with the control. Since the design of the experiment ruled out optical and tactile stimuli as possible influences on the aphids, we suggest that volatile semiochemicals produced by the female *D. carpenteri* caused the increase in reproduction which has been observed. Further experiments are to be carried out in order to identify these semiochemicals.

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Observations on the cocoon of *Oligosita krygeri* Girault (Hymenoptera: Trichogrammatidae) oophagous parasitoid of *Cicadella viridis* (L.) (Homoptera: Cicadellidae)

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Ceresa-Gastaldo, L. & E. Chiappini 1994. Observations on the cocoon of *Oligosita krygeri* Girault (Hymenoptera: Trichogrammatidae) oophagous parasitoid of *Cicadella viridis* (L.) (Homoptera: Cicadellidae). Norwegian Journal of Agricultural Sciences. Supplement 16. 131-140. ISSN 0802- 1600.

The formation of the cocoon of *Oligosita krygeri*, an oophagous parasitoid of *Cicadella viridis* is described.

Key words: cocoon, *Oligosita krygeri*, parasitoids, Trichogrammatidae.

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INTRODUCTION

Among parasitoid Hymenoptera, a cocoon[1] is frequently made by the ectophagous species and those endophagous species which then emerge from the host when they reach larval maturity, to complete their metamorphosis and become adults.

The formation of a cocoon inside the host by endophagous parasitoid Hymenoptera is less common, although it has been noted with both Ichneumonidea and Chalcidoidea.

As an example of the first, we can mention *Pelecystoma harrisinae* (Ashmead) (Hymenoptera: Braconidae), an endoparasitoid of the larvae of *Harrisina americana* (Guér) (Lepidoptera: Pyromorphidae), which places its pupa inside the exoskeleton of its host, in a cocoon made up of two capsules joined by a «plastic-like» substance (Smith et al. 1955).

As Askew (1971) mentioned, it is believed that construction of a cocoon is rare among Chalcidoidea. However, exceptions are found in some families (Encyrtidae, Aphelinidae, Chalcididae). The phenomenon appears to be more widespread in the

[1] By «cocoon» is meant, following the broadest meaning accepted by Grandi (1951), the envelope that mature larvae make to protect the pupal instar, which would otherwise be defenceless, using materials they themselves produce, sometimes along with a greater or lesser quantity of material gathered from outside; the cocoon may be made «either with silk (secreted by the labial glands or by the Malpighian tubules), or of silk but mixed with foreign substances or objects, or of intestinal secretions which harden when exposed to the air, or, finally, of other ingredients».

Encyrtidae family. It has been noted particularly with *Copidosoma* spp. and *Bothriothorax* spp., parasitoids of the larvae of Diptera (Howard 1891), *Ageniaspis fuscicollis* Dalman, oolarval parasitoid of *Yponomeuta* spp. (Bugnion 1891, Silvestri 1908), *Encyrtus infelix* (Embleton) in *Saissetia hemisphaerica* Targioni (= *Saissetia coffeae* (Walker)) (Homoptera: Coccidae) (Embleton 1904), *Litomastix truncatellus* (Dalman) in *Autographa gamma* (L.) (Lepidoptera: Noctuidae) (Silvestri 1906), *Metaphycus melanostomatus* (Timberlake) in *Lecanium capreae* (L.) (= *Eulecanium tiliae* (L.)) (Homoptera: Coccidae) (Imms 1918), *Encyrtus infidus* Rossi in *Eulecanium coryli* (L.) (= *Eulecanium tiliae* (L.)) (Homoptera: Coccidae) (Silvestri 1919), *Metaphycus lounsburyi* (Howard) in *Saissetia oleae* (Bernard) (= *Saissetia oleae* (Olivier)) (Homoptera: Coccidae) (Smith & Compere 1928), *Carabunia myersi* Waterstone in *Clastoptera undulata* Uhler (Homoptera: Cercopidae) (Myers 1930), *Microterys speciosus* Ishii (Ishii 1932), *Ooencyrtus johnsoni* (Howard), ooparasitoid of *Murgantia histrionica* Hahn (Hemiptera: Pentatomidae) (Maple 1937), some species of the *Isodromus* genus (Clancy 1946, Principi 1947) and *Leptomastix dactylopii* Howard, a parasitoid of *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) (Zinna 1959).

At first, this envelope was thought to be a real puparium, made from the hardened remains of the larva (Silvestri 1908, Clausen 1932). Later Silvestri himself (1919) thought they were made from the secretions of the «tergal glands». The same explanation was given by Ishii (1932), who observed that this substance emerged from the oral opening, while Flanders (1938) believed that it came from glands he himself called «iliac».

After a careful biological and morphological study, Zinna (1959) noted that these glands «at the rear are placed» ... «in correspondence with the ileum, and in front continue without interruption with the labial glands»; modifying Flanders's (1938) terminology, he called them «ileo-labial». This same author also showed how the secretion was spread over the parasitoid's body and then hardened in the air, thus forming the cocoon.

There have been numerous studies and hypotheses on its function. Many authors have presumed it is related to the parasitoid's respiration, showing that there is a connection to the host's tracheae (Embleton 1904, Silvestri 1908, Thorpe 1936). Maple (1937) and Zinna (1959) in particular linked the phenomenon to the fact that the respiratory arrangement of the larvae of the first instar is different from that of the mature larva where, passing through a tracheal system of the peripneustic type, it needs protection for the newly-formed stigma: the contents of the egg, not yet entirely consumed, could occlude them.

In some cases it has been estabilished that the protective function of the cocoon is also to avoid squashing by the remains of the victim (Zinna 1959).

Other observations regard the Aphelinidae: *Coccophagus scutellaris* (Dalman) (Timberlake 1913), *Coccophagus rusti* Compere (Flanders 1938), *Coccophagus bivittatus* Compere (Zinna 1961), parassitoids of *Coccous hesperidum* L. (Homoptera: Coccidae), and *Coccophagoides similis* (Masi) (= *Diaspiniphagus moeris* (Walker)) in *Diaspidiotus viticola* (Leonardi) and *Targionia vitis* (Signoret) (Homoptera: Diaspididae) (Zinna 1962).

Among the Chalcididae, the presence of cocoons has been noted in *Brachymeria compsilurae* (Crawford), inside the larvae of Tachinidae, endophagous larval parasitoids of Lepidoptera (Dowden 1935).

Oligosita krygeri Girault (Hymenoptera: Trichogrammatidae) has been noted in Denmark (Bakkendorf 1934, 1943), in Japan (Doutt 1961, Miura & Yano 1987) and in Italy (Arzone 1972, Viggiani 1981) as an oophagous parasitoid of *Cicadella viridis* (L.) (Homoptera: Cicadellidae), and also, in Japan, of *Nephotettix cincticeps* (Uhler) (Homoptera: Deltocephalinae) (Yashiro 1979).

In northern Italy *O. krygeri* has two generations annually, synchronons with those of the leafhopper, spending the winter in the third larval instar; the preimaginal development is completed in the instars of egg, larva of first, second and third instar, eopupa and pupa (Arzone 1974).

MATERIALS AND METHODS

The specimens studied were both collected in the field (canal banks, meadows and uncultivated areas of the province of Piacenza) and bred in the laboratory.

For the first type the eggs of *C. viridis* were removed from leaves of *Carex riparia*, collected periodically from the field between November and February. The plant material was in part preserved[2] for later use and in part opened in the laboratory to remove the leafhopper eggs. Those with *O. krygeri* parasites, which are easily recognized, were isolated in Petri dishes (diameter 3,5 cm), the bottom of which were covered with absorbent paper dampened with a physiological solution (Figure 1). Cleaning of the breeding cell and changing of the solution every two days meant it was possible to follow the development of the parasitoids up to their emergence without any pollution problems.

Those bred in the laboratory were obtained from eggs laid by *O. krygeri* that had become adult in the laboratory. In this case as soon as the adults emerged from the egg, they were moved to Petri dishes with a small quantity of sugar solution and a little bit of leaf containing *C. viridis* eggs. After the time necessary for oviposition, the eggs were replaced; those with parasites were removed from the vegetable tissue and bred using the same procedure described above.

The *C. viridis* eggs used as hosts for the parasitoids came from a mass breeding programme carried out in a climatic chamber at 25°C, 60% relative humidity and 16 hours light, mostly on *Setaria viridis* obtained from seed.

The development of the parasitoid inside the host egg was followed in detail by means of daily observation, carried out using both the stereoscopic and the optical microscope, in the later case making use of drop slides.

Histological sections of host eggs with *O. krygeri* parasites were effected at various instars of their development by means of fixing in Carnoy's liquid, inclusion in paraffin and colouring with hematoxylin-eosin (Beccari & Mazzi 1966), generic colouring for the tissues of the insects (Clark 1981), specific for chitinous material (Clark 1981) and PAS (Beccari & Mazzi 1966).

Pieces of the cocoon, freed from the corium of the host egg, were prepared for observation under the optical microscope both as they were and after colouring with green methyl, with black sudan or specific colouring for chitinous material.

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[2] The leaves were cleaned and wrapped in damp absorbent paper, and then kept in the refrigerator in polyethylene bags.

Samples dried to the critical point with CO_2 and metallized with gold were studied under a Hitachi S 2300 scansion electronic microscope.

RESULTS AND DISCUSSION

In *O. krygeri* the cocoon is made by the larvae of the 3rd instar, which, unlike what Zinna (1959) observed for *L. dactilopii*, are characterized by a noticeable lack of movement when compared with their previous life.

It is found attached to the corium of the host egg, although not fixed there immoveably. In fact, if the corium is cut and raised, it can be detached completely without damaging the cocoon.

As pointed out by Arzone (1974), when the egg containing the cocoon is looked at through the stereoscopic microscope under incident light, it appears to be very turgid and is often crossed by superficial furrows and is at first a yellowish-brown colour and later, when the pupal instar is reached, an opalescent greyish-brown, which makes one suspect the presence of air (Figure 2), as Zinna found concerning the aeroscopic plate of the egg of the Encyrtidae. It must, however, be noted that those eggs infected with parasites and bred in the laboratory always look more-or-less transparent regardless of the presence of the cocoon, so that one can see the parasitoid inside them (Figure 3).

When the cocoon is freed from the corium of the host egg, the outside looks like a smooth membrane of uniform structure and a yellowish-brown colour; inside, on the part that is in contact with the larva, a certain superficial graininess can be noted. This structure becomes much clearer when observed through the optic microscope (Figure 4). Study of the sections makes it clear that this graininess is due to the presence of mushroom-shaped structures sticking up above the surface of the inside (Figure 5).

Observation using the SEM confirms these results. The cocoon (Figure 6) is seen to be formed of a series of interwoven and compressed filaments (Figure 7) which appear to be joined to each other. On the internal surface there are little lumps which are cylindrical in shape to start with (Figure 8), but then enlarge at the ends in contact with the larva's exoskeleton (Figure 9), until in some places they form an unbroken surface (Figures 10 and 11).

When the host egg contains, as is usually the case, two larvae, they lie indifferently with their heads towards an end or towards the centre and, as Arzone (1974) underlined «the outside of the egg is marked by a noticeable furrow, and on the inside there is a corresponding septum» which separates the two larvae. When a larger number of larvae are present, they can all lie in different directions. The septum appears to be formed by the adjacent walls of the cocoons, and this has a mirror-like appearance where the interwoven filaments are in the central part and the mushroom-shaped formations on the two outer sides (Figure 12).

In eggs containing several larvae, the walls connected to the corium of the host egg appear before those touching the other larvae (Figures 13 and 14): if an egg containing larvae that are not yet completely mature is sectioned, it is possible to see how they are not separated by a septum, even though already surrounded by the typical structure of a newly-formed cocoon.

Mature larvae extracted from the host produce a drop of liquid (Figure 15) from the oral and anal regions, which spreads over the surface of their body and later forms

a coveryng layer (Figure 16), following a mechanism which recalls that noted by Zinna (1959) for the creation of the cocoon.

A comparison between the Trichogrammatidae studied and the other parasitoids of *C. viridis* found on the same material gathered from the field, indicated a greater vitality of the wintering larvae of *O. krygeri*, enclosed in their cocoons. They were less likely to suffer from moulds and rot, or to be crushed because of the drying of the eggs inside the leaves.

CONCLUSIONS

This study has allowed us to demonstrate the formation of the cocoon in *O. krygeri*, establishing its protective function.

The way in which it is made is not clear, although the type of structure found by observation through SEM, and the limited movements of the larvae, lead one to suppose that there is no «spinning» operation.

We can therefore hypothesize that the cocoon may be the result of the solidification of the secretions emitted by the oral and anal orifices when exposed to the air, according to a mechanism analogous to that presumed by Zinna for *L. dactilopii* (1959), *C. bivittatus* (1961) and *C. similis* (1962).

So far the creation of cocoons has been established among endoparasitoids Chalcidoidea only for those species that attack adults or preimaginal instars subsequent to the egg. The only mentions concerning ooparasitoids are for the *Ooencyrtus* genus, which is however characterized by tracheate eggs with an areoscopic plate: it is this morpho-functional adaptation, typical of this family, which makes it a special case among oophagous parasitoids.

O. krygeri is a parasite of the eggs of *C. viridis* and so an instar that does not have tracheae which supply free air. On the other hand, it does not possess the typical structures such as those of the *Ooencyrtus*, which connect it to the air outside.

As it has not yet been possible to show how respiration takes place in the egg of *C. viridis* and the preimaginal instars of the parasitoid, it would be premature to ascribe a respiratory significance to the cocoon of *O. krygeri*, even though, in the light of observations carried out so far, we believe it to be probable.

Figure 1. Leafhopper's eggs parasitized by *O. krygeri,* on absorbent paper dampened with a physiological solution.

Figure 2. Leafhopper's eggs parasitized by *O. krygeri,* in a leaf of *Carex riparia* opened for this purpose.

Figure 3. Pupae of *O. krygeri* visible through the cocoon and the corion of the leafhopper's egg.

Figure 4. Part of the cocoon of *O. krygeri* seen through an optic microscope.

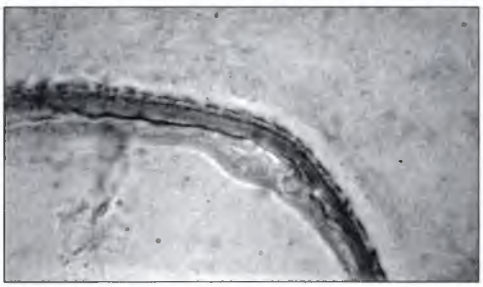

Figure 5. Section of an egg of *C. viridis* in which the corium can be seen on the outside, and just inside, the cocoon; the arrow indicates the mushroom-shaped structure raised above the inner surface.

Figure 6. Cocoon of *O. krygeri* containing a larva that can be seen through the opening. The septum is characterized by its grainy appearance, different from the smooth outher walls.

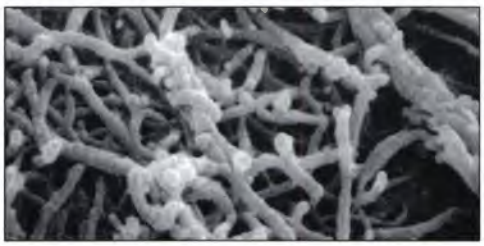

Figure 7. Detail of the structure formed by filaments, which is typical of the cocoon of *O. krygeri.*

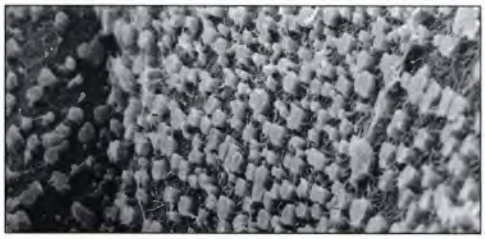

Figure 8. Internal surface of the cocoon of *O. krygeri* with the little lumps of cylindrical shape.

Figure 9. Detail of the small lumps inside the cocoon; it will be noted that some are cylindrical and others are in a more advanced stage. The upper extremity is the one in contact with the larva.

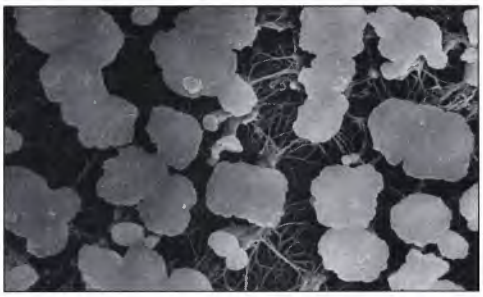

Figure 10. Inner side of the cocoon seen from above; the enlarged small lumps join together in some places.

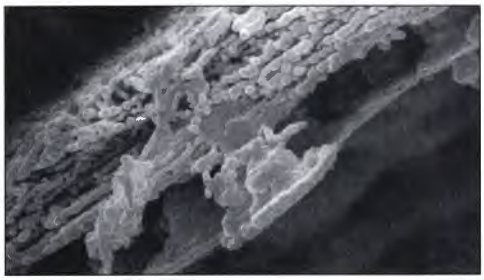

Figure 11. Side view of the same detail of the previous photo.

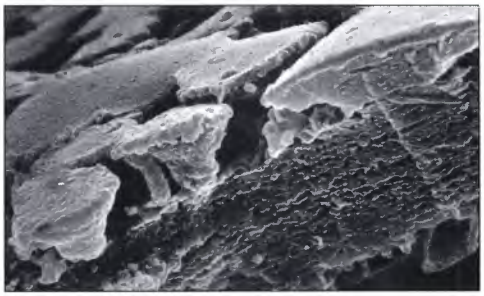

Figure 12. Septum seen side on.

Figure 13. Larvae of the third instar of *O. krygeri* enclosed by the cocoon, still lacking the separation septum.

Figure 14. Larvae of the third instar of *O. krygeri* enclosed by the cocoon, with the separation septum.

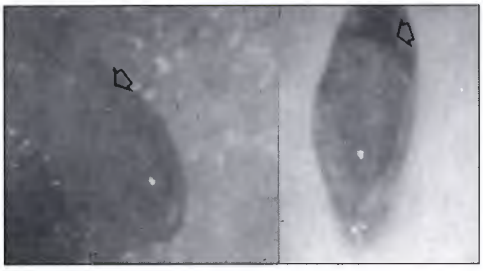

Figure 15. Larvae of the third instar of *O. krygeri* removed from the host. The drops of liquid secreted in the oral and anal areas (a), which will later join to form a single drop (b), can be seen. The arrows indicate the mandibles.

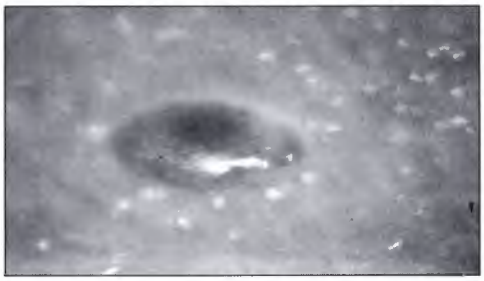

Figure 16. Larva of the third instar of *O. krygeri* removed from the host. The drop of liquid secreted has spread uniformly over the body to form a covering.

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The relation between clutch size and fitness in a larval-pupal endoparasitoid

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The larval-pupal endoparasitoid *Aphaereta minuta* attacks larvae of Diptera species of different sizes. Females oviposit in young larval stages but the eventual size of the host pupa determines host food availability for competing offspring. We studied clutch size decisions by this parasitoid. Previous work showed that females vary their clutch considerably and lay larger clutches in larvae of host species that produce larger pupae. Here we present evidence that females also lay larger clutches in larger larvae than in smaller larvae of the same host species. We measured the consequences of clutch size variation for fitness in different instars of the host *Delia antiqua*. Clutch size was manipulated and the relation between clutch size and fitness was quantified. The calculated Lack clutch size increased with larval host stage. When host encounter rate is low, clutch size approaches the Lack value.

Keywords: *Aphaereta minuta*; Braconidae; clutch size; Hymenoptera; reproductive strategy.

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INTRODUCTION

For an insect parasitoid, the size of the host determines the amount of food available for its offspring. The capacity to allocate different numbers of offspring to hosts of different sizes, and so of different quality, potentially enables polyphagous gregarious parasitoids to optimally exploit host resources. Indeed, as predicted by optimality models (Godfray 1987), literature reports on a positive correlation between clutch size and host size (e.g. Takagi 1986). Koinobiont parasitoids that attack on-growing hosts have a difficult task to make optimal decisions on the allocation of the number of eggs to hosts, as the eventual size of the host has not yet been reached at the moment of oviposition. The parasitoid *Aphaereta minuta* (Nees) (Hymenoptera: Braconidae) is a polyphagous larval-pupal endoparasitoid of many fly species, the larvae of which feed on ephemeral food sources. Young larvae are preferred for oviposition and the parasitoid eggs hatch during or just after the pupation of the host (Evans 1933). Hence, at the moment of oviposition the female is faced with a problem of how to

estimate the amount of resource that, after pupation of the host, is going to be available for its offspring. The foraging female parasitoid can encounter and parasitize larvae that greatly differ in age and size and potential to grow further. How does she deal with such variation in resources and how is she to optimize the allocation of her offspring?

Optimality models have been developed to explain the evolution of clutch sizes and predict variability in this life-history parameter under various conditions such as limited resource (i.e. host) availability or limitation of time or eggs available in the foraging female wasps (Charnov & Skinner 1984, 1985, Iwasa et al. 1984). Overall, when the relation between clutch size and fitness was quantified, observed clutch sizes were found to be lower than the calculated «Lack clutch size» (being the clutch size that leads to the greatest gain in parental fitness when maximizing the fitness gain per clutch is equivalent to maximizing lifetime fitness (Godfray et al. 1991)). The Lack clutch is only expected when animals lay a single clutch in their life or when opportunities to lay additional clutches are very rare. When eggs are limited, for example, females are selected to maximize their fitness gain per egg and so lay clutches of a single egg (under the assumption there is no Allee effect). When time available for oviposition is limited the animal is expected to maximize its overall rate of gain of fitness and clutch size will depend on the time between ovipositions, approaching the Lack clutch size only under long travel time conditions.

In a previous paper (Vet et al. 1993) it was shown that ovipositing females indeed considerably vary their clutch size in response to both inter- and intraspecific variation in host size. (1.) Female *A. minuta* lay more eggs in larvae of host species that will produce larger pupae, even when the larvae of the different species are the same size at the moment of oviposition, (2.) females lay more eggs in older/larger larvae when compared to younger/smaller larvae of the same host species. When parasitized at an older stage, host larvae developed into larger pupae than when parasitized at a younger stage. An explanation for this strategy of laying smaller clutches in younger host larvae is that young dipteran larvae have a lower chance of survival to the pupal stage than older larvae. This can be due to the short period of substrate availability, the strong scramble type competition between the fly larvae and the longer period of being exposed to other natural enemies. In the present study we investigate whether clutch size is influenced by the size (or instar) of the host larva at the moment of oviposition. We manipulate clutch size and analyze how it affects parasitoid fitness in *D. antiqua* larvae of two different ages. Fitness curves are based on clutch size, survival to adult stage, sex ratio and fecundity of offspring. We also study the effect of host-encounter rate on clutch size decisions.

MATERIALS AND METHODS

Parasitoids

The culture of *A. minuta* originated from females that emerged from onion baits containing *D. antiqua* larvae collected near Wageningen, The Netherlands. *A. minuta* was maintained on first and second instar larvae of the onion fly *D. antiqua*.

Hosts

Larvae of different ages of *Delia antiqua* (Meigen)(Diptera: Anthomyiidae), were

used as hosts (first, second and third instar larvae). *D. antiqua* was reared on decaying onions at 23 °C, 70 % RH.

Experiments

Mated females were offered hosts in Petri dishes containing an agar layer with host rearing medium. Parasitoids were observed during oviposition and the number of quiverings of the last abdominal segments and the ovipositor sheath was counted (a direct determiner of clutch size, see Vet et al. 1993). Larvae were incubated individually in a plastic cup (30 ml) containing a surplus of rearing medium. The number, sex ratio and size of their offspring was investigated. Clutch size was manipulated by interruption, superparasitization or low host-encounter rates (inter-oviposition time of 24 or 48 hours).

RESULTS

Clutch size and fitness

For the fitness calculation we used clutch size, survival to adult stage, sex ratio of offspring and fecundity of daughters; $F = c.s.sr.f$, where F = fitness, c = clutch size, s = survival to adult stage, sr = sex ratio (proportion daughters) and f = fecundity of

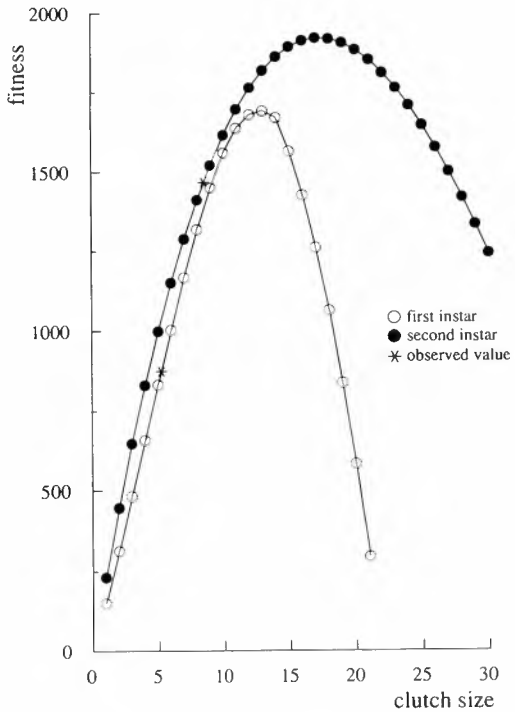

Figure 1. Fitness curves for parasitization of first and second instar *D. antiqua* larvae. Observed values give the mean clutch size of unmanipulated parasitizations.

daughters. Survival to adult stage and sex ratio are expressed as a function of clutch size. To determine fecundity we used the relation between the number of emerging adults and their size. Figure 1 shows the fitness curves for parasitization of first and second instar larvae. Part of the curve is calculated with extrapolated regression lines and so is only an estimation (>11 for the first instar and >20 for the second larval instar). The calculated Lack clutch size for parasitization of first and second instar larvae was 12.88 and 17.27 respectively, being larger than the observed clutch size of unmanipulated parasitizations (5.25±1.99 (n = 180) and 8.50±2.04 (n = 545) respectively).

Effect of host-encounter rate

Clutch size in second instar *D. antiqua* larvae increased with inter-oviposition time and approached the calculated Lack clutch when inter-oviposition times were 48 h (Figure 2).

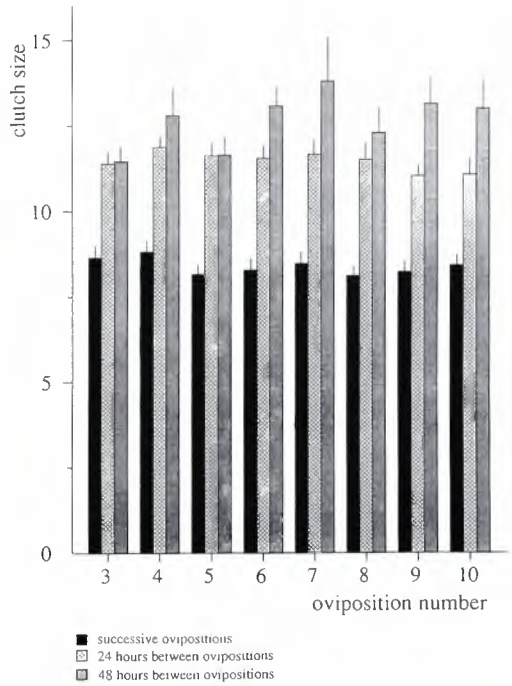

Fig. 2. Clutch size for successive ovipositions of A. minuta in second instar *D. antiqua* larvae for different inter-oviposition times. Mean (bars) and SE (lines).

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Susceptibilty of parasitized insect hosts to predators

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Hosts parasitized by insect parasitoids are ecologically different from nonparasitized hosts. They are traditionally thought to be more vulnerable to predation by insect predators than nonparasitized hosts. However, this assumption is poorly supported by experimental evidence. Because immature parasitoid survival is contingent upon that of the host, natural selection should act to lower the probability of host predation. It is suggested that parasitized hosts are similar to nonparasitized hosts in their vulnerability to insect predators. Components of parasitoid strategy to reduce predation pressure on the host are reliance on host defensive mechanisms and manipulation of host behaviour.

Keywords: host-parasitoid interactions, parasite-induced effects, parasitism, parasitoid, predation, predator selection.

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Parasitism causes biochemical, physiological and behavioural changes in host insects (Beckage 1985, Vinson & Iwantsch 1980). Parasitoid induced alterations may have direct effects on host ecology, thereby modifying the response of the host-parasitoid complex to the environment (Thompson 1990). In particular, parasitism may influence host susceptibility to predation.

Animals which differ in behaviour, appearance and distribution tend to have different levels of susceptibility to predation. Predators normally hunt for more profitable prey and select those that minimize the investment of energy and time (Schoener 1971, Krebs 1978). Parasite induced changes in host characteristics commonly result in a weakening of the host (Thompson 1990). This, and the fact that predators preferentially attack or have greater success capturing weak and diseased prey (Temple 1987) indicates that predators are expected to select parasitized individuals from the host population.

This potential difference in vulnerability of parasitized and healthy hosts would have crucial consequences for the developmental strategies of endoparasitoids, the immature stages being substantially tied to the biology of the host (Price 1973). Specifically, parasitoids die if their hosts are preyed upon, and mechanisms by which immature parasitoids lower host susceptibility may be absolute requirements for successful parasitism.

Functional aspects of the ecology of hosts parasitized by insect parasitoids are important but not fully explored. For one thing, the question as to whether parasitized hosts are really more vulnerable to predation than healthy ones remains open. This paper is intended to stimulate curiosity on interactions between predators and parasitized hosts rather than satisfy it. At present, the number of predictions, often

based on anecdotal observations or derived from the literature on protozoan and metazoan parasites, greatly exceeds the number of experimental investigations. To my knowledge, no study has been designed to directly examine the question of vulnerability of hosts parasitized by parasitoids to insect predators. The consequences have been ignored in population ecology. In this paper I use the general term parasitoid, but most of the discussed aspects refer to koinobiont parasitoids (Askew & Shaw 1986).

CHANGES IN SUSCEPTIBILITY

Insects are constantly endangered by natural enemies and have evolved various means of morphological, physiological and behavioural defenses against them (Evans & Schmidt 1990). Mimicry, camouflage, aposematism, armour, aggressive reactions, escape responses and so forth attest to the strong selective force exerted by predators (Harvey & Greenwood 1978). Insect prey species generally exhibit multiple anti-predator mechanisms by which different types of predators are deterred (Pearson 1990). The outcome of the interaction depends on the nature of the host and the predator.

Several published studies document susceptibility to predation of a wide range of invertebrates and vertebrates infected by typical parasites (Temple 1987, Moore & Gotelli 1990), many involving insect hosts. Changes in host characteristics frequently reflect indirect stress effects, which have no adaptive significance for either the host or the parasite (Minchella 1985). Apart from that, parasite-induced changes of host behaviour may increase vulnerability to predation. Typical examples are those in which parasites with complex life-cycles alter the behaviour of their intermediate host to increase the probability of transmission to the final host (reviewed by Holmes & Bethel 1972, Moore & Gotelli 1990). To promote selective predation, parasites have been shown to reduce stamina, decrease locomotory efficiency, cause disorientation and alter host responses to environmental stimuli such as temperature, light, gravity, and substrate (Holmes & Bethel 1972).

Similarly, a number of subtle developmental and behavioural changes in hosts parasitized by insect parasitoids have been observed. Parasitoids have been shown to affect energy allocation within the host (Slansky 1986) and to interfere with the host's endocrine system (Beckage 1985). This may modify host developmental time, cause the host to pass through supernumerary larval instars, induce premature or prevent host pupation, and modify morphological features such as pigmentation or thickness of the integuments. Parasitized hosts may also become sluggish, increase or decrease their rate of feeding, reproduce and mate at a reduced rate, change their period of activity, change their foraging patterns, or select specific microhabitats (see Slansky & Scriber 1985, Brodeur & McNeil 1992, Horton & Moore 1993).

Predictably, all these changes would influence host defensive behaviours, as well as patterns of predator avoidance, thereby modifying overall susceptibility of parasitized hosts. However, the link between parasitism and vulnerability in predator-parasitized host interactions is by no means inclusive. In a review, Fritz (1982) identified nine studies where evidence of differential predation of parasitized hosts had been reported. Predation was higher on nonparasitized hosts in five cases, higher on parasitized hosts in two cases, and similar in the remaining two. Yet, only one

study involved an insect predator, the others concerned bird or mammal predators. Tostowarick (1972) showed that sawflies parasitized by either a tachinid fly or an ichneumonid wasp were more susceptible to predation by a pentatomid bug than nonparasitized conspecifics. To my knowledge, only two other studies have been published since the review of Fritz (1982). Jones (1987) showed that pierid caterpillars parasitized by *Apanteles glomeratus* were more likely to be attacked and killed by ants than healthy caterpillars. Roland (1990) reported that pupae of the winter moth *Operophtera brumata*, parasitized by a tachinid fly, *Cyzenis albicans*, are less susceptible to predation by beetle larvae than nonparasitized pupae. Here, I deliberately ignore evidence of differential predation of parasitized aphids (and aphid mummies) in systems involving ant-attendance. These should be analysed carefully as differences in host mortality may be a consequence of the mutualistic relation between aphids and ants rather than the result of a difference in aphid vulnerabilty (Vinson & Scarborough 1991).

ARE PARASITIZED HOSTS REALLY MORE VULNERABLE TO PREDATION?

The limited experimental evidence is insufficient to provide a comprehensive pattern for relationships between parasitism and host susceptibility to predators. Predation rate differences do not emerge consistently among studied systems, with parasitized hosts being less, more or as susceptible to predation as nonparasitized hosts. This raises questions about the assumption of increased susceptibility of hosts parasitized by parasitoids to insect predators.

Because immature parasitoid mortality is largely contingent on that of the host (Price 1973), and because predation has been identified as a significant selective force in the evolution of several animal characteristics, natural selection should act to promote parasitoid characteristics that make parasitized hosts less susceptible to predation (Fritz 1982). I would therefore suggest that hosts parasitized by koinobiont parasitoids are similar to nonparasitized hosts in their susceptibility to insect predators. Mortality of parasitized hosts would be likely to increase only at specific stages of parasitoid development, such as during wasp egression or while moving between microhabitats.

Assessing whether parasitized and healthy hosts vary in their level of vulnerability to predation is challenging. First, there is a great variety of means by which parasitoids exploit their host, and it is very unlikely that the outcome of various host-parasitoid associations would be identical with regards to susceptibility of parasitized hosts. Second, predators from different taxa appear to differ in their attitude towards parasitized and nonparasitized hosts (Jones 1987). Third, parasitized host characteristics are not static, and vulnerability is expected to vary during the course of the interaction, primarily in relation to parasitoid ontogeny.

IMMATURE PARASITOID STRATEGIES

To counteract predation pressure on the parasitized host, two main strategies which endoparasitoids adopt have been suggested: reliance on host defensive responses, and manipulation of host behaviour.

As noted by Jones (1987), hosts are already doing their best to avoid predators, and developing parasitoid larvae should exploit the host's defensive mechanisms to promote their own survival (see also Brodeur & Vet 1994). In particular, current theory assumes animals have some capacity to assess predation risks which influence their responses to specific threats (Lima & Dill 1990). It is likely that parasitoid larvae, confined within the host, do not have the capacity to actually perceive predation risk. They may therefore rely on, and benefit from, the behavioural sensitivity of their host to predators.

Consequently, it might be beneficial for endoparasitoids to evolve towards a relationship where immatures have minimal effect on the defensive behavioural patterns of the host. For example, selective tissue destruction by feeding on haemolymph, fat tissues and reproductive organs of the host, as well as selective feeding rate through partial assimilation of the food ingested by the host would contribute to maintain parasitized hosts in relatively good condition during parasitoid larval development (Slansky 1986).

A second strategy has been suggested by Vinson (1975) and Fritz (1982), who hypothesized that immature parasitoids may reduce risk of predation by manipulating the behaviour of the host. One prediction was that the timing of host behaviour manipulation should coincide with a period of high vulnerability (Fritz 1982), such as near the end of parasitoid larval development. At this point host defensive capacities are more likely to be altered. Recent studies have provided experimental evidence that host behaviour manipulation can lower the probability of hyperparasitism (Stamp 1981, Brodeur & McNeil 1989, 1992). However, the adaptiveness of parasitoid-modified host behaviour relative to predation by arthropods has not yet been demonstrated.

Presumably, the most reliable scenario to reduce the impact of predation would be for many koinobiont parasitoids to rely on the host defensive mechanisms as long as they are effective, and thereafter to manipulate the behaviour of the host.

RELATIONSHIP TO COMMUNITY STRUCTURE, HOST RANGE AND BIOLOGICAL CONTROL

Parasitism and predation are among the major biotic determinants of animal community structure. Besides the direct effects parasitism has on population dynamics of the host (Anderson & May 1978), it may interface with predation by modifying parasitized host vulnerability (see above), thereby affecting the distribution, abundance and diversity of both the host and the parasite (Minchella & Scott 1991). Some empirical evidence exists for typical parasites suggesting that the structure of animal communities is, to varying degrees, the result of predator-parasitized host interactions (see Dobson 1988). Such information is lacking for communities involving insect host-parasitoid systems. However, one hypothesis for lower species diversity of Ichneumonidae in the tropics as compared to temperate areas is that, because predator diversity is higher in the tropics and parasitized hosts are assumed to be more vulnerable, predation pressure on parasitized hosts would reduce parasitoid species diversity (Rathcke & Price 1976).

Predator-parasitized host interactions may also play a role in determining whether or not a potential host can be successfully utilized by parasitoids. The

diversity and abundance of arthropod predators are high in most types of terrestrial habitats (Shoenly 1990). Predictably, parasitized hosts encounter a wide range of predatory species and differ in their relative vulnerability. Thus, all other aspects being equal, if immature parasitoids experience different levels of predation when developing in different host species, they should evolve a preference for the less vulnerable host species.

Understanding patterns of vulnerability of parasitized and nonparasitized hosts might also be meaningful for implementing effective biological control strategies, as interference between beneficial organisms should be avoided (van Lenteren & Woets 1988). A growing number of biological control programs tend to combine two or more species of both predators and parasitoids to reduce populations of pest species. However, potentially detrimental effects of predatory species on parasitoids, through predation of parasitized hosts, are unknown. For example, the parasitic wasp, *Aphidius matricariae*, is commonly used together with aphid predators, such as ladybird beetles, lacewings, and gall midges to control the green peach aphid, *Mysus persicae*, on greenhouse tomatoes in Canada. Unfortunately, we are unaware of the intrinsic vulnerability of parasitized aphids to each of the predators. Consequently, it prevents a determination of the optimal conditions under which predators and parasitoids can be used in conjunction to suppress aphid populations.

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Phylogenetics and biological transitions in the Braconidae (Hymenoptera: Ichneumonoidea)

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Phylogenetic analysis of cyclostome and selected non-cyclostome subfamilies of Braconidae employing several novel character sets in addition to traditional adult external morphology shows the cyclostome subfamilies to be a paraphyletic basal lineage. Some analyses support the hypothesis that endoparasitism in the Rogadinae, Opiinae, Alysiinae, Aphidiinae and in the non-cyclostome group of subfamilies may have had a single origin, whilst others do not.

Keywords: Braconidae, cyclostomes, endoparasitism, parasitoids, phylogeny.

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The Braconidae is a large family of parasitic Hymenoptera with some 40 subfamilies which collectively encompass a wide range of biologies and display great morphological variation. For some time now the family has been viewed as comprising two rather distinct groups of subfamiles, the so-called cyclostomes and the non-cyclostomes, the former being characterized for the most part by possession of a ventrally recessed clypeus and a concave glabrous labrum and a number of features of their internal anatomy (Edson & Vinson 1979; Barlin & Vinson 1981; Maeto 1987; Quicke et al. 1992). Whilst the non-cyclostomes, in the sense of Quicke & Achterberg (1990), are entirely koinobiont endoparasitoids, both idiobiont ectoparasitism and koinobiont endoparasitism are found in the cyclostome group of subfamilies (Rogadinae, Opiinae, Alysiinae and Aphidiinae). Many different opinions concerning the evolutionary relationships of these subfamilies, and in particular the relationships of the endoparasitic Rogadinae, Opiinae, Alysiinae, Aphidiinae and non-cyclostomes, have been expressed over the years.

Quicke & Achterberg (1990) presented a detailed phylogenetic analysis of more than 40 braconid taxa and employing 96 characters. The cladograms obtained were surprising to many workers in that they suggested that endoparasitism had evolved only once in the family (discounting its occurrence in specialist lineages within the Braconinae and Doryctinae). Whilst this result was drawn into some doubt by Wharton et al (1992) who pointed out the existence of some errors in the analysis, Achterberg & Quicke (1992) were unable to agree on all the points raised and Wharton et al.'s reanalysis still showed the same relationships. Subsequently, Whitfield (1992) presented an analysis of a restricted set of cyclostome taxa as an attempt to resolve the number of times that endoparasitism had arisen in this group.

However, again imprecision in the methodology employed and some erroneous data entries were shown to compromise this conclusion (Quicke 1993). Thus there still remains considerable uncertainty about the evolutionary history of the Braconidae and it seems likely that the incorporation of new data sets as well as careful reappraisal of previously used data will be required to obtain a robust phylogeny. Here I present some preliminary reanalyses incorporating new or previouly little-used characters in an attempt to throw additional light on the evolution of endoparasitism in the Braconidae.

METHODS

Many features, particularly of larval morphology and biology, that had been used by Quicke & Achterberg (1990) but which might be associated functionally with endoparasitism were excluded from the analysis so as to minimize possible bias in the results. In addition to cyclostome taxa, data are also incorporated for small but diverse set of representatives of non-cyclostome braconids (Agathidinae, Euphorinae, Helconinae, Meteorinae, Microgastrinae and Trachypetinae). Data were analysed using the maximum parsimony program PAUP version 3.1.1 (Swofford, 1993). All addition sequences were tested and most parsimonious trees found using the tree-bisection-reconnection option. Characters were polarized using the Ichneumonidae to construct a hypothetical outgroup taxon.

CHARACTERS AND CHARACTER STATES

1. Venom gland reservoir: 0 = undivided; 1 = subdivided.
2. Venom glands inserted on to reservoir: 0 = anteriorly or medially; 1 = posteriorly.
3. Number of venom gland insertions on to reservoir or primary duct: 0 = 2; 1 = 1; 2 = many. (Unordered)
4. Venom primary duct: 0 = not terminating in an unsculptured, weakly chitinized bulb; 1 = terminating in a bulb.
5. Primary venom duct intima: 0 = completely with spiral or annular sculpture; 1 = partly spirally sculptured (to beyond level of gland inserts) but unsculptured for at least the posterior quarter of its length; 2 = not spirally sculptured (sculpture not reaching level of gland insertions). (Ordered)
6. Venom gland reservoir: 0 = weakly muscularized; 1 = highly muscularized.
7. Number of pairs of ovarioles: 0 = fixed at 1; 1 = fixed at 2; 2 = 3-6; 3 = more than 6. (Ordered)
8. Number of valvilli on each lower ovipositor valve: 0 = 2; 1 = 1; 2 = 0 (Unordered)
9. Location of ovipositor valvilli: 0 = on distal third of lower ovipositor valve; 1 = on medial third; 2 = on basal third.(Ordered)
10. Valvillus: 0 = simple; 1 = with a fringe.
11. Valvillus: 0 = petal-shaped, with petiole smoothly merging with anterior face; 1 = cup-shaped, with petiole located sub-marginally.
12. Ventral margin of dorsal ovipositor valve: 0 = deeply cleft in cross-section (i.e. considerably less deep medially than submedially); 1 = more or less flat or weakly emarginate.

13. Dorsal ovipositor valve, pre-apically: 0 = more broadly connected with a mid-longitudinal septum.; 1 = with two halves joined by only a thin bridge.
14. Lumen of dorsal ovipositor valve: 0 = divided by a mid-longitudinal septum; 1 = undivided.
15. Egg tube: 0 = closed dorsally be lower valves; 1 = closed dorsally by upper valve.
16. Eighth metasomal sternite of male: 0 = produced medio-anteriorly (usually sharply pointed); 1 = simple, more or less straight.
17. Cuspidal lobe of male genitalia: 0 = well developed; 1 = reduced or absent.
18. Cuspidal lobe of male genitalia: 0 = articulated with volsella; 1 = immovably fused with volsella.
19. Parameral processes: 0 = long, extending well beyond middle of digitus; 1 = short, not only just reaching level of middle of digitus.
20. Basal ring (gonobase): 0 = short and transverse (concave, straight or hardly produced anteromedially); 1 = moderately elongate (distinctly produced anteromedially); 2 = very elongate. (Ordered)
21. Hagens glands (on metasomal tergum 8 + 9): 0 = absent; 1 = present.
22. Vas deferens insertion on accessory glands: 0 = posterior; 1 = anterior.
23. Testes: 0 = fused dorsal to gut; 1 = separate or fused ventral to gut.
24. Mature spermatozoa: 0 = 60 – 150 μm long, normal structure; 1 = 25 – 50 μm long, normal structure; 2 = 5 – 20 μm long, highly modified structure. (Ordered)
25. Axoneme of mature spermatozoan: 0 = with a pair of central microtubules; 1 = with only 1 or with no central microtubules.
26. Mitochondrial derivatives of mature spermatozoa: 0 = medium-sized to large (approximately as deep as the axoneme); 1 = reduced (approximately half diameter of axoneme or less).
27. Placodiform sensillae on peribasal flagellomeres: 0 = not extending along whole length of flagellomere, usually irregularly distributed or with strongly staggered pattern; 1 = entire, extending more or less along whole length of flagellomere, in a regular arrangement.
28. Internal opening of antennal placode sensillae (Barlin & Vinson, 1981): 0 = round to short eliptical, not more than 0.25 times external length of sensillum; 1 = elongate, between 0.25 and 0.8 length of sensillum; 2 = occupying entire length of sensillum. (Ordered)
29. Hypostomal carina (if its position can be determined): 0 = joining occipital carina before mandibular insertion; 1 = reaching mandibular insertion separately from occipital carina.
30. Labrum: 0 = flat or convex; 1 = distinctly concave.
31. Labrum: 0 = largely setose; 1 = with setae restricted to periphery.
32. Dorsally rounded hypoclypeal depression: 0 = absent; 1 = present.
33. Hypoclypeus: 0 = without a pair of clusters of setae; 1 = with a pair of clusters of setae.
34. Propleuron: 0 = with a distinct posterior flange; 1 = without a flange.
35. Prepectal carina: 0 = present (at least partly); 1 = absent.
36. Anterior subalar depression: 0 = carinate; 1 = smooth.
37. Scuto-scutellar sulcus: 0 = absent ; 1 = partially developed; 2 = complete. (Ordered)
38. Propodeum: 0 = areolate, with at least two enclosed median areas; 1 = areolate but with only one enclosed median area; 2 = without distinct enclosed median area. (Ordered)

39. Setae on distomedial part of hind tibia: 0 = simple; 1 = apically lanceolate and (usually) flattened; 2 = entirely flattened and longitudinally striate (often but not always forming a comb). (Ordered)
40. Hind tarsus: 0 = longer than or subequal to length of hind tibia (at least 0.9 x hind tibia length); 1 = considerably shorter than hind tibia (0.5 – 0.7 x length).
41. Forewing vein 2-SR+M: 0 = transverse; 1 = longitudinal.
42. Forewing vein r arising: 0 = beyond the middle of the pterostigma; 1 = medially or submedially on pterostigma.
43. Ratio of length of forewing veins SR1:3-SR: 0 = <1.5:1; 1 = >1.5:1.
44. Forewing vein 3-M: 0 = tubular for more than 0.7 times distance to wing tip; 1 = tubular for 0.4 – 0.7 times distance to wing tip; 2 = tubular for less than 0.4 times distance to wing tip. (Ordered)
45. Hindwing basal (costellar) catch bristles located: 0 = on a well developed trace of vein C and all located beyond the point of emergence of vein 1-SC+R; 1 = on a small stub of vein C but overlapping with origin of vein 1-SC+R; 2 = entirely on vein C+SC+R without any appreciable development of vein C. (Ordered)
46. Proper hamules: 0 = 'S'-shaped or at least with a recurved or hooked tip; 1 = shaped as a simple sickle-like hook.
47. Hind wing vein m-cu: 0 = present; 1 = absent.
48. Hindwing vein 2-CU: 0=absent; 1=present.
49. 1st metasomal tergite: 0 = simple; 1 = with a pair of posteriorly diverging grooves arising from a short medial groove just posterior to the insertion of the ligament.
50. 2nd metasomal (3rd abdominal) spiracle: 0 = in notum; 1 = in epipleuron.
51. 2nd metasomal tergite: 0 = without a mid-longitudinal carina; 1 = with a mid-longitudian carina (rarely reduced).
52. Mature larva with first spiracle: 0 = in prothoracic segment (posterior part); 1 = in mesothoracic segment (anterior part).

RESULTS

Analysis of the data matrix presented in Table 1 yielded 96 equally parsimonious trees of length 158, consistency index 0.42 and retention index 0.65, which were used to construct strict and Adams consensus trees (Figs 1a, b). As with previous results (Quicke & Achterberg 1990), the cyclostome subfamilies do not appear as a monophyletic clade but rather they form a basal grade with the Apozyginae the most basal subfamily. Unfortunately, the critical question as to whether endoparasitism in the non-cyclostomes and in the Opiinae, Alysiinae and Rogadinae had a common origin cannot be answered due to lack of resolution in this part of the cladogram though the Adams consensus tree (Fig. 1b) showed the Opiinae + Alysiinae as being paraphyletic respect to a clade comprising the Aphidiinae together with the non-cyclostome subfamilies. In order to resolve this part of the tree further, three further analyses were performed. Using the rescaled consistency index as the weighting function, two rounds of reweighting gave three trees whose strict consensus was fully resolved (Fig. 1c). In these, endoparasitism appears to have evolved twice, once in the Rogadinae (including Clinocentrini) and once in the Opiinae+Alysiinae+Aphidiinae-+non-cyclostomes. Using the consistency index as the reweighting function gave similar results. However, using the retention index after one cycle gave three trees

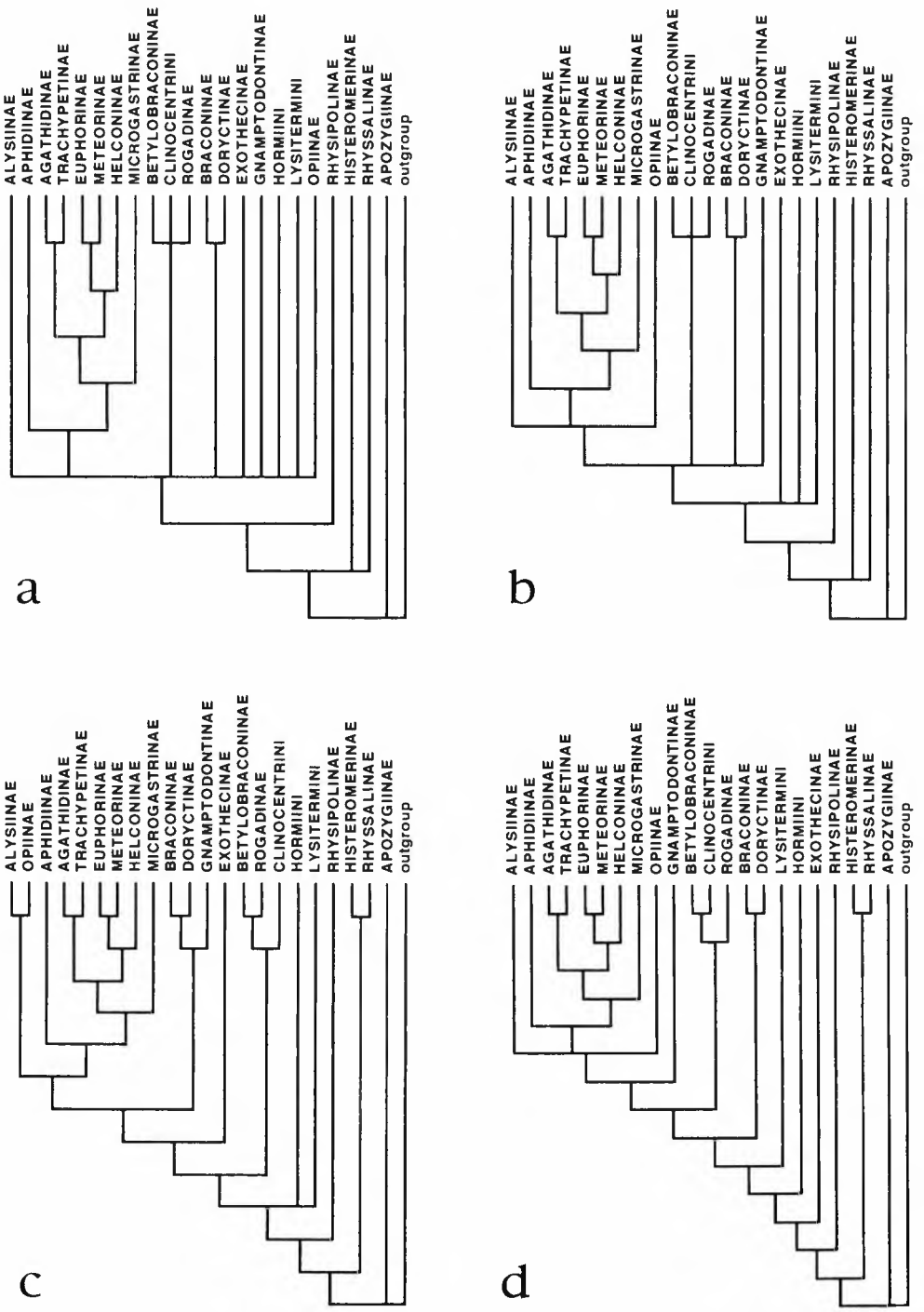

Fig. 1. 1a, b. Strict and Adams concensus of 96 trees obtained by parsimony analysis of data matrix: length 158, ensemble consistency index 0.42, ensemble retention index 0.65; 1c. Strict consensus of 3 trees obtained after two cycles of reweighting using maximum value of rescaled consistency index; 1d. Strict consensus of 3 trees obtained after reweighting using maximum value of retention index.

whose fully resolved strict consensus (Fig. 1d) showed a single origin of endoparasitism (the biology of the Gnamptodontinae is not yet known).

In conclusion, we are still not in a position to say definitively how many times endoparasitism has evolved within the Braconidae and incorporation of more datasets will no doubt be required. Molecular studies are now underway and it is hoped that a total evidence approach will finally provide the answer.

SUMMARY

Incorporation of new data still does not permit the construction of an unequivocal phylogenetic hypothesis for the Braconidae and the question of how many times endoparasitism has evolved remains unanswered. However, the new analyses reconfirm the view that the cyclostome braconid subfamilies constitute a paraphyletic basal grade with respect to the non-cyclostome ones.

Table 1. Data matrix.

ALYSIINAE	???0?11100	0000010112	1011001070	1000112?00	????20?101	00
APHIDIINAE	01?0?102??	?101110100	0111011201	1001010120	01?1211101	00
APOZYGINAE	???????00?	?0?0?00002	010???0101	1110000?1	0011011000	0?
BETYLOBRACONINAE	001001?12?	?100011101	000???1001	??00001220	10?0100100	1?
BRACONINAE	0120211100	1100?111?2	0000001071	?111110210	110?201110	00
CLINOCENTRINI	0010011107	?100111111	0?????1001	1100001120	11?2010100	10
DORYCTINAE	1100211?00	11000??1?2	0?0???1001	?1100?2?10	????20?10?	00
EXOTHECINAE	1110111110	1100001111	0000001071	1100102100	????200100	00
GNAMPTODONTINAE	011011?100	0101011111	1?????10?1	1100112210	111220?100	0?
HISTEROMERINAE	0111001010	1010000000	0??0001201	1111000200	0001110111	0?
HORMIINI	0110017100	1000011111	0?????1001	1100002110	?01??00100	0?
LYSITERMINI	0110?1?100	?010?11110	??????1001	1100012170	0010?00100	0?
OPIINAE	?110111107	0100010110	101?001010	1?00112?10	?1???0?10?	00
RHYSIPOLINAE	0110017000	?000001111	000???1011	1110001170	10?22?0100	00
RHYSSALINAE	0111007000	?0?0001?10	0??0001001	1110000001	0001200101	00
ROGADINAE	0?10011101	0?00011110	0000001001	1100002220	11?200?100	10
AGATHIDINAE	0110203101	0101117000	0110110100	00?0100??0	1112000001	??
EUPHORINAE	0?10002111	0101110100	011201?1?0	00?0000?20	?012101101	01
HELCONINAE	0010203101	0101110??0	0112110170	00?0000??0	10??001101	01
METEORINAE	0010202121	0101100000	0112010100	00?0000??0	1012101101	01
MICROGASTRINAE	0110201021	0101100100	0112110170	00??000??0	1?12001171	01
TRACHYPETINAE	0110?132??	??0??11100	011???0100	00?0002110	?102001001	0?
outgroup	000000200?	0000000000	0000000100	00?0000?0	0?0000?000	00

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Němec and Starý's «Population Diversity Centre» Hypothesis for Aphid Parasitoids Re-visited

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The results of studies on the host preference behaviour and population genetics of the aphid parasitoid *Aphidius ervi* Haliday are considered in the context of the «population diversity centre» hypothesis proposed by Němec & Starý (1984a). Available data are consistent with the concept and with the designation of the pea aphid *Acyrthosiphon pisum* as the population diversity centre for *A.ervi*. Implications of the hypothesis in terms of aphidiine speciation and population dynamics are discussed.

Keywords: Aphid parasitoids, Braconidae, behaviour, electrophoresis, genetics.

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Němec & Starý (1984a) proposed the «Population Diversity Centre» hypothesis to explain differences in genetic diversity between populations of the same parasitoid species occurring on different aphid hosts. The host species associated with the greatest genetic diversity in the parasitoid was regarded as the main, and probably the original, host and they called this host the Population Diversity Centre; the normal host range of the parasitoid was termed the Population Diversity Web.

Němec & Starý (1984a) used *Diaeretiella rapae* (M^{c}Intosh) parasitising *Brevicoryne brassicae* (L.), *Myzus persicae* (Sulz.) and *Hayhurstia atriplicis* (L.) to illustrate the hypothesis. Genetic variability was assessed by measuring allozyme variability on electrophoretic gels stained for esterase enzymes (Němec & Starý 1984a). Variability was greatest in samples collected from *B.brassicae* and so this host was designated the population diversity centre. A similar study was also done on *Aphidius ervi* Haliday parasitising *Acyrthosiphon pisum* (Harris), *Sitobion avenae* (F.) and *Microlophium carnosum* (Buckton) (Němec Starý 1984b). In this case samples from *A.pisum* had the greatest genetic diversity.

The hypothesis implies that species such as *D.rapae* and *A.ervi* have, during their evolutionary history, expanded their host range from a single, original host (the population diversity centre) onto one or more alternative hosts, thus forming the population diversity web. Tremblay & Pennacchio (1988) suggested that such an expansion to new hosts could be a response to intraspecific competition, and that the processs could lead to adaptive divergence (Templeton 1981) between populations on different hosts. Over time, speciation would occur through the development of ecological and ethological barriers to gene flow.

In this paper the results of a series of studies done at Rothamsted, mainly on *A.ervi*, are considered in the context of the population diversity centre hypothesis.

HOST PREFERENCE

The ability of *A.ervi* to transfer between different host species was examined in a series of laboratory experiments (Cameron et al. 1984, Powell & Wright 1988). Parasitoids reared from *A.pisum* produced very few mummies when confined with either *M.carnosum* or *S.avenae* whereas those reared from *M.carnosum* produced mummies on *A.pisum* at the same rate as on *M.carnosum*. Differences in mummy production on different hosts could be caused by varying attack rates by female parasitoids. Alternatively, differences in host physiology could affect either host acceptance after ovipositor penetration or egg encapsulation. Subsequent studies of parasitoid attack rates revealed differences between hosts consistent with the varying level of mummy production (Powell & Wright 1988). This implies that a lack of host recognition by female parasitoids was the cause of the low mummy production which sometimes accompanied transfers to a new hots.

If *A.pisum* is the population diversity centre for *A.ervi*, and therefore the host on which most genetic diversity occurs, populations on *M.carnosum* and *S.avenae* probably arose when some individuals from the original parasitoid population became adapted to attack these new hosts. They could have achieved this either by developing attack and acceptance responses to semiochemical and physiological cues associated with the new hosts or by overcoming their defence mechanisms, or both. Such adaptation is likely to have involved only part of the genotypic spectrum present in the original *A.ervi* population. This would explain the reduced genetic diversity recorded in *A.ervi* populations from *M.carnosum* and *S.avenae* (Němec & Starý 1984a). Thus, the assymetry observed in host transfer trials (Powell & Wright 1988, Hopper et al. 1993) is consistent with the population diversity centre hypothesis if we assume that only a proportion of the population on *A.pisum* possessed the genotype required for successful parasitisation of the other two hosts (resulting in a low average mummy production upon transfer). However, all individuals in populations from *M.carnosum* and *S.avenae* were genetically adapted to recognise and accept *A.pisum* as a host (Powell & Wright 1992, Hopper et al. 1993).

However, data which seem to conflict with this reasoning were obtained by Němec & Starý (1983) in some of their transfer trials. When they transferred *A.ervi* from *M.carnosum* to *A.pisum* very few mummies were formed, in direct contrast to our results. Nevertheless, the female parasitoids from *M.carnosum* «vigorously oviposited» into *A.pisum* (Němec & Starý 1983), as observed in our trials, so that the problem was not due to lack of host recognition. Therefore, the parasitoids in the trials of Němec & Starý (1983) either did not accept the new host and so did not release eggs after ovipositor penetration, or their eggs and/or larvae could not overcome the physiological defense mechanisms of the new host. The latter explanation was proposed by Starý et al. (1985). This difference between our results and those of Němec & Starý (1983) suggests that strains of the aphid host differ in their ability to defend themselves physiologically against parasitism.

GENETICS AND INTRASPECIFIC VARIABILITY

Starý et al. (1985) concluded that *A.ervi* occurs as a number of «biological races», each attached to a particular host and that genetic differences exist between populations. More recently, genetic and biological differences between populations of *A.ervi* on the same host have been documented (Gonzalez 1988, Botto et al. 1988). The term «biotype» has been used in both situations to denote these infraspecific categories but Tremblay & Pennacchio (1988) warn against the current ambiguity of this term and advocate the development of more appropriate terminology. Němec & Starý (1986) distinguished between host biotypes and genetic strains, pointing out that the latter can occur on more than one host, having become adapted to new hosts from populations originally parasitising the population diversity centre host.

Host preference in aphid parasitoids may have a genetic basis. To investigate this, *A.ervi* from different hosts were cross-mated during host preference experiments (Powell & Wright 1988, Powell, Atanassova & Wright, unpubl.). In these experiments host recognition behaviour, as measured by attack rates, was strongly influenced by genotype. Although most *A.ervi* females from a population in which both parents were also reared on *A.pisum* had very low attack rates against *M.carnosum*, their attack rates were high on both hosts if male parents were reared on *M.carnosum*. Olfactory responses to semiochemical cues play a major role in host recognition by aphid parasitoids (Decker 1988, Powell et al. 1991) and so these responses are probably genetically determined. This is consistent with the assumption that genetic strains which were able to respond to semiochemical recognition cues from other aphids such as *M.carnosum* and *S.avenae* developed in populations of *A.ervi* on the population diverity centre host, *A.pisum*. Once populations had become established on these new hosts, they would retain the ability to recognise and attack *A.pisum* unless new genotypes evolved which were specific to the new host. Such new genotypes would then form a behavioural barrier between populations on the new and original hosts, leading to sympatric speciation.

ELECTROPHORETIC STUDIES

Studies of intraspecific genetic variability in populations of *D.rapae* and *A.ervi*, based on isozyme variability, led to the concept of population diversity centres (Němec & Starý 1983, 1984a, 1984b). Transfers of *A.ervi* from one host species to another are often accompanied by reductions in isozyme variability (Němec & Starý 1983, Cameron et al. 1984). When parasitoids were transfered from *A.pisum* to *S.avenae* they produced very few mummies and it took four generations on the new host for mummy production to reach levels similar to those on the original host (Cameron et al. 1984). Electrophoretic studies of esterase isozymes were done during these transfer trials and revealed that only one of a pair of alleles present in the laboratory population on *A.pisum* occurred in the new population on *S.avenae*. This supports the assumption that only some of the genetic strains present in *A.ervi* popultions on *A.pisum* (the population diversity contre host) were adapted to parasitise alternative hosts such as *S.avenae* and *M.carnosum*. However, when *A.ervi* was transfered from *M.carnosum* to *A.pisum* in the laboratory only one of seven esterase isozymes occurred in the new population (Němec & Starý 1983). This suggests that the

population on *M.carnosum* in Czechoslovakia had evolved genetic strains which were no longer able to parasitise *A.pisum*, and therefore sympatric speciation was in progress.

Field populations of *A.ervi* have much greater isozyme variability than do laboratory populations (Unruh et al. 1983, Němec & Starý 1985). Recent electrophoretic studies of field populations sampled in England and in Bulgaria looked at a range of isozymes (Powell, Atanassova & right, unpubl.). Isozyme banding patterns were more similar in populations on *A.pisum* from Bulgaria and England than they were between sympatric populations on *A.pisum* and *M.carnosum* in England.

This again suggests that ecological or behavioural isolating mechanisms have developed between populations of *A.ervi* on different hosts.

SPECIATION

Once barriers to gene flow between sympatric populations have developed, following adaptation to alternative host species by genetic strains originating on the population diversity centre host, speciation by adaptive divergence would be expected (Tremblay & Pennacchio 1988). Evidence exists to suggest that this has occurred in *A.ervi* to such an extent that populations on *M.carnosum* can be regarded as a distinct species, *Aphidius microlophii* (Pennacchio & Tremblay 1987). In the laboratory, parasitoids from populations on *M.carnosum* cross-mate with those from populations on *A.pisum* and produce fertile hybrids (Powell, unpubl.). Also, as discussed above, parasitoids from *M.carnosum* populations in England will transfer to *A.pisum* in the laboratory without any reduction in mummy production. However, it is now essential to demonstrate whether or not individuals move between sympatric field populations on different host species, since ecological and even behavioural barriers existing between field populations could disappear in the laboratory.

A commonly used method for detecting significant gene flow between conspecific animal populations is to compare isozyme frequencies between samples from these populations (Loxdale & Den Hollander 1989). If significant gene flow is occurring then they could be assumed to be sub-populations of the same population and would have identical, or very similar, isozyme frequencies. However, in the case of aphid parasitoids, populations occurring on different hosts could have significantly different isozyme frequencies even in the presence of significant gene flow. This would occur if only some of the genotype within the population on host «a» were able to transfer to host «b» but all genotypes from the population on host «b» could transfer to host «a». This is exactly the situation predicted by the population diversity centre hypothesis and supported by some of the data outlined above. Alternative methods of detecting gene flow between parasitoid populations, such as the mark-release-recapture of individuals, are therefore urgently needed in order to confirm this situation in the field.

CONCLUSIONS

A series of studies on the host preference behaviour and population genetics of *Aphidius ervi* provided data which were consistent with the concept of population diversity centres, as proposed by Němec & Starý (1984a). *Aphidius ervi* has received considerable attention because of its worldwide importance as a biological control agent, but similar studies on other oligophagous species are required to establish whether or not *A.ervi* is an unusual case. For example, detailed studies of host preference in truly polyphagous species would demonstrate the extent to which the population diversity centre hypothesis could be applied within the aphidiinae.

The aphidiine parasitoids, especially the genus *Aphidius*, present taxonomic difficulties when examined using traditional morphological criteria. One possible reason for this is that some species may be currently undergoing active speciation, either by adaptive divergence on different hosts or as a result of expansion of their geographic range. The problem is deciding when to «draw the line» and to designate populations on different hosts as distinct species. Further work on the field population ecology of aphid parasitoids is urgently needed to quantify gene flow between sympatric populations on different host species.

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Detection of genetic variability in *Trichogramma* populations using molecular markers

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Estimates of diversity between and within natural populations are necessary for the optimization of rearing strategies of *Trichogramma* to improve their efficiency in biological control programs.The PCR-based polymorphic assay procedure called RAPD has been used to survey genetic variability within a *Trichogramma evanescens* population. Extensive variability was detected. A screening of 40 isofemale lines using a single primer revealed 20 different RAPD banding patterns. The use of RAPD markers in population genetics and ecology studies is discussed.

Keywords: Genetic diversity, Insect parasitoid, RAPD markers, *Trichogramma.*

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Trichogramma are minute egg parasitoid hymenoptera particularly important as biological control of phytophagous insects that feed on crops. The taxonomy of the genus has been based on morphological traits. However these characters may display a great level of plasticity and do not always reflect the phylogenetic relationships among species. Efforts have to be devoted to identification and classification of *Trichogramma*. Moreover, despite their commercial use under inundative release in fields, very little is known of the basic biology of *Trichogramma* natural populations. There is a need for new types of markers that are species- diagnostic and that are also suitable for analyzing the intraspecific genetic polymorphism. This would be useful for practical management purposes.

Molecular techniques provide efficient tools for the study of natural population genetics. Restriction fragment length polymorphism (RFLP) of the DNA was the most frequently used marker, requiring however large amount of pure DNA, until the development of the polymerase chain reaction (PCR) (Saiki et al. 1988) that amplifies DNA from a few nanograms. Recently, genetic differences were demonstrated between *Trichogramma* species using DNA amplification of the variable first internal transcribed spacer (ITS1) of the ribosomal DNA (Orrego & Agudelo-Silva 1993). However PCR depends on knowing the DNA sequence to be amplified. The random amplified polymorphic DNA (RAPD) procedure developped by Williams et al. (1990) and Welsh & McClelland (1990) has revolutionized the detection of genetic diversity, since it is based on the random amplification of unknown DNA regions by PCR, using

a single ten to twenty nucleotide-long primer. In a preliminary study, Landry et al. (1993) used RAPD markers for *Trichogramma* species identification.

The present study defines the conditions for the successful application of RAPD to *Trichogramma* DNA using a 17-bp primer and demonstrates the great level of genetic polymorphism in a natural population of *Trichogramma evanescens*.

MATERIAL AND METHODS

The population under study was made of forty lines of *Trichogramma evanescens* (kindly provided by B. Pintureau). These lines were established from four wild-trapped parasitized egg clusters of a noctuid, from the vicinity of Lyon (France). The four egg clusters were labelled A to D and ten isofemale lines were made per egg-cluster when *Trichogramma* adults emerged. The forty lines were reared separately in the laboratory.

Total genomic DNA was extracted from about fifty adults from a single isofemale line according to the procedure described in Mac Ginnis et al. (1983). RAPD reactions were also carried out on single individuals whose DNA was extracted using a chelating resin, as described in Walsh et al. (1991). An adult parasitoid was placed into a 1.5 ml microfuge tube and crushed with a flame-stopped up Pasteur pipette. 20 ul of a 5% (w/v) Chelex solution (Bio-Rad Laboratories) was added to the specimen, heated at 56°C for 30 minutes then 100°C for 5 minutes, vortexed, centrifuged for a few seconds and stored at -20°C.

Amplification reactions were performed as reported by Williams et al. (1990), either with 10 ng of *Trichogramma* DNA or with 2 ul of Chelex supernatant and 0.5 unit of *Taq* DNA polymerase (Appligene). DNA was amplified by PCR in a ThermoJet thermal cycler (Equibio s.a., Angleur Belgium). An oligomer containing 17 nucleotides (CCCTGGACGTCTACAAT) with a 53% G-C content (kindly provided by D. Fournier and designed for other purposes) served as primer for amplification reactions. As described in Akopyanz et al. (1992), cycling program consisted in three low stringency amplification cycles of (94°C, 5 min ; 40°C, 5 min ; 70°C, 5 min) and 30 high stringency amplification cycles of (94°C, 1 min ; 55°C, 1 min ; 70°C, 2 min) followed by a final incubation for 10 minutes at 70°C. Products of amplification were electrophoresed in 1.4% agarose gels, stained with ethidium bromide and photographed under UV lights.

RESULTS

Ten offsprings of an isofemale line (line C9) were individually tested with RAPD system using the seventeen nucleotide primer, to ascertain the level of genetic homogeneity in the line. Figure 1 reveals that the ten individuals display the same RAPD banding pattern.

Total genomic DNA was extracted from each of the forty lines and was randomly amplified with the same seventeen nucleotide primer. Figure 2 shows the great level of polymorphism in RAPD banding patterns among some of the *T. evanescens* lines. Each pattern displays four to six bands (Figure 2). Isofemale line D9 displayed a RAPD banding pattern very different from the other lines (data not

shown). Actually this line appeared to belong to an other *Trichogramma* species (pers. obs.) and was not considered in further analysis. A total of twenty different bands of amplification was scored out of the thirtynine RAPD reactions. Two bands were considered different as long as they had not the same molecular weight. This means that I did not test for size variations and differences in band intensity were not taken into account. Out of these twenty bands, eighteen were polymorphic for presence-absence. For each of the thirtynine lines, the twenty bands of amplification were coded 1 for presence and 0 for absence (Table 1). This allowed us to define twenty different RAPD patterns. Table 1 clearly shows that bands are shared by several lines from different egg clusters, while some other bands are very rare in the population.

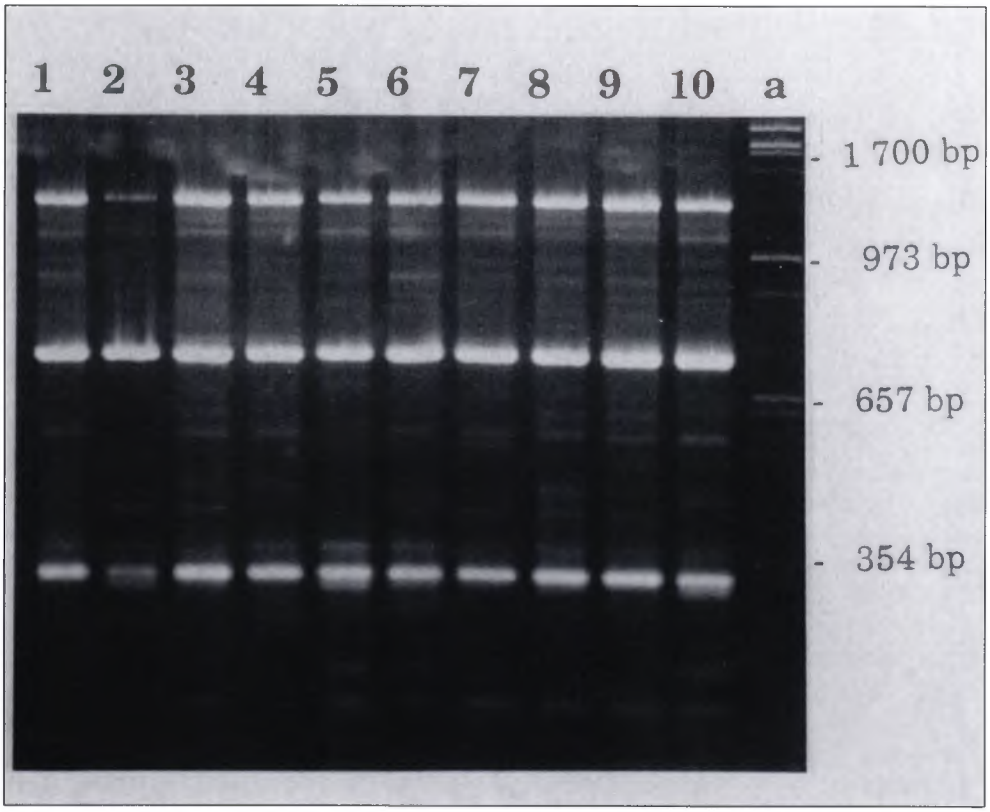

Figure 1: RAPD banding pattern of ten sibs of an isofemale line (C9) of *Trichogramma evanescens*. Lane (a) contains Cla I digests of the DNA of the phage Lambda that are used as molecular weight markers.

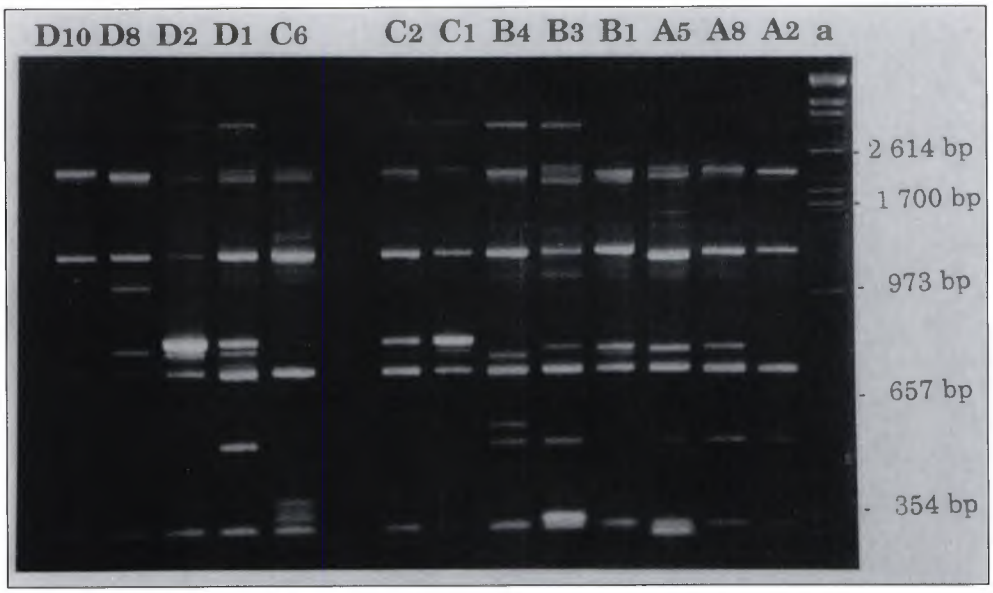

Figure 2: DNA fingerprints of some of the forty *Trichogramma evanescens* lines obtained through RAPD procedure. Lane (a) contains Cla I digests of the DNA of the phage Lambda that are used as molecular weight markers.

DISCUSSION

The aim of the present study was to identify discrete genetic markers to assess the genetic variablity in a population of forty isofemale lines of the species *T. evanescens.* A major advantage of RAPD analysis is that it provides multiloci informations in a single procedure for both the identification of the species and the detection of intraspecific variations.The first result of this study was that for a given DNA preparation, the primer used generates a characteristic and repeatable pattern of DNA bands. Moreover offsprings of an isofemale line display the same banding pattern. Therefore the DNA bands generated by the selected primer can be considered as genetically defined characters.

When DNA preparations from different lines of the same population are subjected to analysis, the RAPD patterns obtained with the same primer show a high level of similarity within the population as compared to populations from other species (data not shown). So, the second result of this study was the power of the RAPD procedure for *Trichogramma* species identification, as demonstrated with the particular banding pattern of line D9 which actually belongs to an other species of *Trichogramma.*

The great level of polymorphism detected among thirtynine lines added to the great intraline homogeneity strongly suggests that genetic variability potentially existed in the natural population of *T. evanescens* that parasitized the four egg clusters.

The next step in this study was to evaluate how many *Trichogramma* females effectively parasitized one egg cluster, a single female or several females? Six RAPD

Table 1: Definition of twenty RAPD patterns from the polymorphism (presence/absence) of the twenty bands of amplification revealed in a sample of forty lines of *T. evanescens* labeled A, B, C and D.

1	**1**0000000001000001001	A1-A3-A9
2	**1**0000000001000100001	A2-B7
3	**11**000001101000000001	A4
4	**1**0000001001000110001	A5
5	**1**0000100010100100001	A6
6	**1**0000001001000100001	A7-A8-A10-B6-B8-D5
7	**1**0000001001000000001	B1-D6-D10
8	**1**0000001001000100011	B2-B3-D3-D7
9	**1**0000000101001100001	B4-B5
10	**1**0000001001010100001	B9-B10
11	**1**0010001001000000001	C1
12	**1**0110000001000000001	C2
13	**1**0010000001000000001	C3-C8
14	**1**0000001001001000001	C4-C7-C10
15	**1**0000000001000000111	C5-C9
16	**1**0010000001000000111	C6
17	**1**0010010000100100001	D1
18	**1**0001001001000000001	D2
19	**1**0100001001000100001	D4
20	**11**000001101000000001	D8

patterns were distinguished among the ten lines A, six among lines B, six among lines C and seven among lines D. However, as mentionned previously, except maybe for lines C, lines from one egg cluster are not characterized by a particular genetic structure as compared to the other ones.

Therefore, whether only one female parasitized one egg cluster (A, B or D) seems unlikely. However, this hypothesis can not be totally excluded, particularly for egg cluster C. RAPD loci are supposed to be not linked and are dominant systems. This means that a RAPD locus that is heterozygous for presence absence will display the same pattern as a locus homozygous for presence. Depending on the rate of recombination between loci and knowing that the number of chromosomes in *Trichogramma* is equal to five, the offspring of a founding female could eventually display a high level of polymorphism. However, as mentionned previously, we tested RAPD patterns among ten individuals of the same line and no variability was observed.

From Table 1 it appears that a total of 9 to 12 different bands can be counted per egg cluster. If only one female parasitized an egg cluster, either she should display many more bands of amplification than each of the lines she generated and she should be heterozygous for most of the loci, or she should have mated with a male that has a

genotype very different from hers. Whatever the case, polymorphism at nine RAPD loci gives a possibility of 2^9 RAPD patterns in the progeny. This suggests that the probability that two or three sibs out of ten sibs sharing the same patterns is low. This has however often be the case in our analysis (cf Table 1). One way to test this hypothesis would be to breed two lines showing a low percentage of common bands, knowing which loci is heterozygous in the female parent (*Trichogramma* male being haploid) and to analyse the offspring RAPD patterns.

In conclusion, results argue in favor of egg cluster parasitization by more than a single female : first, each RAPD pattern displays four to six bands while a single mated founder female should display a possibility of nine to twelve bands. Most of these loci being heterozygous, they should generate more than six to seven genotypes out of ten offsprings. Second, some RAPD patterns are common to lines from different egg clusters.

This analysis proves that molecular markers can be a very interesting tool to study some particular aspects of the ecology of a natural population of *Trichogramma*.

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Maternal inheritance of infestation efficiency in a parasitoid wasp, *Trichogramma bourarachae* : the role of symbionts

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Two strains of *Trichogramma bourarachae* differ in their infestation efficiencies : 60 eggs/female/5 days in the High strain (H), 25 in the Low one (L). Back-crosses demonstrate the maternal cytoplasmic inheritance of these variations. Antibiotic and heat treatments reduce efficiency in the H line, whereas the Lower one is not affected. Microscopic observations confirm the responsibility of microorganisms in the reproductive superiority of the higher line. The reduction in the offspring production in the antibiotic-treated H strain is temporary, and after the treatment stops this strain progressively recovers its higher efficiency.

Keywords: Antibiotic treatment; heat treatment; infestation efficiency; maternal inheritanc; parasitoid insects; symbiont; *Trichogramma*.

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The variability in the infestation efficiency of Moroccan strains of *Trichogramma bourarachae* and *T. voegelei* was reported by Mimouni (1992), who further demonstrated the maternal inheritance of the trait, thus suggesting the role of cytoplasmic factors (microorganisms). Such a result is not uncommon in insects, but up to now it had not been reported in parasitoid species, where cases of symbiotic association have been reported with regard to immunological processes (Stoltz & Vinson 1979, Dover et al. 1987), and to induction of thelytoky (Werren 1983, Stouthamer et al. 1990, Rousset et al. 1992, Stouthamer et al. 1993).

Here we confirm the maternal inheritance of variations in infestation efficiency in *T. bourarachae*, and we demonstrate that symbiotic microorganisms are responsible for the better infestation efficiency in the higher strain.

MATERIAL AND METHODS

Quantification of infestation efficiency. Females (less than 24 hours old) were individually exposed for 5 days to about 500 hosts (UV-killed *Ephestia* eggs). The number of host eggs blackering 4 days later measures the 5 day infestation efficiency,

which is highly correlated to the total infestation capacity of *Trichogramma* females (Chassain 1988).

Strains. The High strain (H) was founded from females caught in 1988 in Tadla (central Morocco, near Marrakech), and the Low one (L) originated (1984) from the Atlantic coast (El Oualidia, near Casablanca). Since their capture, the strains have been reared in the lab on *Ephestia kuehniella* (Pyralidae) eggs. All the experiments and rearing were run at 22°C, 50% RH under L.D. 12:12 photoperiod.

Back-crosses. In both the H and L strains, 15 females were individually crossed with 15 males of the two strains. In each of the 15 G1 progenies, one female (issuing from one-day-old mothers) was individually back-crossed with one male of the same strain as her father. In each G1 and G2 progeny, 15 females were tested for their infestation efficiency. These back-crosses replace the maternal genome with the paternal one : crossing females from the H line with L males over several generations creates females with paternal genome (L) and maternal cytoplasm (H), and reciprocal crosses (L females with H males) create females with H genome and L cytoplasm.

Curative treatments. Antibiotic and heat treatments were applied to Go mothers, and the effect was sought on the infestation efficiency of their untreated daughters.

Two antibiotics were studied, added to honey (0.25%) : tetracycline (a first generation antibiotic ; Sigma®) and doxycycline (a recent antibiotic ; Vibraveineuse, Pfizer®). In each strain (H and L), young females (generation Go) were fed either pure honey (10 females / strain) or antibiotic-added honey (10 females / strain) for one day, then provided with unlimited host eggs for 5 days.

For heat treatment, ten females (Go mothers) were exposed for 24 hours to 30°C, then transferred to 22°C. In each strain ten control females were not heat-treated. All females were fed honey and received host eggs on the second day.

In both experiments (antibiotic and heat treatments), G1 daughters were chosen within the first day offspring of each treated or untreated mother (one daughter per mother), and individually tested for their infestation efficiency.

Long-term antibiotic treatments. Only doxycycline was tested in this experiment. Six sub-cultures of the H line were founded. Two were treated only once, two were treated every second generation, two every 4th generation. Every second generation, alternating with treatment, 10 (untreated) females were tested in each sub-culture for their infestation efficiency.

Staining. Eggs from H, L, and tetracycline-treated H strain were stained with a lacmoid solution (Stouthamer & Werren 1993) before microscopic observation.

RESULTS

Inheritance of infestation efficiency. In the control (HxH, LxL) and hybrid strains (HxL, LxH), the infestation efficiency is stable over generations, and only depends on the origin of the founding female (Figure 1). The partial substitution of the maternal genome by the paternal one does not affect infestation efficiency. The maternal

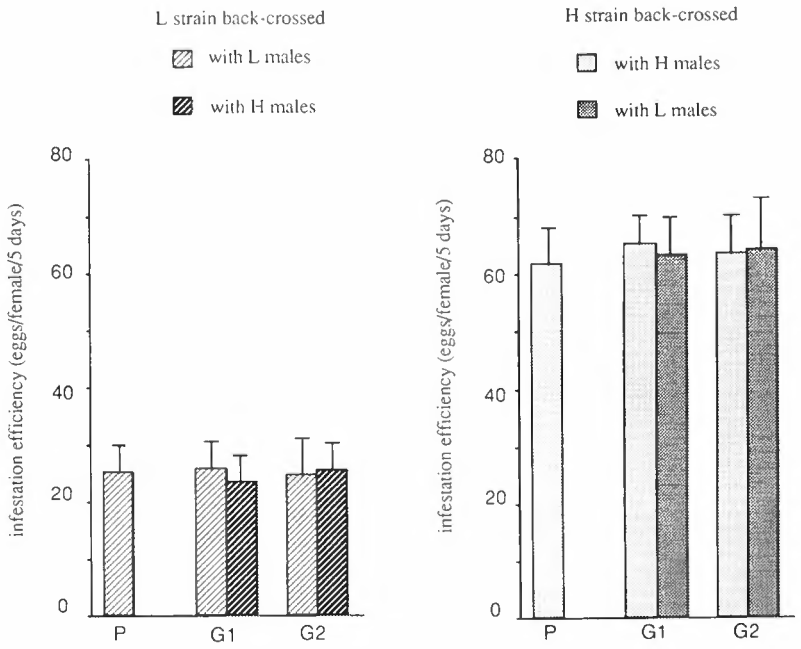

Figure 1. Maternal inheritance of infestation efficiency. The infestation efficiency (mean ± standard error) is measured on parents (P), on the first (G1) and on the second (G2) generations of reciprocal back-crosses. Every stick is the average of 15 females infestation efficiency.

cytoplasmic inheritance of the trait, originally reported by Mimouni (1992) is totally proved.

Curative treatments. In the two strains, the infestation efficiency of the treated mothers is not affected by antibiotics, but it is reduced by heat treatment (Table 1 and 2).

In the L strain, the infestation efficiency of daughters is not affected by the antibiotic treatment that their mothers have received. By contrast, the H strain is much more sensitive (Figure 2), and the infestation efficiency of daughters is significantly reduced by the antibiotic treatment of their mothers. Doxycycline is more efficient than tetracycline, probably due to its better cellular penetration ability and to the lower microbial resistance. Heat treatment of mothers significantly reduces the infestation efficiency of daughters in the H line (Figure 3).

Both antibiotics and heat treatments are well known for their curative effects on symbionts, and they have quite different effects on the two strains. The three treatments tested here, heat, doxycycline and tetracycline, are not equally efficient, but recent experiments indicate that prolonged treatments with tetracycline or heat have a stronger effect, while interfering with ageing of the mothers.

Figure 4 shows that the long-term antibiotic treatment with doxycycline (every 2nd or 4th generation) results in the stability of the low infestation efficiency (about 30 eggs) of the four subcultures, whereas in the subcultures treated only once (Go generation), the infestation efficiency increases regularly from generation 7 onwards.

Table 1. Effect of antibiotics on the treated mothers (Go). Two antibiotic treatments (tetracycline and doxycycline at a dose of 0.25%) are applied to females (Go mothers) in the H and the L strains for 24 hours. The mean (± standard error) of each group (control, tetracycline-treated and doxycycline-treated) is calculated on 10 females, the 3 means are compared by analysis of variance.

	Control	Tetracycline	Doxycycline	Analysis of variance
Go mothers H	57.6 ± 1.67	56.5 ± .91	58.3 ± 1.53	NS
Go Mothers L	22.0 ± 1.34	23.4 ± .75	23.1 ± .81	NS

Table 2. Effect of heat on the mothers (Go). Heat treatment (30°C) is applied to females (Go mothers) in the H and the L strains. Means (±standard error) are calculated on 10 females, and compared by analysis of variance.

	Control (22°C)	Treated (30°C)	Analysis of variance
Go mothers H	54.1 ± 1.87	45.7 ± 1.76	p = .0043
Go mothers L	26.3 ± 2.11	20.1 ± 1.26	p = .021

Microscopic observations. Microscopic observations (Figure 5) establish that the higher infestation efficiency of the H strain is associated with the presence of cytoplasmic symbiotic agents, which are absent in the eggs of the L strain and in the eggs of the tetracycline-treated H strain.

DISCUSSION

Variations in infestation efficiency, maternal inheritance of the trait, and microscopic observations confirm that symbionts are responsible for the higher infestation efficiency of the H strain. Their sensitivity to tetracycline and their stainability by lacmoid solution make it likely that these symbionts are Rickettsia, probably *Wolbachia* (Stouthamer et al. 1990, Stouthamer 1990).

Antibiotic or heat treatments destroy most symbionts within oocytes (Louis et al. 1993), but a few resistant forms can subsist and become active again in the absence of further treatment, thus explaining the increase in infestation efficiency after the treatment stopped.

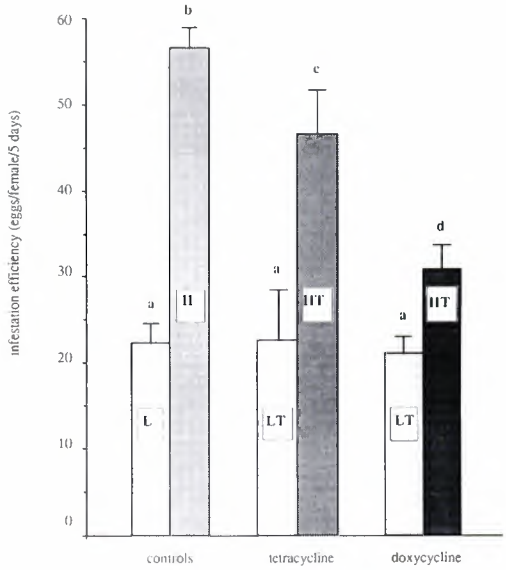

Figure 2. Effect of maternal antibiotic treatments on the infestation efficiency of untreated daughters. Means (±standard error) are calculated on 10 females, and compared by variance analysis. (L, H : daughters of untreated control mothers from low and high strain; LT, HT : daughters of treated mothers in low and high lines). The different lower-case letters show significantly different means.

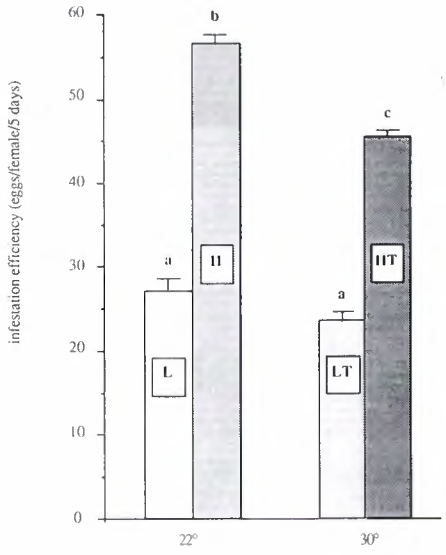

Figure 3. Infestation efficiency of daugthers of untreated mothers from the H and L lines, and of treated mothers (HT, LT). Daughters G1 (offspring of Go mothers) are all untreated. Means (±standard error) on 10 females, test by variance analysis, the different lower-case letters show significantly different means.

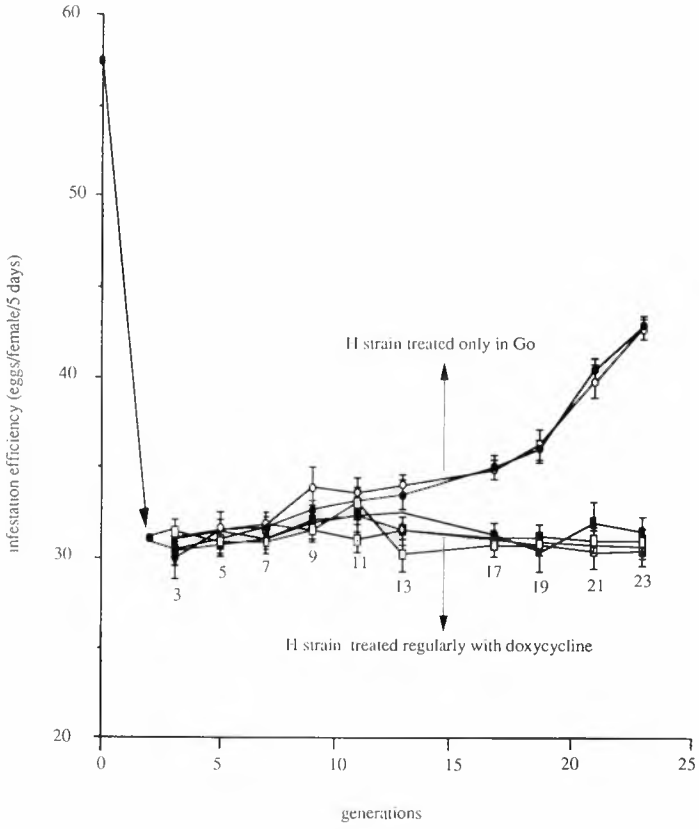

Figure 4. Long-term treatment with doxycycline. Circles : lines treated only once. Open and black squares: lines treated every 2nd or 4th generation, respectively. Measures on untreated females.

Since in *Trichogramma* species vitellogenesis is mostly preimaginal (Anunciada & Voegelé 1992, Fleury & Boulétreau 1993), symbionts certainly act on the female's physiology during the larval or pupal stages. Thus the offspring of older mothers would receive fewer symbionts, and that could explain the interaction between prolonged treatments and the ageing of mothers.

The between-strains variations can be due either to their different laboratory rearing times, or to their different genetic makeup: current experiments indicate that the H genotype favours the stability of the symbiont-parasitoid association.

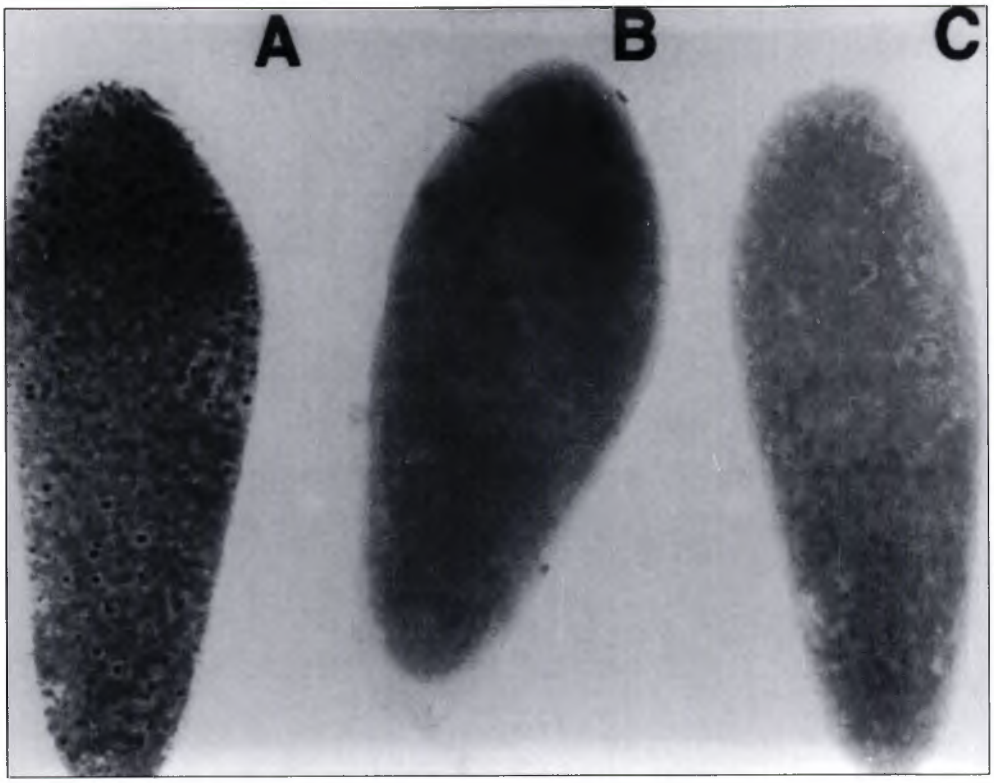

Figure 5. Microscopic observations of *Trichogramma bourarachae* eggs stained by lacmoid solution (Stouthamer & Werren 1993). H strain eggs with microorganisms (A), L strain eggs (B) and tetracycline-treated H strain eggs (C) free of microorganisms.

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Locomotor behaviour in females of two *Trichogramma* species: description and genetic variability

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The daily variations of locomotor activity and locomotor behaviour measured by linear speed, angular speed and sinuosity are described in females of two *Trichogramma* species, using automatic video equipment. The genetic bases of the variability of these traits are investigated by analysing isofemale lines in each species. The analysis of phenotypic resemblance between relatives strongly suggests the existence of genetic factors responsible for the variability of the locomotor behaviour and for the variability of its circadian variations. Parameters describing locomotor behaviour are correlated between lines, and their temporal variations are also correlated at the daily scale. These results may have important consequences in fundamental and applied research on insect parasitoids.

Keywords: circadian rhythm, genetics, locomotor behaviour, *Trichogramma.*

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Locomotor behaviour plays an important role in the life cycle of insect parasitoids : it is involved in the encounter of sexual partners after emergence, in adult dispersion and in the finding of hosts by females. Thus, locomotor activity is directly related to infestation efficiency (Vinson 1984). As is the case for different activities (nutrition, mating, oviposition, etc.), locomotor activity presents circadian variations in many insect species (Saunders 1982), and, for parasitoids, different species are known to express a circadian rhythm of locomotor activity (Fleury et al. 1991, Pompanon et al. 1993).

In *Trichogramma* species, inter and intra specific variability was observed at two levels : variability of the activity (i.e. daily amount of activity), and variability of the characteristics of the rhythmic variations (i.e. activity pattern, phase) (Pompanon et al. 1993). In order to check the existence of genetic bases explaining a part of this variability, the females of two *Trichogramma* species were studied. Their locomotor behaviour was first described by different parameters and their temporal variations were analysed using an automatic video system allowing individual recordings. Then, in each species, isofemale strains were studied to compare between and within families variations.

MATERIAL AND METHODS

Biological material. Two *Trichogramma* (Hymenoptera: Trichogrammatidae) species were studied : *T. brassicae* Bezdenko (a strain from Moldavia, used in France for biological control) which is arrhenotokous ; *T. cacoeciae* Marchall (a French strain) which is thelytokous. They were reared at 22°C under photoperiod LD 12:12, on *Ephestia kuehniella* Zeller eggs (Lepidoptera: Pyralidae) .

Experimental conditions. Experiments were performed at 22°C and 75% R.H., under photoperiod LD 12:12 (daylight intensity of 1200 lux, provided by 4 fluorescent tubes). Insects were continuously lighted by infrared light (wavelength > 730 nm) to which they are not sensitive (Saunders 1982), thus allowing measurements under dark conditions. During all the experiments, the insects were individually placed in circular arenas (diameter 1 cm, height 1mm) with sufficient quantity of a honey solution.

Parameters recorded and measurements. An automatic video system allows the real-time analysis of the path of each insect for 5 seconds every 10 minutes under light or dark conditions, during 10 to 15 days (see Allemand et al. 1994) for more details). During each measurement, 4 parameters were calculated : activity (percentage of time spent moving), linear speed (in mm/s), angular speed (in °/s) and sinuosity (% of angles > 5°). The last three parameters are instantaneous parameters which are only defined when the insect is moving.

80 insects were individually studied, with 6 measurements of 5 s every hour. At the end of the experiment, for each parameter, hourly means were calculated to study their evolution with time. Two parameters were calculated to describe the rhythm : Median Hour of Activity (MHA : time when 50% of the activity of the day occurs) and a pattern index (daily activity / [duration of activity x maximum activity]).

Study of isofemale lines. 20 *T. brassicae* and 13 *T. cacoeciae* females were isolated after emergence and after mating for *T. brassicae*. Each female was placed in a glass tube with a host patch and a honey solution, for 24 hours. Then they were moved to individual arenas, and analyzed by the video system for 5 days. Hosts parasitized by these females were kept, and 4 daughters in each line were isolated after emergence, and analyzed in the same conditions as their mother (after 24 h on a host patch in a glass tube).

RESULTS

Characterization of locomotor behaviour. *T. brassicae* and *T. cacoeciae* females show a daily rhythm of activity, with a concentration of displacements during the light phase (Figure 1). A main difference between the two species is observed : there is high activity of *T. cacoeciae* throughout the photophase while *T. brassicae* show an earlier decrease of activity. On the other hand, *T. brassicae* presents a higher maximum of activity (about 80% versus 70% for *T. cacoeciae*).

The means of the parameters describing locomotor behaviour are only calculated when activity is sufficiently high (more than 5% of individuals moving). Like activity, these parameters show temporal variations which are significant

(Friedman's test) except for sinuosity (Figure 2). In both species, linear speed is positively correlated to activity, while angular speed and sinuosity are negatively correlated to it. Moreover, angular speed and sinuosity which are positively correlated are both negatively correlated to linear speed (Table 1).

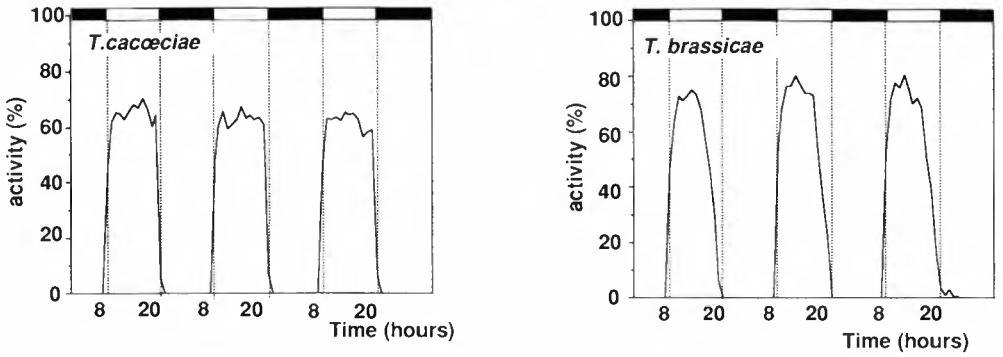

Figure 1. Circadian variations of locomotor activity in *T. brassicae* and *T. cacoeciae* females.

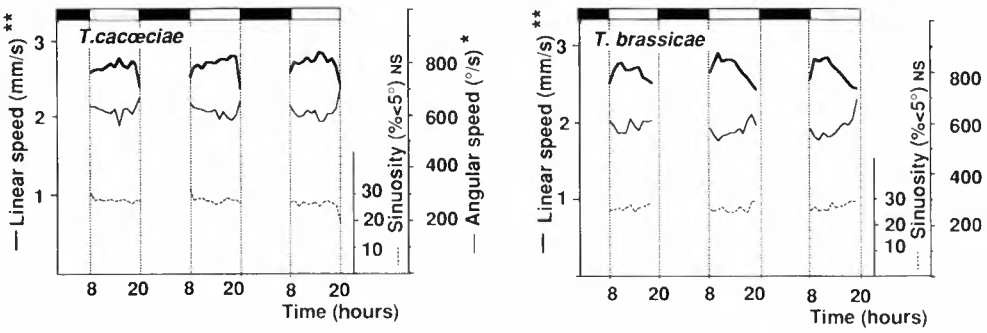

Figure 2. Circadian variations of parameters describing locomotor behaviour in *T. brassicae* and *T. cacoeciae* females. Significance of the temporal variations tested by Friedman's test. * : $p \leq 0.01$, ** $p \leq 0.005$, NS : no significant.

Analysis of isofemale lines.

Resemblance between sisters. For each parameter, within- and between-lines variability are compared by analysis of variance (Table 2). In both species, for parameters describing locomotion (except for sinuosity in *T. cacoeiae*), the between-lines variability is significantly higher than the within-lines variability, which means that females of the same family have a similar locomotor behaviour compared with females of different families. Concerning the parameters describing the rhythm, the

differences between families are almost significant at a 5% level in *T. brassicae*, and only MHA is significant in *T. cacoeciae*.

Resemblance between mother and daughters. The correlations between the mother of each family and the mean daughter are not significant whatever the parameter studied.

Correlation within lines. In both species, correlations between parameters are significant for all considered couples (Table 3).

Table 1. Correlations within hours of the photophase

		Linear speed	Angular speed	Sinuosity
T. cacoeciae	Activity	0.473[2])	- 0.338[1])	- 0.466[2])
	Linear speed		- 0.818[3])	- 0.573[2])
	Angular speed			0.414[2])
T. brassicae	Activity	0.882[3])	- 0.787[3])	- 0.621[3])
	Linear speed		- 0.826[3])	- 0.629[2])
	Angular speed			0.518[3])

[1]) $p \leq 0.05$, [2]) $p \leq 0.01$, [3]) $p \leq 0.001$

Table 2. Significance of differences between isofemale lines tested by analysis of variance

		T. brassicae	*T. cacoeciae*
Locomotion	Activity	0.002	0.04
	Linear speed	0.05	0.02
	Angular speed	0.02	0.05
	Sinuosity	0.01	ns
Rhythm	Activity pattern	0.06	ns
	MHA[4])	0.08	0.02
Number of lines studied		20	13

[4]) MHA : Median Hour of Activity

Table 3. Correlation within lines

		Linear speed	Angular speed	Sinuosity
T. brassicae	Activity	0.729[3])	- 0.739[3])	- 0.425[1])
	Linear speed		- 0.940[3])	- 0.553[2])
	Angular speed			0.438[1])
T. cacoeciae	Activity	0.933[3])	- 0.935[3])	- 0.835[3])
	Linear speed		- 0.947[3])	- 0.777[2])
	Angular speed			0.835[3])

[1]) $p \leq 0.05$, [2]) $p \leq 0.01$, [3]) $p \leq 0.001$

DISCUSSION

Isolated individuals of the two *Trichogramma* species studied express a circadian rhythm of activity without external stimulus except photoperiod. This rhythm persists in free-running for *T. brassicae* (Allemand et al. 1994) which demonstrates its endogenous bases. The more accurate study of the parameters involved in the locomotor behaviour demonstrates the occurrence of temporal variations, at the daily scale, for linear and angular speed. More than this, correlations between these parameters are significant at two different levels. First, the more active lines are those expressing the highest linear speed and the lowest angular speed as well as the lowest sinuosity. Secondly, daily variations of these parameters are strongly correlated to one other : when an individual is more active (increase of the length and/or the frequency of displacements), its linear speed increases and its angular speed decreases. Thus, on the one hand the daily means of studied traits are strongly correlated within lines independently of circadian variations, and on the other hand temporal variations of the parameters are correlated at the daily scale.

The existence of daily variations with a significant amplitude could have hidden the between-lines differences if they had not been taken into account. So the comparison of isofemale lines over 5 days is justified to distinguish temporal variations from between-lines variability.

The study of isofemale lines by analysis of resemblance between sisters strongly suggests the existence of genetic bases to the variability of locomotor behaviour and less clearly to the variability of the characteristics of its rhythm, which has already been demonstrated for another parasitoid, *Leptopilina heterotoma* (Fleury 1993). This is not consistent with the absence of significant correlation between mothers and daughters which suggests that there is no transmission of these traits from one generation to the next. The most probably hypothesis explaining the absence of relation between mothers and daughters, is the strong influence of external factors acting differentially on the two consecutives generations, thus obscuring the genetic relationship. In particular, the variation in the quality of the host, which is not completely controlled, could modify locomotor behaviour in the adult. The quality of the host is actually known to influence a lot of aspects of the physiology of adult *Trichogramma* (Bigler et al. 1982).

These results suggesting the existence of genetic bases to the variability of locomotor activity will be completed by other experiments like the analysis of consanguine lines of *T. brassicae*, making possible the study of the stability of the traits, generation after generation.

In conclusion, different aspects may be taken into account in the study of the behaviour of insect parasitoids for applied and fundamental research. On the one hand, the occurrence of daily variations must be considered in any trajectometric study, in order to avoid confusion between the variability between individuals and the variations due to circadian rhythmicity. On the other hand, the existence of genetic factors controlling a part of the variability of locomotor behaviour may be considered at two levels : for selection of more efficient strains in biological control, and for the study of natural populations in which competition or the impact of external factors could act as selection pressures on locomotor behaviour which contributes to the fitness of insect parasitoids.

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Geographic variation in locomotor activity rhythms of *Leptopilina heterotoma*: inheritance and role in species richness of the *Drosophila* parasitoid community

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Fleury F., R. Allemand, P. Fouillet & M. Boulétreau 1994. Geographic variation in locomotor activity rhythms of *Leptopilina heterotoma* : inheritance and role in species richness of the *Drosophila* parasitoid community. Norwegian Journal of Agricultural Sciences. Supplement 16. 191-197. ISSN 0802-1600.

Under laboratory conditions (22°C, LD 12:12), *Leptopilina heterotoma* females show a diel rhythmicity in their locomotor activity controlled by an endogenous timing mechanism (circadian rhythm) suggesting that females show in nature a real temporal organization of their behaviour. However, wide variations exist in the daily patterns of distinct geographic populations. Mediterranean populations have two peaks of activity at the beginning and end of the photophase, whereas more northern populations are mostly active during the afternoon. Crosses between French and Tunisian strains demonstrate the genetic basis of these differences and the biparental inheritance of the pattern of activity. For different populations of *L. heterotoma*, comparisons of female activity rhythms with those of their local competitors indicate that competing species are asynchronous and can share time. The activity rhythms probably play an important role in interspecific competitive interactions and thus could contribute to species diversity in the *Drosophila* parasitoid community.

Keywords: circadian rhythm, community structure, *Drosophila* parasitoids, genetic variability, interspecific competition.

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INTRODUCTION

In recent years, increasing attention has been paid to the cause of variation in host foraging behaviour (Lewis et al. 1990) but, until now, most investigations have focused on the learning ability of parasitoid females (Turlings et al. 1993) and only a few studies have reported genetic variations in their behaviour (Chassain & Boulétreau 1987, Prévost & Lewis 1990). Among the behavioural traits involved in host location, circadian activity rhythms remain almost unstudied despite the essential role they play in the resource-searching process (Bell 1990). Moreover, variations in

the daily pattern of activity are expected in response to the external factors which vary with a 24 h periodicity and may be either abiotic (light, temperature) or biotic (host availability, activity of competitors).

A genetic study was carried out on the activity rhythm of *Leptopilina heterotoma*, a generalist parasitoid of *Drosophila* that is widely distributed in Europe. Comparison of the locomotor activity rhythms among different populations of *L. heterotoma* shows that wide variations exist in the daily pattern of activity in females, and crosses demonstrate that these differences are genetically transmitted. The adaptive significance of this variability is discussed in the light of experimental results suggesting the role of activity rhythms in interspecific competitive interactions.

CIRCADIAN NATURE OF LOCOMOTOR ACTIVITY RHYTHMS

The locomotor activity rhythm was measured using a new automatic actograph based on the video image analysis technique (Allemand et al. 1994). Parasitoids are isolated in a circular arena without hosts but with honey as food. Every five minutes, a video recording of a few seconds is analysed by a computer which detects the occurrence of any movement for each individual. The hourly activity is then calculated as the percentage of records (12 per hours) where the parasitoid was found to be active.

In laboratory conditions (22°C and LD 12:12), females of *L. heterotoma* show a diel rhythm of locomotor activity which persists under continuous darkness (free running experiment). Figure 1 gives 3 examples of individual activity measured in a free running experiment after 3 days under photoperiodic conditions (Tunisian strain). Most female activity occurs during the photophase with two peaks at light-on and during the afternoon. Under continuous darkness, a periodic activity persists. The persistence of the rhythm in the absence of any external temporal information demonstrates that the daily pattern of activity is controlled by an endogenous timing mechanism (biological clock). The endogenous periods are not equal to 24 hours, thus indicating that the daily rhythm of *L. heterotoma* is a true circadian rhythm. The circadian nature of the daily pattern of activity indicates that *L. heterotoma* females show in nature a real temporal organization of behaviour. Parasitoids are not active at all times of day, and the host-searching process is the result of a complex interaction between the endogenous rhythm, the physiological state of females, their individual experience and their response to external stimuli. Activity rhythm is then a trait which should be taken into account in any behavioural study of host parasitoid interactions. For example, circadian rhythms are probably involved in the temporal synchronization between parasitoids and their hosts, which is as important as spatial coincidence in the success of parasitism.

GEOGRAPHIC VARIATION IN THE DAILY PATTERN OF ACTIVITY

Wide variations in the locomotor activity rhythm were found in females according to their geographical origin, whereas all the males show the same pattern of activity. Among the five strains studied, two kinds of patterns were observed, with a clear opposition between the mediterranean and the northern populations. The strains from Tunisia and Antibes (south of France) show two peaks of activity at the beginning and

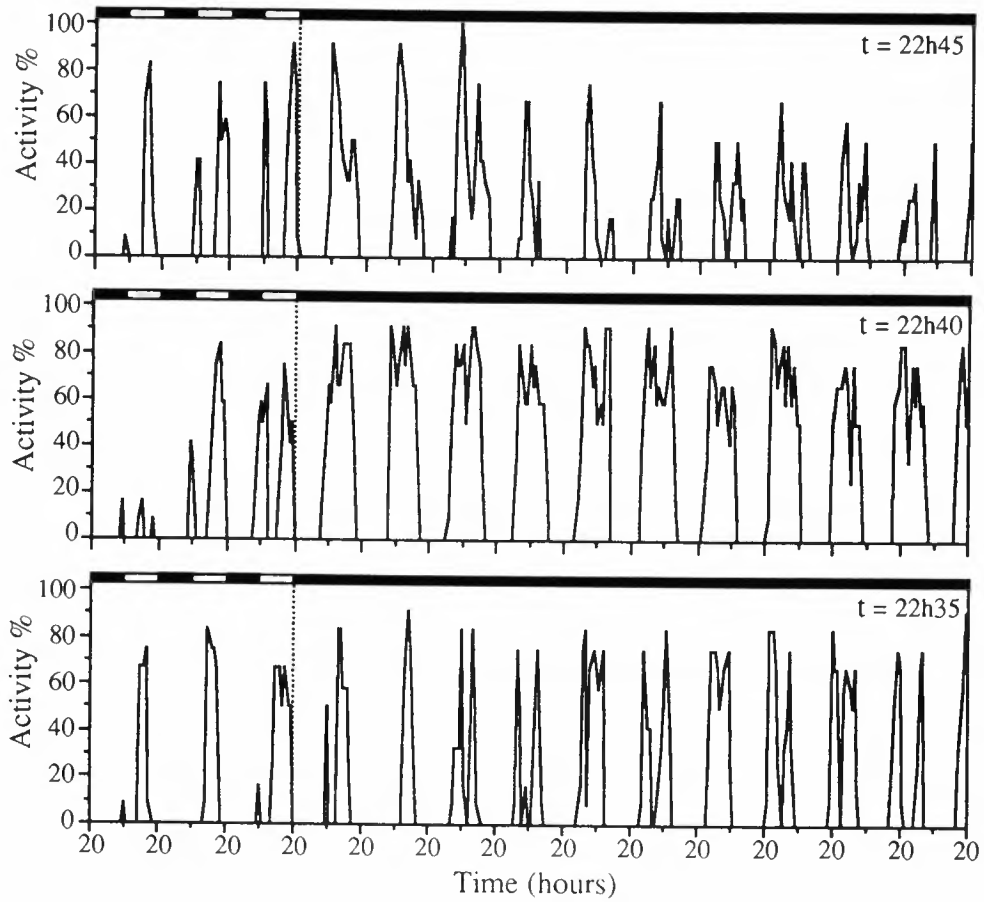

Figure 1: Locomotor activity of 3 *L. heterotoma* females (Tunisian strain) measured for 10 days under continuous darkness after 3 days under photoperiodic conditions (LD 12:12). t = endogenous period calculated according to Sokolove and Bushell (1978).

end of the photophase, whereas more northern populations (two originating from the area of Lyon and one from The Netherlands near Leiden) are mostly active during the afternoon. This difference in the daily pattern of activity is presented in Figure 2 by the 24-hour average curves of the rhythm of females originating from Tunisia and Lyon. The profiles of the rhythms but also the rates of activity differ between these two strains. Reciprocal crosses show that these differences have a genetic basis (Figure 2). Females were crossed with males of the other strain for two generations (F1 hybrids and backcross). The two reciprocal F1 show an intermediate pattern of activity, which is roughly the average between the two parental ones. These results demonstrate the absence of any maternal effect and the biparental inheritance of the patterns of activity which are further supported by the backcrosses. After two generations, the French female line became similar to the Tunisian one and conversely.

This genetic variability between populations of *L. heterotoma* suggests that selective pressures led parasitoid females to adapt the temporal organization of their behaviour to the local environmental conditions. Variations in the rhythms could be only a response to the latitudinal variation in abiotic factors such as the daylength or other associated cycles, or the consequence of complex interactions between the species belonging to the same trophic levels or not (Daan 1981). For example, variations between populations could result from local variations in the species diversity of the hosts, or in their daily patterns of activity (movement, pheromonal emissions involved in host location by parasitoids). Competitive pressures constitute another selective factor which could modify the daily pattern of activity of parasitoids. The adaptive significance of variations in circadian rhythms was investigated within the framework of this last hypothesis.

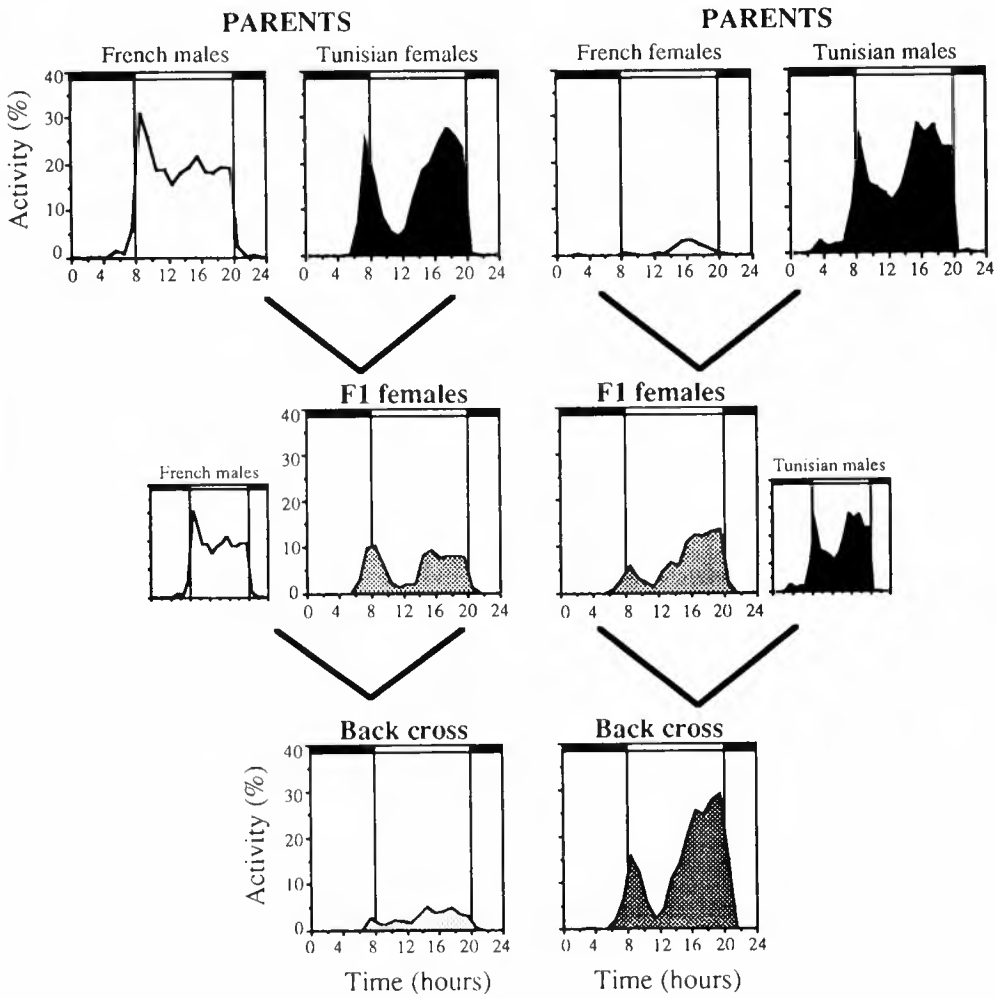

Figure 2: Variability and genetic determinism of the locomotor activity pattern in *L. heterotoma*. Each curve represents the mean of 30 individuals. The grey levels show the relative proportion of French (white) and Tunisian (black) genomes.

ROLE OF CIRCADIAN RHYTHMS IN THE SPECIES RICHNESS OF A *DROSOPHILA* PARASITOID COMMUNITY

Circadian rhythms constitute an important element of the structure of the communities since the coexistence of competing species could result from resource partitionning on a daily temporal basis (Schoener 1974). A comparative study of activity rhythms between species competing for the same hosts was carried out to determine whether such phenomena exist in the *Drosophila* parasitoid community. Three sympatric situations involving populations of *L. heterotoma* were studied. In all cases, the activity of competing species is asynchronous. In Tunisia, *L. heterotoma* and *L. boulardi* females have an opposite rhythm : the former are diurnal whereas the latter are mostly nocturnal with a peak of activity just after light-off. A 3 to 4-hour difference was found between the phases of their two main peaks of locomotor activity (Figure 3). The same difference was observed between *L. heterotoma* and *L. boulardi* originating from Antibes (south of France). In the area of Lyon, *L. boulardi* is absent and *L. heterotoma* competes with *Asobara tabida,* which shows its main peak of activity in the morning. Asynchronous activity was observed again in this system since *L. heterotoma* females originating from Lyon are only active during the afternoon and do not show the first peak of activity of the mediterranean populations (Figure 3).

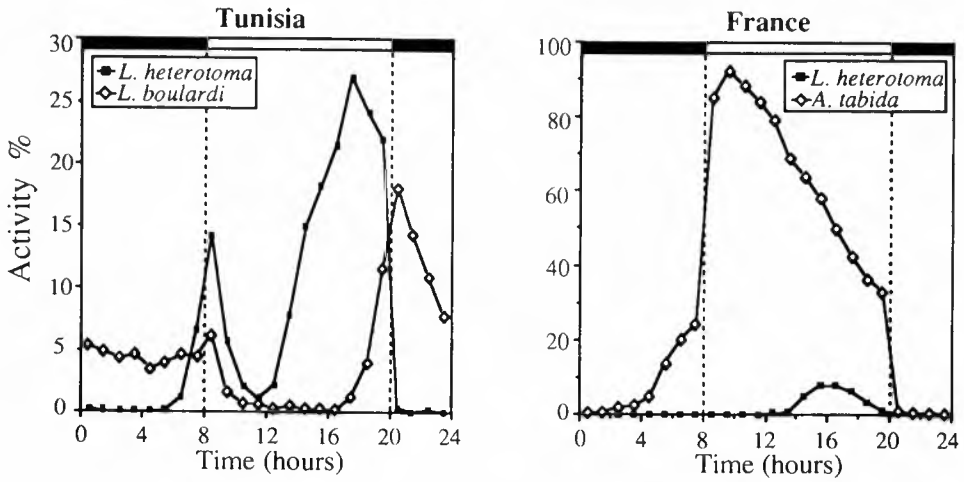

Figure 3: Comparison of the daily patterns of activity of competing species (n=30): *Leptopilina heterotoma* and *L. boulardi* females in Tunisia and *L. heterotoma* and *Asobara tabida* females in France (Lyon).

The fact that competing species are active at different times of day suggests that parasitoid females can share their hosts on a daily basis, thus reducing interspecific competition. This difference in the daily pattern of activity must also be considered when multiparasitism occurs, since the issue of intrinsic competition depends on the sequence of the two ovipositions and of the time between them (Mackauer 1990). The

success of the second infestation is reduced when parasitization occurs too long time after the first one, and it could be an advantage for the weaker competitor if it is active several hours before the better one. It is then likely that the temporal segregation observed in the activity of the competing species can contribute to the species diversity of parasitoids. As a consequence, interspecific competition could partly account for the genetic variability in circadian rhythms between populations of *L. heterotoma*. The difference in the profiles of mediterranean and more northern populations could correspond to a shift in their temporal niches in response to competitive interactions, since the variation in the daily pattern of activity correlates with a change in the structure of the *Drosophila* parasitoid community (*L. heterotoma* competes with *A. tabida* in the north but with *L. boulardi* in the south).

CONCLUSION

The variations in the daily pattern of activity at both intra- and between-species levels emphasize the importance of circadian activity rhythms in host-parasitoid associations. The genetic basis of intraspecific differences demonstrates that local adaptations in activity rhythms can occur in nature, and confirms the existence of genetic variability in the behaviour of parasitoid insects. That suggests further genetic studies on other behavioural traits involved in host-searching processes. Among species, the wide diversity of the diel pattern of activity and the temporal segregation of activity that occur between competing species could partly explain the coexistence of parasitoids attacking the same hosts. Differences between species can also arise from interactions between parasitoids and their hosts. For example, the differences between *L. heterotoma* and *A. tabida* could result from the different searching strategies of these two parasitoids, which do not use the same cues for host location.

From an agricultural point of view, circadian rhythms appear as an important element in the selection of the most suitable natural enemy for biological control. The comparison of activity rhythms of potential candidates might make it possible to choose the species which is active at the right time of day for host parasitization. Moreover, plurispecific releases should use asynchronous species to avoid competition between them, and to prevent any kind of temporal host refuge.

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Evolution of antennal cleaner structure in the Hymenoptera (Insecta)

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The morphology of fore tibio-tarsal antenna cleaner of 250 species of Hymenoptera representing nearly all families was investigated using scanning electron microscopy. In most symphytan families as well as in most Apocrita, distinctive articulated modified setae occur on the basitarsus. In most Symphyta these appear to form an integral part of the antennal cleaner. However, these do not appear to be homologous with the setae that constitute the basitarsal comb in Apocrita, the first indication of which was observed in the Anaxyelidae, Orussidae and Cephidae.

Keywords: antenna cleaner, Hymenoptera, morphology, phylogeny.

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Grooming behaviour has been described in detail for several insects (Hlavac 1971; Valentine 1973; Greene 1975), and has been shown to have two main functions: transferring material from one body part to another and cleaning (Hlavac 1975; Jander 1976). It has been claimed that grooming behaviour could provide a significant indicator of phyletic relationships (Farish 1972; Jander 1976) and consequently, the structure of specialized grooming structures might likewise be expected to provide evidence of relationships. Here we provide a preliminary survey of antenna cleaner morphology in the Hymenoptera so as to determine its possible phylogenetic significance.

The primitive pattern of antennal cleaning involves lowering one antenna at a time, then pulling it with the ipsilateral foreleg while passing it from base to tip through the mouthparts. This type of behaviour occurs in the beetles, the only holometabolous insects in which plesiomorphous antenna cleaning is known (Jander 1976). The apomorphous pattern of antenna cleaning is to scrape one antenna by the ipsilateral foreleg without pulling it into the mouth but rather clamping it between the apical foretibial spur and modified basitarsus. A transitional behavioural pattern has been found in the common soldier beetle *Chauliognathus marginatus* (Cantharidae) (Jander 1976). In the Hymenoptera, the antenna is cleaned by the ipsilateral foreleg which has a specialised organ called the antenna cleaner or strigil. Farish (1972) defined three different antenna cleaning movement in the Hymenoptera; single antennal grooming, double antennal grooming, and alternative antennal grooming. During antennal grooming, the antenna bends down and is clasped by the antenna cleaner at its proximal end, and the foreleg is then run along the antenna from base to tip, and specialized setae scrape dirt towards the apex.

Whilst the morphology of the antenna cleaner has been used in a number of taxonomic works including interpretation of interrelationships at lower taxonomic levels, there have been few detailed studies on antenna cleaner morphology and what there have been have mostly dealt with aculeate Hymenoptera (Brothers 1975; Teodorescu 1981; Schonitzer 1986; Schonitzer & Lawitzky 1987).

METHODS

Specimens used included both dry museum specimens and alcohol-preserved material. Most were prepared for scanning electron microscopy; forelegs were transferred to dry ethanol (>18 hours), cleaned by sonication and air dried before being mounted on a microscope stub and sputter-coating with gold. Some rarer specimens were examined without coating with a Environmental Chamber Mark ABT 55 electron microscope.

MATERIAL EXAMINED

Taxa examined are listed below according to superfamily. Sexes are indicated by M or F in parentheses.

Xyeloidea (Xyelidae): *Xyela* 2 spp. (F); *Macroxyela* 2 spp.(F); *Xyelecia nearctica* (M); *Pleroneura coniferarum* (F)

Megalodontoidea (Pamphilidae): *Pamphilius* 2 spp. (F); *Acantholyda erythrocephala* (F); *Cephalcia arvensis* (F); *Neurotoma saltuum* (F); *Cephalcia abietis* (F); *Celidoptera maculipennis* (M); *Itycorsia posticalis* (M). (Megalodontidae) *Megalodontes turcicus* (F); *Tristactus judaicus* (F); *Rhipidioceros spireae* (F).

Tenthredinoidea (Blastocotomidae) *Blasticotoma filicetti pacifica* (F). (Argidae) *Arge* cyanocrocea (F); *Arge* 2 spp. (F); *Atomacera coerulescens* (F); *Zenarge* (M); *Sterictiphora geminata* (F); *Manaos filicornis* (F). (Cimbicidae) *Trichiosoma* sp., (F); *Abia candens*, (F); *Cimbex* sp., (F) (Diprionidae) *Monoctenus juniperi* (F, M); *Diprion pini* (F); *Neodiprion sertifer* (F); *Diprion* sp. (M). (Pergidae) *Perga dorsalis* (F); *Lophyrotoma zonalis* (F); *Phylacteophaga* sp. (F); *Philomastix nancarrowi* (F). (Tenthredinidae) *Euura* sp. (F); *Nematus sp.* (F); *Croesus* sp. (F); *Priophorus* sp. (F); *Trichiocampus* sp. (F); *Nematus tibialis* (F); *Halidamia* sp. (F); *Empria alector* (F); *Allantus* sp. (F); *Monophadnoides* sp. (F); *Selandria* sp. (F); *Aneugmenus* sp. (M); *Pseudohemitaxonus* sp. (F); *Rhogogaster* sp. (F); *Macrophya* sp. (F); *Tenthredopsis* spp. (F); *Tenthredo* sp. (F); *Macrophya annulata* (M).

Siricoidea (Anaxyelidae) *Syntexis libocedrii* (F). (Siricidae): *Sirex* sp. (F); *Urocerus gigas* (F); *Xeris* sp. (F). (Xiphydriidae) *Xiphydria* 2 spp. (F); *Derecyrta jakowlewi* (F).

Cephoidea (Cephidae): *Trachelus tabidus* (F); *Cephus pygmaeus* (F); *Calameuta filiformis* (F); *Hartigia* sp. (F); *Janus femoratus* (F).

Orussoidea (Orussidae): *Guiglia schauinslandi* (F); *Orussus* 2 spp. (F) *Ophrynopus wagneri* (F); *Stirocorsia kohli* (F).

Trigonalyoidea (Trigonalyidae): *Trigonalis hahnii* (F); *Poecilogonalos costalis* (F); *Orthogonalys pulchella* (F).

Megalyroidea (Megalyridae): *Dinapsis oculohirta* (F); *Megalyra fuscipennis* (F).

Stephanoidea (Stephanidae): *Stephanus froggatti* (F); *Diastephanus* sp. (F); *Parastephanellus* 2 spp. (F).

Evanioidea (Aulacidae): *Aulacus* sp. (F); *Pristaulacus* sp. (F). (Evaniidae): *Evania* 2 spp. (F); *Evaniella* sp. (F); *Szepligetella sericea* (F). (Gasteruptiidae): *Gasteruption* 2 spp. (F); *Hyptiogaster avenicola* (M).

Cynipoidea (Charipidae): *Alloxysta victri* (F). (Cynipidae): *Diplolepis rosae* (F); *Neuroterus* sp. (F); *Synergus apicalis* (F). (Figitidae): *Anacharis eucharioides* (F); *Callaspidia* 2 spp. (F); *Figitis anthomyiarum* (F); *Melanips opacus* (F). (Eucoilidae): *Rhoptromeris heptoma* (F); gen. sp. (F). (Ibaliidae): *Ibalia leucospoides* (F). (Liopteridae): *Paramblynotus* sp. (F).

Ceraphronoidea (Ceraphronidae): *Ceraphron* 2 spp. (F). (Megaspilidae): *Lagynodes pallidus* (F); 2 gen. (F).

Proctotrupoidea (Diapriidae): *Belyta* sp., F-U.K.; *Psilus* sp. (F); gen. sp. (F). (Heloridae): *Helorus* 2 spp. (F). (Monomachidae): *Monomachus* sp. (F). (Pelecinidae): *Pelecinus* sp. (M). (Proctotrupidae): *Codrus* sp. (F); *Exallonyx ligatus* (F); *Phaenoserphus pallipes* (F); *Proctotrupes* sp. (F). (Roproniidae): *Ropronia* sp. (F). (Vanhorniidae): *Vanhornia* sp. (F).

Scelionoidea (Scelionidae): *Scelio* (F). (Platygasteridae): *Platygaster* sp. (F); gen. sp. (F).

Ichneumonoidea (Ichneumonidae): *Metopius* sp. (F); *Triclistus podagricus* (F); *Ichneumon deliratoris* (M); *Megarhyssa* sp. (F); *Pseudorhyssa* sp. (F, M); *Rhyssa persuasoria* (F); *Ganodes innetaii* (F); *Theronia* sp. (F); *Diacritus* sp. (F); *Hymenoepimecis* sp. (F); *Deuteroxorides albitarsus* (F); *Acrotapnus tibialis* (F); *Labena* sp. (F); *Labium longiceps* (M); *Brachycyrtus* sp. (F); *Coleocentrus* sp. (F); *Arotes amoenus* (F); *Phrudus* sp. (F); *Agriotypus armatus* (F); *Xorides fuligator* (F); *Pion fortipes* (F); *Lamachus eques* (F); *Megastylus* sp. (F); *Phytodietus fumiferanae* (F); *Vernamalon spilopterum* (F); *Agrypon flaveolatum* (F); *Ophion* sp. (F). (Braconidae): *Meteorus versicolor* (F); *Zele deceptor* (F); *Pygostolus sticticus* (F); *Stantonia* sp. (F); *Orgilus pimpinellae* (F); *Charmon cruentatus* (F); *Microdus clausthalianus* (F); *Rhyssalus* sp. (F); *Rhamnura* sp. (F); *Bracon fulvipes,* (F); *Histeromerus* sp. (F); *Microgaster subcompleta* (F); *Alphomelon* sp., (F); *Doryctes fartus* (F).

Chalcidoidea (Agaonidae): *Brachyscelidiphaga* sp. (F); *Sycotetra serricornis* (F); *Trichilogaster acacialongifoliae* (F). (Aphelinidae): *Aphelinus* sp. (F); *Encarsia*

partenopea (F); *Marietta* sp. (F). (Chalcididae): *Brachymeria* sp. (F); *Conura* sp. (F). (Elasmidae): *Elasmus* sp. (F). (Encyrtidae): *Copidosoma* sp. (F); *Choreia inepta* (F). (Eucharitidae): *Orasema texana* (F); *Pseudochalcura gibbosa* (F). (Eulophidae): *Eulophus* sp., (F); *Pediobius furvus* (F); *Tetrastichus schoenobii* (F). (Eupelmidae): *Calosota metallica* (F); *Macroneura* sp. (F); *Metapelma spectabile* (F). (Eurytomidae): *Bephratelloides* sp. (F); *Eurytoma natalensis* (F). (Leucospidae): *Leucospis* sp. (M). (Ormyridae): *Ormyrus punctiger* (F). (Perilampidae): *Monacon tricorne* (F); *Perilampus hyalinus* (F). (Pteromalidae): *Plutothrix* sp. (F); *Pteromalus* 2 spp. (F); *Semiotelus* sp. (F). (Signiphoridae): *Signiphora* sp. (F). (Tanaostigmatidae): *Tanaostigmodes* sp. (F). (Tetracampidae): *Epiclerus* sp. (F). (Torymidae): *Torymus* sp. (F); *Apocryptophagus gigas* (F); *Physothorax* sp. (F). (Trichogrammatidae): gen. sp. (F).

Mymaromatoidea (Mymarommatidae): *Paleomymar* 2 spp. (F).

Mymaroidea (Mymaridae): *Mymar* sp. (M); gen. sp. (F).

Chrysidoidea (Scolebythidae): *Ycaploca evansi* (F); gen. sp. (F). (Sclerogibbidae): *Sclerogibba* sp. (F); gen. sp. (F). (Bethylidae): *Bethylus fuscicornis* (M); *Goniozus legneri* (F). (Chrysididae): *Adelphe anisomorphae* (F); *Chrysis ruddii* (F); *Loboscelidia* sp. (F); *Pseudochrysis neglecta* (F). (Dryinidae) *Aphelopus* sp. (F); *Chelogynus cameroni* (F); Gonatopodinae gen. sp. (M); *Prenanteon* sp. (M). (Embolemidae): *Embolemus* sp. (F). (Plumariidae): gen. sp. (F).

Vespoidea (Tiphiidae): *Tiphia femorata* (F); Thynninae gen. sp. (F). (Sapygidae): *Sapyga clavicornis* (F). (Scoliidae): *Scolia hirta* (F); gen. sp. (M). (Bradynobaenidae) gen. sp. (F). (Sierolomorphidae): *Sierolomorpha* sp. (M). (Rhopalosomatidae): *Rhopalosoma* sp. (F). (Pompilidae): *Anoplius aigerrimus* (F); *Arachnospila spissa* (M); *Batozonellus fuliginosus* (M); *Caliadurgus fasciatelus* (F); *Cerapoles maculatus* (F); *Pompilius anceps* (F). (Eumenidae): *Ancistrocerus gazella* (F); *Delta* sp. (F); *Eudynerus foraminatus* (F). (Vespidae): *Dolichovespula arenaria* (F); *Vespa crabro* (F); *Vespula consobrina* (F); *Polistes dominulus* (F). (Formicidae): *Dorylus* sp. (F).

Apoidea (Apidae): *Melipona beecheii* (F); *Trigona fulviventris* (F). (Sphecidae): *Passaloecus singularis* (F); *Ectemnius continuus* (F); *Bembix* sp. (F); *Philanthus* sp. (F); *Tachysphex pompiliformis* (F).

GENERAL MORPHOLOGY AND TERMINOLOGY

Terminology follows that of Schonitzer & Lawitzky (1987). The antenna cleaner (strigil) consists of two main parts: an apical foretibial spur and a modified basitarsus. In most symphytans and all Apocrita, the inner spur is modified and is called a calcar. In non-aculeates and less derived aculeate Hymenoptera (Chrysidoidea), the calcar consists of a trunk which is strong and hard, and a transparent velum (or lamella). In many aculeates there is a trunk and velum and also a distinct apex. The basitarsus bears modified setae, often in the form of a fine comb, and the inner surface of the proximal part of the basitarsus is often notched opposite the spur.

RESULTS

Two distinct types of setal structures were found on the basitarsus. One comprising a row of posteriorly-directed, paddle-shaped, articulated setae which is located anteriorly and extends from near the base to the middle or end of the basitarsus. These setae may form single or multiple rows (Fig. 1). The other structure, referred to here as the comb, comprises a row of usually closely-spaced or adpressed simple setae located on the posterior of the basitarsus.

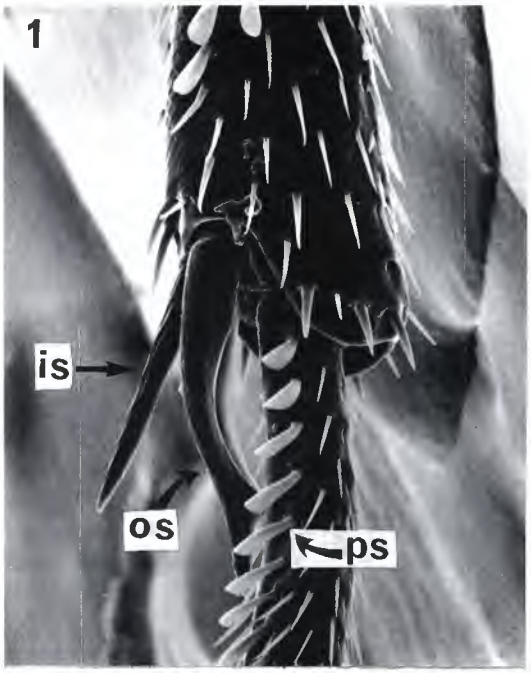
In the least derived and probably most ancient hymenopteran family, the Xyelidae, there is a well developed antennal cleaner with a modified inner spur and an unmodified outer one. The calcar is flattened, slightly inwardly curved and apically bifurcate. It has a trunk and lamella along the posterior-dorsal rim. The basitarsus lacks an obvious notch but there is a well developed row of large articulated paddle-shaped setae along the anterior edge that are quite distinct from the more typical setae distributed over the rest of the basitarsus. Similar paddle-shaped setae are also distributed on the anterior face of the tibia (Fig. 1).

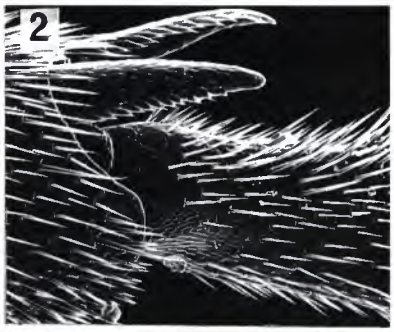
Some Symphyta (Argidae, Cimbicidae, Diprionidae, most Pergidae, and some Tenthredinidae) have two unmodified or only slightly modified spurs and their basitarsi are covered with either ordinary setae (Fig. 2) or with slightly modified setae but in some, distinct paddle-shaped setae are also present. The Pamphilidae, Blastocotomidae, Siricidae, Xiphydriidae and most Tenthredinidae have a similar structure to that of the Xyelidae. In the Megalodontidae, the inner spur is not bifurcate at the apex and also the basitarsus bears more than one row of paddle-shaped setae almost to the end.

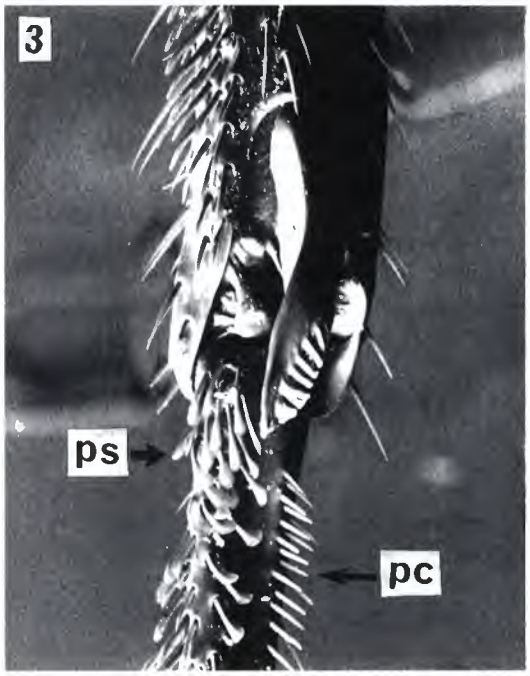
The Anaxyelidae, in addition to possessing more than one row of paddle-shaped setae, show the 'first' indication of a primitive comb of normal setae opposite the calcar though these are not very closely spaced (Fig. 3). An indication of a simple comb of fine setae is also found in the Cephidae (Fig. 4). In the Orussidae there is a well-developed basitarsal comb comprising a single row of closely spaced but otherwise normal setae. In addition, however, orussids also have more than one row of distinctly paddle-shaped setae on the basitarsus (Fig. 5).

In the Apocrita, the antenna cleaner almost always has a basitarsal comb of fine setae (though this is absent in some Chalcidoidea) and a modified inner spur (only known exception being the masarid wasp *Pseudomasaris vespoides*). Many Apocrita also have paddleshaped setae either on their basitarsi or anterior surface of tibiae (Fig. 6).

In the «Parasitica» and in the Chrysidoidea the basitarsus has is a weak notch whereas in other aculeate Hymenoptera the notch is deeper and more sharply defined. The comb which is located at the posterior of the notch, usually consists of a single row of closely adpressed setae but in some less derived Ichneumonoidea (e.g Rhyssinae) there are two comb rows. The basitarsal comb in some Chalcidoidea has a markedly different orientation on the basitarsus being orientated obliquely rather than longitudinally though the phylogenetic and functional significance of this has yet to be determined.







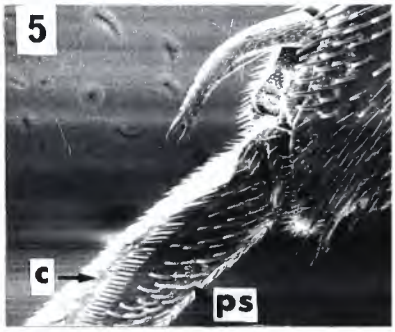


Fig. 1. Xyela julli (Xyelidae), Female. Left foreleg anterior aspect. os: outer spur; is: inner spur; ps: paddle-shaped setae. *Fig. 2. Lophyrotoma zonalis* (Pergidae), Female. Right foreleg anterior aspect. *Fig. 3. Syntexis libocedrii* (Anaxyelidae), Female. Right foreleg anterior-ventral aspect. ps: paddle- shaped setae; pc: indication of primitive comb. *Fig. 4. Trachelus tabidus* (Cephidae), Female. Right foreleg anterior aspect, showing paddle-shaped setae and primitive comb. *Fig. 5. Guiglia schauinslandi* (Orussidae), Female. Left foreleg anterior aspect. ps: paddle-shaped setae; c: basitarsal comb. *Fig. 6. Torymus sp.* (Torymidae), Female. Left foreleg anterior aspect, showing paddle-shaped setae and basitarsal comb.

DISCUSSION

The relationships of the Apocrita to the Symphyta and also between the Aculeata and the other «Parasitica» have been studied by many authors. Königsmann (1977) suggested that the Symphyta excluding the Cephoidea is a holophyletic group, treating the Cephoidea as a sister group of the Apocrita. Rasnitsyn (1988) claimed that the Orussoidea is the extant sister group of traditional Apocrita and thus he accorded the Orussoidea infraorder rank (Orussomorpha) within the Apocrita.

Possession of articulated paddle-shaped setae on the anterior of the basitarsus and tibia appears to be the plesiomorphous condition in the Hymenoptera. Schonitzer & Lawitzky's (1987) claim that such setae are an autapomorphy for the Formicidae is therefore rejected and rather, we consider it to be plesiomophous. The presence of more than one row of articulated paddle-shaped setae on the basitarsi may be derived condition, however, the Megalodontidae, Anaxyelidae and Orussidae displaying this condition in the Symphyta and it is also widely distributed among the Apocrita. The basitarsal comb of the «Parasitica» is a distinct character formed from simple setae on the posterior of basitarsus. Within the Symphyta, structures resembling a simple comb are found only in the Anaxyelidae and Orussidae and perhaps in a less derived state, in the Cephidae. These findings suggest that the Anaxyelidae, Orussidae and possibly also the Cephidae may belong to a lineage (grade) that is more closely related to the Apocrita than to the other symphytan families. It remains to be determined whether in the Apocrita, the paddle-shaped setae have a different function from the comb, but one possibility is that the former are important in cleaning body parts other than the antennae and that this is their main function in those «Parasitica» and Aculeata in which they are retained.

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SUMMARY

Antenna cleaner morphology in the Hymenoptera has been investigated from a phylogenetic point of view. Articulated paddle-shaped setae on the anterior of the basitarsus is postulated to be plesiomorphic condition in the Hymenoptera. Whilst the occurrence of a basitarsal comb of normal adpressed setae in the Apocrita is suggested to be apomorphous feature that first developed in the Cephidae, Anaxyelidae and Orussidae.

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Identification of different compounds from different plants responsible for the orientation of *Campoletis sonorensis* to potential host sites

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Campoletis sonorensis, an ichneumonid attacking several species of noctuid larvae that attack a group of plants, is attracted to volatiles released by these plants. Although several attractive sesqueterpenes have been isolated and identified from cotton we designed a study to determine whether these or different compounds were responsible for the attraction to tobacco. The results revealed two compounds not identified in cotton as attractive synamones from tobacco along with one sesquiterpene common to both plants.

Keywords: Host-location, parasitoid, semiochemicals, synamone.

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INTRODUCTION

The Ichneumonid *Campoletis sonorensis* (Cameron) attacks several species of lepidoptera occurring on a select group of plants (Krombein et al. 1979) and it has been established that many species of parasitoid attacking herbivores respond to chemicals released by plants on which herbivores occur (McAuslane et al. 1991a, Leconte & Thibout 1986, Turlings et al. 1990, 1993, Reed et al. 1970, Camors & Payne 1973). Further, it has been established that damaged plants are considerably more attractive due to changes in the composition of the volatiles released by the damaged plant (Vinson & Williams 1991, McAuslane et al. 1991b, Turlings et al. 1991). But whether the chemicals to which these parasitoids respond are the same in the different plants to which they are attracted is not clear. This question is particularly relevant for those species of parasitoid attacking different herbivores on different plants. However, the question is not simple because parasitoids readily learn to associate many chemicals with the presence of herbivores and respond (Vet et. al. 1984, Vet & Groenewold 1990). Therefore, it is important to determine the response of naive females to the different plants and to utilize naive females in the isolation of the responsible factors.

Elzen et al. (1983) demonstrated that naive *C. sonorensis* would respond to several plants known to serve as food plants for the host. In contrast, plants that do not support known hosts for *C. sonorensis* were not attractive. Vinson & Williams (1991) confirmed that *C. sonorensis* would fly to both tobacco and cotton in a wind

tunnel which serve as food plants for *Heliothis virescens* (F.). The major attractive compounds isolated from cotton were shown by Elzen et al. (1984) using a "Y"-tube olfactometer to be a series of sesquiterpenes, of which α-Bisabolene, ß-Caryophyllene oxide and gossonoral were the most active. Chemicals responsible for the attraction of *C. sonorensis* to tobacco are unknown and are the subject of this report.

METHODS

Using a wind tunnel described by Elzen et al. (1986) we exposed naive mated females between 4-8 days old to tobacco leaf discs or filter paper discs treated with extracts or isolated and identified compounds determined by bioassay-driven isolation (Vinson, Lu & Williams, unpubl.). A minimum of three replicates of 15 females were individually exposed and observed for five min. or until they either contacted a treatment or control (solvent treated 7 cm filter paper disc).

RESULTS

Over half (52.5%) of the females released responded to a tobacco leaf compared to only 5% responding to the control. A similar response was obtained using ether-pentane extracts of tobacco leaves. This ether-pentane extract was fractionated by gas chromatography using a 3% OV-101 or Chromosorb 750 column programmed from 80°C to 260°C at 15°C/min.). Fractions were collected in glass capillary tubes as described by Brownlee & Silverstein (1968) and bioassayed. Only three fractions showed significant activity. These were identified as follows: caryophyllene, nicotine, and farnesylacetone. The response of *C. sonorensis* to synthetic samples of these three compounds is shown in Table 1.

Table 1. Response of naive female Campoletis sonorensis to filter paper discs treated with 100 µg of synthetic samples of the identified active fractions.

Chemicals	Number	% responding
Caryophyllene	45	51.1
Nicotine	45	33.3
Farnesylacetone	45	60.0
Ether-pentane	60	6.2

DISCUSSION

The results of this study demonstrate that *C. sonorensis* not only initially responds to a series of sesquiterpenes present in cotton, but to at least two compounds present in tobacco that are not reported from cotton, i.e. nicotine and farnesylacetone. These results suggest that *C. sonorensis* does not just respond to a certain blend of chemical odors and, therefore, will only search plants with a particular blend, but responds to a group of compounds any number of which may or may not be present in a particular plant. Although the full complement of chemicals to which *C. sonorensis* may respond is unknown, the identification of the attractive compounds from both sorghum, *Sorghum bicolor* (L.), Moensch (Elzen et al. 1984), and Sesame, *Sesamum indicum* L. (McAuslane et al. 1991b) would be of interest.

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The Active Role of Plants in the Foraging Successes of Entomophagous Insects

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Recent research shows that plants respond to herbivore-inflicted damage with the release of specific volatiles. These volatiles emissions are exploited by parasitoids and predators as cues to locate herbivorous hosts and prey. Research with maize seedlings has shown that they emit far more volatiles when they are damaged by caterpillars than when they are mechanically damaged. One critical factor in the induction of the volatiles is the regurgitant of the caterpillar. Furthermore, it was found that the plant response is systemic (even undamaged leaves of an injured plant will emit volatiles) and that peak production of volatiles is during daytime. It is argued that further research should focus on the specificity of the plant reponses and on the plant's physiological mechanisms that are involved.

Keywords: host location, maize, parasitoids, plant volatiles, tritrophic interactions.

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INTRODUCTION

Price (e.g. 1981) set the tone for ecologists to think in terms of multitrophic level interactions. He was one of the first to suggest that «plant resistance to herbivores may involve adaptations to favor the action of the herbivore's natural enemies» (Price 1981). His realization that plants may actively foster the presence of parasitoids and predators has now been substantiated in several studies that show how plants under herbivore-attack may emit signals that attract natural enemies.

One well studied example of plant-produced signals is the odor emitted by spider mite-infested plants (Dicke & Sabelis 1988, Dicke et al. 1990a, b, Takabayashi et al. 1991a). These odors contain specific volatiles, mostly terpenoids, that are used by predatory mites to locate their prey (= the spider mites). The coevolutionary «cat and mouse game» between parasitoids and their hosts has also resulted in a variety of fascinating strategies used by the parasitoids to locate their hosts through olfaction (Vet et al. 1991, Tumlinson et al. 1992) and vision (Wäckers 1994). I will here review research that studied synomone-mediated host habitat location in a maize-caterpillar-parasitoid system and discuss some of the questions that still remain to be answered to fully understand the interactions in this and comparable tritrophic systems.

HOW *COTESIA MARGINVENTRIS* BENEFITS FROM SIGNALS FROM MAIZE

In 1985 a study was started on the host searching behavior of the parasitic wasp *Cotesia marginiventris* Cresson. This endoparasitoid can successfully develop in the larvae of a wide variety of lepidopterous species. Several of its hosts, such as beet armyworm, fall armyworm, corn earworm, and soybean looper, are serious pests on many crops. It is anticipated that a better understanding of *C. marginiventris*' foraging behavior will allow a more effective use of this parasitoid as a biological control agent against these pests.

Initial experiments conducted in olfactometers and flight tunnels soon revealed that *C. marginiventris* largely relies on odor cues to locate host habitats (Turlings et al. 1989, 1991a). The attractive odors were emitted when caterpillars feed on maize seedlings. To determine the exact source of the odors a complete plant-host complex was divided into its three main components: caterpillars, caterpillar by-products (mainly feces), and the damaged seedlings. When given a choice between to components in flight tunnel tests, the wasps showed a clear preference for the damaged seedlings. Moreover, in single choice test almost 80% of the wasps would fly to the damaged plants (similar to the attractiveness of the complete complex) (Turlings et al. 1991a). Healthy plants that had never been subject to herbivore-attack were found to be only marginally attractive; the wasps strongly preferred plants that had been damaged by caterpillars. In fact, it was observed that wasps landed most frequently on damaged sites. This discovery that odors of damaged plants are important in the host finding process of this wasp led to a more detailed study of the airborne plant chemicals that might be involved.

IDENTIFICATION OF MAIZE VOLATILES

To determine what volatiles were actually released by the maize plants, caterpillar-damaged seedlings were placed in a volatile collection system in which pure air was passed over the plants and the emanating volatile molecules were trapped on an adsorbent. Traps were extracted with methylene chloride and the extracts were analyzed by gas chromatography. Figure 1b shows the typical chromatographic profile of volatiles that are released by maize seedlings on which beet armyworm caterpillars have been feeding for half a day. The eleven compounds that consistently were detected in such collections were identified (Turlings et al., 1991b) as the four typical «green leafy» volatiles (Z)-3-hexen-1-al, (E)-2-hexen-1-al, (Z)-3-hexen-1-ol, and (Z)-3-hexen-1-yl acetate, the terpenoids linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene, α-trans-bergamotene, (E)-β-farnesene, (E)-nerolidol and (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, and indole, an aromatic.

When volatiles were collected from the caterpillars, caterpillar feces, and caterpillar-damaged seedlings separately, the plants were found to be the source of all compounds mentioned above. As selection will favor caterpillars that avoid detection by their natural enemies it can be expected that they are not only visually inconspicuous, but that they limit emission of olfactory cues as well. They obviously have less control over the cues that may be given off by the plants they feed on. This is confirmed by the results of the volatile collections. As discussed in detail by Vet et al. (1991) and Vet & Dicke (1992), it seems that the wasps, in order to overcome the

problem of limited availability of cues from hosts, are «forced» to make use of less reliable but readily available plant-provided cues.

ACTIVE RELEASE OF TERPENOIDS BY MAIZE AFTER CATERPILLAR DAMAGE

Volatile collections also revealed that maize plants do not release all compounds immediately upon damage. The terpenoids and indole are only released several hours after the initial injury caused by the caterpillars (Turlings et al. 1990, Turlings & Tumlinson 1991). This is illustrated in Figure 1 were chemicals released by freshly damaged maize (Figure 1a) are compared with those from maize that has been damaged overnight (Figure 1b). Both release the «green leafy» odors (that are typically released by freshly cut plants) but only the plants with the older damage release the terpenoids and indole in detectable amounts. This dramatic difference suggested that an active process can be induced in the plants by caterpillar-inflicted injury.

The terpenoids could be reliable indicators of the presence of hosts if they are specifically released in response to caterpillar damage and not just any type of damage. This was tested by allowing caterpillars to feed on a group of seedlings for a period of two hours, while another group of seedlings was mechanically damaged with micro-scissors and razor blades during the same period of time. Volatiles from the plants were collected the following day (16 hours later). Plants damaged by the caterpillars released far more of the terpenoids than the plants that had been mechanically damaged (Turlings et al. 1990). The conclusion therefore must be that there is something about caterpillar-inflicted damage that greatly enhances the production of the terpenoids. One possibility is that oral secretions from the caterpillars cause a reaction in the plants. That oral secretions do play a role was found when the wounds of mechanically-damaged seedlings were treated with caterpillar regurgitant. The following day the plants released volatiles in amounts equivalent to amounts released by caterpillar-damaged seedlings (Turlings et al. 1990). To induce this plant response the caterpillar regurgitant had to actually be in contact with damaged plant tissue; healthy undamaged plants over which regurgitant was smeared did not release detectable amounts of volatiles.

THE RESPONSE OF THE WASPS

To determine if *C. marginiventris* makes use of the actively released terpenoids, plants that were subjected to the above four treatments (caterpillar-damaged; mechanically-damaged; mechanically-damaged + treated with regurgitate; undamaged + treated with regurgitate) were tested for attractiveness to the wasp. Thus, sixteen hours after treatment, instead of collecting volatiles from the maize seedlings, they were placed in a flight tunnel and *C. marginiventris* females were allowed to fly to them. In choice tests the wasp showed a clear preference for the caterpillar-damaged seedlings and those seedlings that had been mechanically damaged and concurrently treated with regurgitate. Hence, they preferred the plants that were releasing the largest amounts of terpenoids (Turlings et al. 1990).

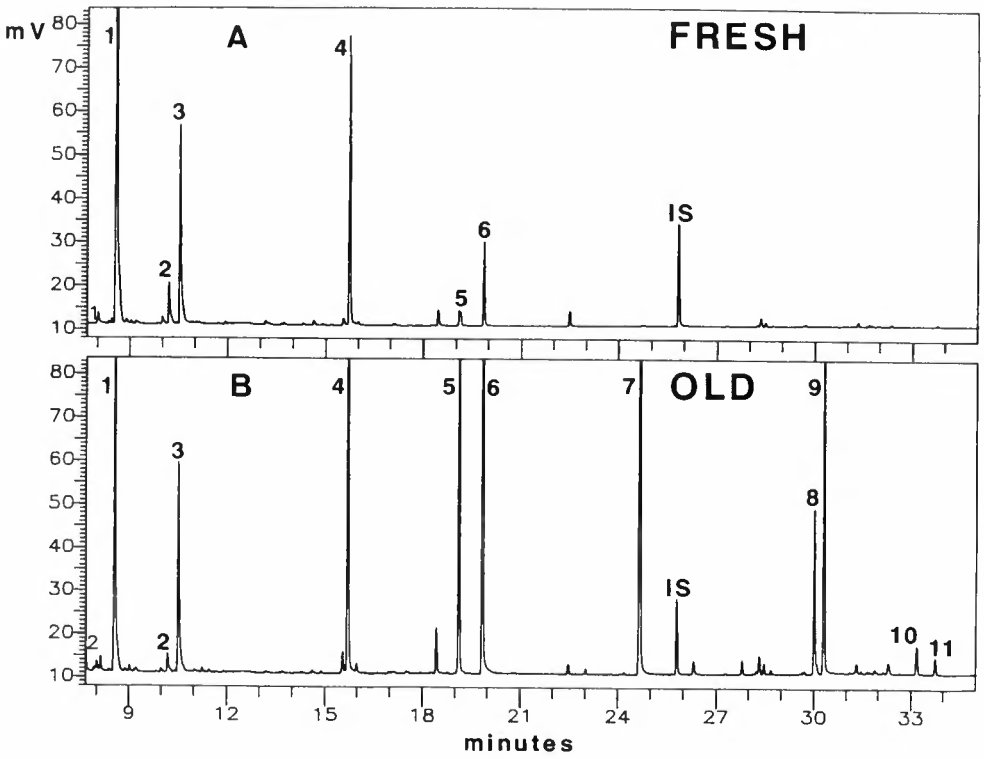

Figure 1. Chromatographic profile of volatiles collected from corn seedlings damaged by beet armyworm larvae, *Spodoptera exigua*. Volatiles were collected for a two hour period on traps containing 25 mg of Super Q and then extracted with methylene chloride. The extract was analyzed on a Quadrex fused silica capillary (50 m x 0.25 mm id) column with a 0.25 µm thick bonded methyl silicone film. A) Volatiles collected from seedlings for two hours starting as soon as larvae were placed on seedlings. B) Volatiles collected from seedlings for two hours 16h after the larvae initially started feeding on the plants. The identified compounds are: 1, (Z)-3-hexenal; 2, (E)-2-hexenal; 3, (Z)-3-hexenol; 4, (Z)-3-hexen-1-yl acetate; 5, linalool; 6, (3E)-4,8-dimethyl-1,3,7-nonatriene; 7, indole; 8, α-trans-bergamotene; 9, (E)-ß-farnesene; 10, (E)-nerolidol; and 11, (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. IS stands for internal standard, which was the added reference compound n-nonyl-acetate (300 ng).

THE SYSTEMIC NATURE OF THE PLANT RESPONSE

During discussions of the possible function(s) of the caterpillar-induced plant response (see below) «signalling» was a frequently heard term. If the plant is indeed purposely signalling chemical information out into its environment than it can be expected that not just the damaged sites but all leaves are emitting the volatiles. Such a systemic action by the plant would distribute energy investments more evenly over the whole plant and allow it to release more of the signal.

To determine whether the plant response was systemic, two leaves of seedlings that carried three leaves were scratched with a razor blade and treated with beet armyworm spit (Turlings & Tumlinson 1992). Control seedlings that were growing in

trays next to the treated seedlings were left unharmed. Twelve hours later the plants were cut from the trays and placed in a collection system. Volatiles were collected from the damaged leaves and the undamaged leaves of the treated plants separately, as well as from the control plants. As is shown in Figure 2, not only did the damaged leaves release the terpenoids, but so did the undamaged leaves of the same plants. Timing of damage is very important in this respect. When we first reported on the systemic release of the volatiles (Turlings & Tumlinson 1992) some of the sesquiterpenes α-trans-bergamotene, (E)-β-farnesene, and (E)-nerolidol were barely detected in the undamaged leaves of the treated plants. But as can be seen in Figure 2, when treatment and subsequent collection are timed right, all terpenes and sesquiterpenes can be found to be released systemically in significant amounts. This timing aspect is discussed in the next paragraph.

THE DYNAMICS OF THE PLANT RESPONSE

In the above experiments plants were damaged or otherwise treated while growing in trays in the greenhouse, but to use them in bioassays and volatile collections they were cut low at the stem and taken into the laboratory. To determine if this handling of the plants resulted in abnormal volatile releases, a new collection system developed by Manukian & Heath (1993) was used to collect volatiles from plants that were still growing. Maize plants were grown in pairs in bottles. When they had reached the

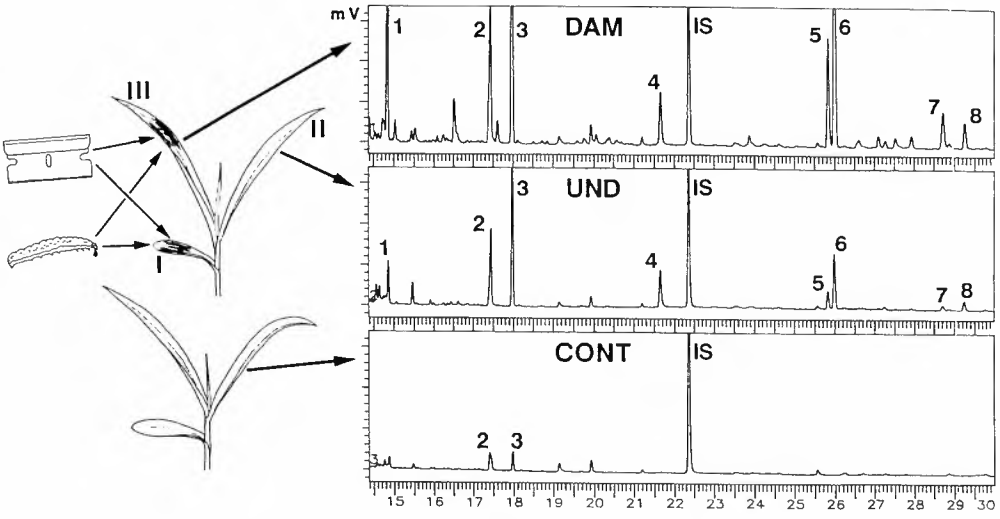

Figure 2. Chromatographic profiles of volatiles collected from corn seedlings. Approximately fourteen hours after treatment, volatiles were collected from leaves that had been damaged and treated with caterpillar regurgitant (DAM); undamaged leaves of the seedlings with other leaves that had been damaged and treated with regurgitant (UND); control leaves of plants left unharmed (CONT). The identities of the various compounds are 1, (Z)-3-hexen-1-yl acetate; 2, linalool; 3, (3E)-4,8-dimethyl-1,3,7-nonatriene; 4, indole; 5, α-trans-bergamotene; 6, (E)-ß-farnesene; 7, (E)-nerolidol; and 8, (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene.

three leaf stage, their leaves were scratched with a razor blade and the damaged sites were treated with beet armyworm regurgitate. The seedlings were immediately placed in a collection system and the volatiles were trapped on filters for a period of three days, whereby every two hours a new trapping filter was used. The main conclusions from this study were that the volatiles released by the treated plants were the same as we found for the previous experiments *and* that the releases showed a clear diurnal rhythm. As before, the «green leafy» volatiles were emitted only immediately after damage, but the terpenoids appeared only after several hours. The induced volatiles were most abundant during midday; at night emissions were minimal. Volatile releases increased again during daylight hours, even two days after initial damage, but overall production decreased gradually (Turlings, Manukian, Heath & Tumlinson, unpubl.).

THE ROLE OF CATERPILLAR REGURGITATE

The previous studies indicated that caterpillar regurgitate plays a role in the induction of volatile releases in maize. To study this in more detail, a test was designed that enabled us to characterize the effects of regurgitate on the plants without damaging the leaves. Seedlings were cut at the stem and incubated in small vials. The vials either contained caterpillar regurgitate (20 x diluted with distilled water) or only distilled water (control). When volatiles were collected from these seedlings 12 hours after they were placed in the vials, the seedlings that had been standing in regurgitate released large amounts of terpenoids, while the control plants remained virtually odorless (Turlings et al. 1993a). In flight tunnel bio-assays, the terpenoid-emitting seedlings were again highly attractive to female parasitoids.

In all previous experiments regurgitate was used from beet armyworm larvae that had fed on maize leaves. Using the above test, regurgitate from a variety of caterpillar species was tested, and in all cases the regurgitate was active (Turlings et al. 1993a). Even regurgitate collected from grasshoppers showed high activity, suggesting that the plant response is of a general nature and can be induced by a large variety of herbivores.

The factor in the regurgitate that triggers the volatile releases is not diet related. It was found that caterpillars that were fed a variety of diets all produced active regurgitate. Even when the caterpillars were forced to eat filter paper their regurgitate was active (Turlings et al. 1993a). Investigations to isolate and identify the active compound(s) in the regurgitate are in progress.

DISCUSSION

It is clear that the plants respond to herbivore-inflicted injury with the release of substantial amounts of volatiles. It was also shown that the volatile emissions are exploited by natural enemies in order to locate prey or hosts. Besides the extensively studied plant-mite and maize-caterpillar interactions other studies have also demonstrated or indicated similar interactions (e.g. Nadel & van Alphen 1988, Ramachandran et al. 1991, Dicke & Minkenberg 1991, Steinberg et al. 1992, 1993, Petitt et al. 1992, McCall et al. 1993). However, we have to be careful in interpreting

the function of the plant-produced volatiles as being signals specifically emitted to attract natural enemies. It is possible for instance, that the terpenoids and other induced compounds are toxins (or byproducts of toxins). As such, they may primarily function to directly defend the plant against the herbivorous arthropods, and perhaps as antibiotics against pathogens that may otherwise easily invade the injured plants (Turlings & Tumlinson 1991).

Additional studies will have to be conducted that investigate the specificity of the plant responses, before we conclude that the sophisticated interactions between plant and parasitoid or predator is a direct communication. It has already been indicated that apple leaves respond differently to different mite species (Takabayashi et al. 1991b). For maize too, chromatographic profiles may differ when different species of caterpillars feed on the same plant species, but this is only true in some cases for the «green leafy» volatiles and not for the truly induced compounds (Röse et al. unpubl.). The wasps show no or a very limited ability to distinguish odors emitted by plants in response to different herbivore species, even when they have a choice between plant odors that are induced by hosts and non-hosts (Turlings et al. 1993a, b, McCall et al. 1993).

In cases where the plant response is fairly general and can be induced by a wide variety of insects, the cues are only weak indicators for the potential presence of host or prey. This would give even more meaning to the hypothesis introduced by Vet et al. (1991), that parasitoids and predators, which make use of plant-produced odors, have the benefit of searching for something that is easily detected, but these cues my not always be as reliable in indicating the presence of a specific host or prey. For the plants to truly «communicate» the presence of certain hosts or prey to natural enemies, it might be necessary that the signals are specific enough to inform the predator or parasitoid if there is really a suitable host or prey present.

For a full understanding of the interactions, more detailed behavioral and chemical studies are required. It is also pertinent that the biochemical processes that control the plants' responses are understood, so that it can be determined whether or not such processes are flexible enough to allow for variability in odor profiles.

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Field manipulation of *Praon* populations using semiochemicals

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An aphid sex pheromone, (+)-(4a*S*,7*S*,7a*R*)-nepetalactone, acts as a kairomone for aphid parasitoids of the genus *Praon*. Behavioural bioassays using a Pettersson olfactometer demonstrated the attraction of female, but not male, *P. volucre* to nepetalactone. The effect of nepetalactone on levels of parasitism by *Praon* spp. was also investigated by placing barley seedlings infested with larvae of the English grain aphid, *Sitobion avenae*, in the field. The presence of nepetalactone increased the proportion of aphids parasitised compared to aphids on plants where nepetalactone was absent. The possible application of nepetalactone in aphid control is discussed.

Key words: – Aphid, field, kairomone, parasitoid, *Praon*, semiochemical.

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Aphid parasitoids respond to a variety of semiochemicals generated by their host or their host's habitat (see Hågvar & Hofsvang (1991) for a review). Laboratory studies have shown that female *Praon volucre* (Haliday) respond to aphid and associated plant odours as well as to aphid honeydew (Wickremasinghe & van Emden 1992). *Praon* spp. females also respond to an aphid sex pheromone, (+)-(4a*S*,7*S*,7a*R*)-nepetalactone, in the field (Hardie et al. 1991, 1994, Lilley et al. 1993). In the present investigation, a Pettersson olfactometer was used to study the attraction of *P. volucre* to this aphid pheromone in the laboratory. Furthermore, barley seedlings infested with young *Sitobion avenae* (F.) larvae were used to evaluate the impact of nepetalactone on *Praon* parasitism levels in the field.

MATERIALS AND METHODS

1) Insect rearing

A clone of the English grain aphid, *S. avenae*, was established from aphids supplied by C. Höller, Kiel University, Germany. Asexual aphids were reared on barley (*Hordeum vulgare* L. var Igri) at 20 ± 2°C in long days (LD 16:8). Parasitoids were maintained on this aphid culture, individual wasps were collected at the mummy stage and isolated until emergence. Virgin wasps, 2–3 days old, were used in the laboratory bioassay.

2) Aphid sex pheromone
(+)-(4a*S*,7*S*,7a*R*)-Nepetalactone (98.4 % pure) was extracted from cat mint, *Nepeta cataria* L. (Dawson et al. 1989).

3) Laboratory bioassay
A modified Pettersson olfactometer (Vet et al. 1983) was used to assess parasitoid responses to diluted nepetalactone. 10 ng samples of nepetalactone in 10 µl hexane were placed on glass coverslips and allowed to disseminate through two opposing arms of the olfactometer while the same quantity of hexane passed through the two remaining arms. Groups of five wasps were released into the centre of the olfactometer and their positions noted every two minutes for a total of 24 minutes at 20 ± 1°C. Many wasps spent long periods in the centre section of the olfactometer and only those which moved out of the centre were scored. The olfactometer was thoroughly cleaned after each replicate and rotated through 45° to remove any directional bias.

4) Field trial
Each weekly trial required twelve pots containing nine ten-day-old barley seedlings on which first and second stadia *S. avenae* larvae were feeding. Larvae were obtained by placing five adult *S. avenae* on each pot, three days prior to the beginning of trial. Before plants were placed in the field the adult aphids were removed and the larvae counted. A glass vial, with a 1 mm hole drilled in the plastic lid, (08-CPV, Chromacol, U.K.) and containing 10 mg nepetalactone in 50µl diethyl ether was placed at the base of the plants. Six pots received vials containing nepetalactone and six, vials without nepetalactone.

Two sites were selected, approximately 40 m apart, in deciduous woodland at Imperial College, Silwood Park. Site A was established at the edge of the woodland, and site B was situated in a clearing. At each site, six wire-mesh (14 cm square), rabbit-exclusion cages (50 cm diam. x 75 cm) were placed 3 m apart in a 3 x 2 arrangement. A nepetalactone or control pot was placed into a cage at random.

Plants were left in the field for four days and then recovered. Any aphids remaining on plants were counted and reared in the laboratory at 20 ± 2°C until mummies formed. *Praon* or non-*Praon* mummies were identified according to the position of the parasitoid cocoon (Starý 1970) and isolated in glass vials until adult parasitoids emerged. Adult females were identified after Starý (1976), however, it was not possible to separate males to species. Trials were run for twelve consecutive weeks from the beginning of July 1993.

RESULTS

1) Laboratory bioassay
Female *P. volucre* were observed in nepetalactone-releasing arms significantly more often than control arms (Table 1). Males, on the other hand, showed no preference for treatment or control arms.

2) Field trials
Approximately 25 % of experimental aphids were recovered at the end of the trials

(Table 2). Similar numbers of aphids were retrieved from treated and control plants at both experimental sites, however, the majority (75 %) of aphids developing into mummies were from site A. Results are presented for both sites combined and over 99 % of mummies were identified as *Praon* spp., the remainder were *Aphidius* spp. (Table 2 and Figure 1). Figure 1 shows that more *Praon* mummies were found on nepetalactone plants than on controls in seven trials, equal numbers occurred once and controls collected more mummies on two occasions. Overall more *Praon* mummies formed on nepetalactone than on control plants (χ^2=19.1, df=1, P<0.001; Table 2). Females of two *Praon* spp.; *P. dorsale* (Haliday) and *P. volucre* emerged from mummies, the majority were *P. volucre* (Table 3).

Table 1. Response of *Praon volucre* to 10ng (+)-(4a*S*, 7*S*, 7a*R*)-nepetalactone in 10 µl hexane (T1 and T2) and 10 µl hexane (C1 and C2) in a Pettersson olfactometer.

No. of Replicates	Wasps / Replicate	Mean cumulative number of wasps/arm/replicate (± S.E.).				
		T1	T2	C1	C2	Centre
8	5	9.4±1.9*	9.9±1.5*	4.5±0.8	5.5±1.9	31.1±17
8	5	5.5±1.4	5.8±0.9	9.5±0.9	6.4±0.8	32.9±1.2

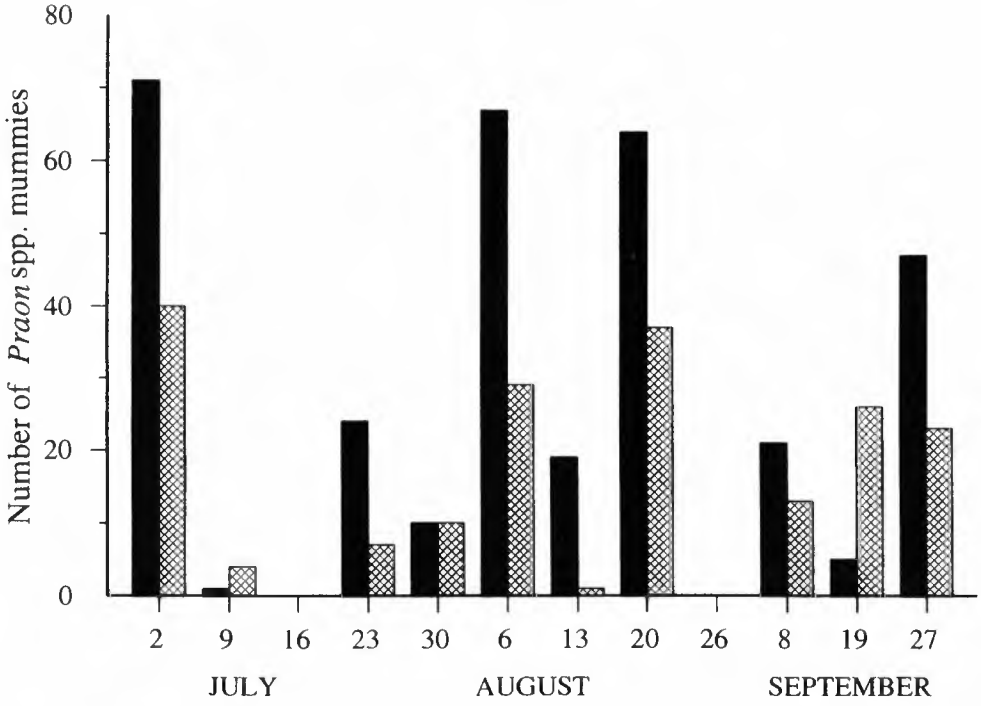
*P < 0.01 (Chi-square test of cumulative totals for all trials)

Table 2. Numbers of *Sitobion avenae* exposed, recovered and parasitised on barley plants associated with (+)-(4a*S*, 7*S*, 7a*R*)-nepetalactone or control.

	Nepetalactone	Control
Larvae exposed	2013	1930
Larvae recovered	579 (29 %)	540 (28 %)
Parasitism		
Praon spp.	331 (57 %)	190 (35 %)
Aphidius spp.	7 (0.02 %)	0

Table 3. Adult *Praon* spp. emerging from parasitised *Sitobion avenae* on plants associated with (+)-(4a*S*, 7*S*, 7a*R*)-nepetalactone or control vials.

Females	Nepetalactone	Conntrol
Praon volucre	64	44
Praon dorsale	4	6
Praon males	71	30
Unemerged	192	110
Total	331	190



Praon spp. mummies recovered from *Sitobion avenae* on barley plants from July to September 1993, dates indicate the last day of each weekly trial. No aphids were recovered on July 16 or August 26. Solid bars – nepetalactone, hatched bars – control.

DISCUSSION

The aphid sex pheromone, (+)-(4a*S*, 7*S*, 7a*R*)-nepetalactone, released from water traps at Silwood Park attracted three aphid parasitoid species, *Praon abjectum* (Haliday), *P. dorsale* and *P. volucre* (Hardie et al. 1991). Only female parasitoids were found in the traps although males were also present in the field (Hardie et al. 1994). The current study shows that females of one of these species, *P. volucre*, also spends longer in olfactometer arms into which nepetalactone is released than in control arms, but male *P. volucre* do not. These findings indicate that the parasitoid females might use nepetalactone to locate oviposition sites, namely sexual female aphids releasing this sex pheromone, but the experiments did not address parasitoid behaviour beyond location of the nepetalactone source. It was also of interest whether the presence of nepetalactone would affect parasitoid behaviour towards asexual aphids which do not release sex pheromones.

Confining asexual aphids on plants, in the presence or absence of nepetalactone, allows the examination of parasitoid behaviour after odour location, reflected by the subsequent levels of parasitism. The results are broadly similar to the water trap experiments in that mainly *Praon* parasitoids were collected. Parasitism was, indeed,

increased on nepetalactone plants, an indication that female *Praon* arrived in greater numbers and/or oviposited more frequently in aphids on these plants.

In the present study, both *P. dorsale* and *P. volucre* were successfully reared from *S. avenae* exposed in the field but the majority (91 %) of females were *P. volucre* (males were not identified to species). Many of the developing *Praon* parasitoids produced brown mummies, often associated with an aestivating or diapause state, rather than white, non-diapausing mummies (Starý 1976, Polgár et al. 1991). All the unemerged mummies (Table 3) were brown. The collection of identical numbers of *P. dorsale* and *P. volucre* in concurrent water trap trials (Lilley et al. 1993) suggests that similar numbers of these species would have approached the experimental plants. The difference between numbers of *P. dorsale* and *P. volucre* emerging from mummies may be due to differences in behaviour immediately prior to oviposition or that many *P. dorsale* failed to develop fully. Records of host suitability indicate that *P. volucre* can utilise *S. avenae* as a host, but there is no record of *P. dorsale* parasitising this aphid species (Starý 1976). Consequently, the relatively large numbers of *P. volucre* successfully reared in comparison to *P. dorsale* could reflect the differential suitability of *S. avenae* as a host for these parasitoids. *P. abjectum* females, reported in earlier field trials (Hardie et al. 1991, 1994) were not recorded in this or other experiments during 1993 (Lilley et al. 1993).

Only a small number of *Aphidius* spp. were recovered from two nepetalactone plants, although *Aphidius* spp. were observed on nearby vegetation throughout the study period (Lilley pers. obs.). *Aphidius ervi* (Haliday) and *A. matricariae* (Haliday) possess olfactory receptors for nepetalactone and laboratory bioassays have indicated that these aphid parasitoids are attracted to nepetalactone (Hardie et al. 1993). However, numbers of *Aphidius* mummies recorded concurs with the few adults caught in a water trap trial (Hardie et al. 1994).

Previous water trap trials have demonstrated the attraction of female *Praon* spp. to nepetalactone sources (Hardie et al. 1991, 1994). The present results indicate that a nepetalactone source will also increase the levels of parasitism in nearby asexual aphid populations. Such behaviour may allow manipulation of *Praon* spp. to increase parasitism levels in asexual pest aphid populations. It should also be possible to draw parasitoids to non-pest aphids, seeded close to crops, to establish localised areas with high parasitoid numbers. These parasitoids may then disperse to target aphids on crops.

SUMMARY

Previous studies (Hardie et al. 1991, 1994) have demonstrated that *Praon* females are attracted to the aphid sex pheromone, (+) - (4a*S*,7*S*,7a*R*)-nepetalactone, released from water traps in the field. In the present study, female but not male, *P. volucre* were also attracted to nepetalactone in a Pettersson olfactometer. Experimental populations of young, asexual *S. avenae* were used to assess the affect of nepetalactone on *Praon* spp. parasitism levels in the field. A higher proportion of aphids recovered from nepetalactone plants were parasitised, when compared to aphids on control plants. Nepetalactone could be used to manipulate field populations of aphid parasitoids with a view to reducing aphid damage.

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Parasitoid of *Drosophila* larvae solves foraging problem through infochemical detour: conditions affecting employment of this strategy

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Parasitoids that forage for herbivorous hosts by using infochemicals may have a problem concerning the reliability and detectability of these stimuli. One solution to this problem is to learn to link highly detectable stimuli, e.g. stimuli from the host's food, to reliable but not very detectable stimuli. In this paper we report on another solution to the reliability-detectability problem. *Leptopilina heterotoma*, a parasitoid of *Drosophila* larvae, spies on the communication system of adult *Drosophila* flies to locate potential host sites. Naive parasitoids strongly respond to a volatile aggregation pheromone, *cis*-vaccenyl acetate (cVA), that is deposited in the oviposition site by recently mated female flies. Thus, *L. heterotoma* resorts to using highly detectable information from a host stage different from the one under attack. The parasitoids may integrate the use of these two solutions to the reliability-detectability problem. The function and ecological implications of these findings are discussed.

Keywords: Drosophila simulans, ecology, Eucoilidae, foraging behaviour, host aggregation pheromone, kairomone, *Leptopilina heterotoma*, windtunnel

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Parasitoids are well-known for using volatile cues during searching for hosts. However, during the first phase of foraging, i.e. at a distance, parasitoids are hypothesized to encounter a foraging problem (Vet & Dicke 1992). Chemicals produced by their host are obviously the most reliable cues in indicating host presence, identity, suitability and accessibility. However, such cues usually have a low detectability because herbivores are small components in a complex environment and if they produce odours at all, this will be in minute quantities. Moreover, herbivores have been selected to minimize the production of cues that attract their enemies. In contrast, cues produced by the food of the host are more detectable because of the larger biomass, but their presence does not necessarily indicate the presence of the host, nor its identity or suitability. This reliability-detectability problem may be solved in different ways, e.g. by associatively linking the presence of a herbivore or its products such as faeces to well-detectable cues such as odours of the food of the host (Vet & Dicke 1992). However, this leaves the question of how the

parasitoid finds the *first* herbivore or its faeces. This may be achieved by employing herbivore-induced synomones that are produced by the food plant of the herbivore in response to herbivore damage. Such cues have a high detectability, are more reliable than odours of undamaged plants and may even be specific for the herbivore species damaging the plant (Vet & Dicke 1992). However, this option is not available to parasitoids that attack hosts in dead or decaying substrates. Another option that parasitoids have in finding their first host is to employ volatiles that their host disseminates to be detectable to conspecifics. This may occur at certain moments in the host's life, albeit by a stage different from the stage attacked by the parasitoid. E.g., *Trichogramma* egg parasitoids use the sex pheromones of their adult hosts to locate areas where reproducing hosts are present (Noldus 1989). This strategy has been termed the infochemical detour (Vet & Dicke 1992).

In this paper we present data on long-distance location of hosts by a larval parasitoid of *Drosophila*. This parasitoid, *Leptopilina heterotoma*, has never been shown to be able to distinguish between odours of fermenting substrates without hosts and substrates to which host larvae had been added (Dicke et al. 1984, Vet 1988). After finding a substrate with hosts and ovipositing in them, the parasitoid associatively learns the substrate odour and is subsequently attracted to it (Vet & Groenewold 1990). In this paper we show that the parasitoid employs the infochemical detour in finding its first patch with hosts. The cues used are adult aggregation pheromones that are deposited onto the oviposition site. We discuss the use of the infochemical detour in relation to another solution to the the reliability-detectability problem, i.e. associative learning.

MATERIAL AND METHODS

Flies. *Drosophila simulans* (laboratory culture established from flies collected in fruit orchards in the Netherlands) was reared on a medium of water, sugar, yeast and agar. Pupae were washed and placed individually in small glass vials. The emerging flies were maintained on water and honey at 20°C and used approximately 1 week after emergence.

Parasitoids. *Leptopilina heterotoma* (laboratory culture established from wasps collected from fermenting fruits in an apple orchard in Wageningen, 1990) were reared on *D. simulans*. We used 8 to 12 days old, naive females for the experiments. Parasitoids were maintained on water and honey in a 12.5 °C incubator from which they were transferred to 20°C several hours prior to an experiment.

Windtunnel. In a windtunnel (100 * 60 * 40 cm), 2 odour sources were placed 10 cm apart on the glass floor. Female parasitoids were individually released from a glass vial, that was laid on the windtunnel floor 35 cm downwind from the 2 patches. A female was considered to have made a choice, when she had arrived on a patch and started probing the substrate with her ovipositor. Parasitoids that did not leave the vial within 5 minutes, that did not arrive on a patch within 10 minutes after leaving the vial or that flew away were recorded to be non-responsive. For each experiment 50 females were tested.

Experimental set-up. For all treatments we used apple-yeast (AY) patches as a substrate. AY-substrate was prepared by mixing finely ground pulp of fresh apples (Golden Delicious) with fresh baker's yeast in a weight ratio of 15:1. These patches,

with a diameter of 2 cm and a height of 0.5 cm, were placed on a circular piece of plastic with a diameter of 5 cm.

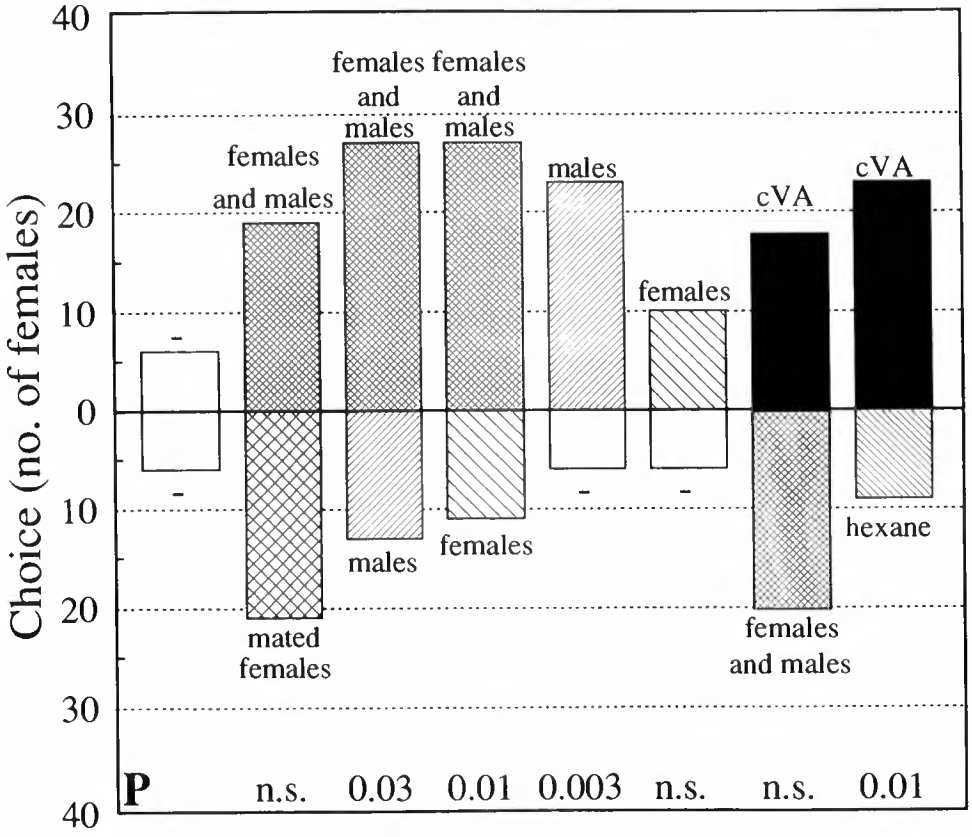
We investigated the response of *L. heterotoma* females toward patches on which different categories of *D. simulans* flies had been present prior to the experiment. Five types of patches were used: (1) 12 virgin *D. simulans* females and 12 virgin *D. simulans* males were placed on an AY-patch. Mating and oviposition occurred on this patch; (2) 12 recently mated *D. simulans* females were placed on an AY-patch. Oviposition took place on this patch; (3) 12 virgin *D. simulans* males were placed on an AY-patch; (4) 12 virgin *D. simulans* females were placed on an AY-patch; (5) a plain AY-patch.

cVA is deposited on patches of categories 1, 2 and 3 (Schaner et al. 1987). The amount of cVA deposited by recently mated females is however about 10 times larger than the amount deposited by virgin males (Schaner et al. 1987). The flies were removed after 6 hours, because a *D. simulans* female deposits the majority of the transferred cVA into the substrate within 6 hours after mating. Furthermore, we compared the response of females to 'natural' cVA with that to synthetic cVA (99% pure; Sigma Chemical Company), dissolved in 15 µl hexane. As a control 15 µl of pure hexane was added to an AY-patch. Directly after preparation, the patches with synthetic cVA were used in the windtunnel.

RESULTS AND DISCUSSION

Naive parasitoids hardly walk upwind when two plain yeast patches are offered (Figure 1). However, the percentage wasps doing so increases dramatically when one or both of the patches have been previously visited by mated females. Moreover, the parasitoids clearly discriminate between a patch on which mated females and males have been present over a patch on which only males or virgin females have been present. A patch on which only virgin females have been present is not preferred over a control patch, but a patch on which virgin males have been present is preferred over a control patch on which no flies have been present. These data correspond with the responses of adult *Drosophila simulans*. Males possess an aggregation pheromone, *cis*-vaccenyl acetate (cVA), that is transferred to females during copulation. The females deposit the pheromone in the substrate during oviposition. Virgin males also deposit some of the pheromone in the substrate (Schaner et al. 1987).

An individual *D. melanogaster* female emits approximately 0.30 µg of cVA within 6 hours after mating (Bartelt et al. 1985). When naive parasitoid females were offered synthetic cVA in amounts that are in the same order of magnitude as that deposited by the 12 female flies, the parasitoids were significantly attracted and they did not discriminate between a patch with synthetic cVA and one on which female and males flies had been present (Figure 1). Thus, these data show that the larval parasitoids employ the infochemical detour by using the aggregation pheromone of their adult host to locate sites where mating and oviposition takes place. This host pheromone is a relatively well-detectable chemical that mediates intraspecific communication. For naive female parasitoids the reliability of this chemical, as a source of information, is higher than microhabitat volatiles: it is associated with oviposition activity of host flies because recently-mated female flies deposit it in the oviposition substrate (Bartelt et al. 1985, Schaner et al. 1987). Different *Drosophila*



Response of naive *L. heterotoma* females toward patches on which different categories of *D. simulans* had been present prior to exposure in the windtunnel, patches with synthetic cVA in 15 µl hexane and patches to which 15 µl hexane had been added. Choices were statistically analyzed by Chi-square test (n = 50 for each bar).

stages (i.e. mating and ovipositing flies, eggs and larvae) often co-exist in the same microhabitat or macrohabitat (Spieth 1974, Shorrocks & Charlesworth 1982). On the other hand the reliability of the information may not go beyond the host species level, because cVA is used by several *Drosophila* species. *Drosophila* flies respond to heterospecific aggregation pheromones although qualitative differences apart from cVA may exist (Schaner et al. 1989a,b). It remains to be investigated whether the polyphagous *L. heterotoma* is able to discriminate between aggregation pheromones of different *Drosophila* species, which could be functional as host species differ in their suitability for survival (Janssen 1989). It remains to be investigated whether generalist and specialist parasitoid species differ in the specificity of their responses to these pheromones. If so, this could have interesting and as yet overlooked implications for microhabitat selection and niche differentiation in the *Drosophila* parasitoid complex.

Leptopilina heterotoma has two solutions to the reliability-detectability problem when searching for larval hosts. Naive females, showing generally low responses to microhabitat odours, employ the infochemical detour by responding to the adult host's aggregation pheromone. After a successful oviposition, the females use associative learning during which the highly detectable and specific microhabitat volatiles are linked to an oviposition experience which was shown to increase their foraging efficiency in the field (Papaj & Vet 1990). However, these learned responses wane if continuation of reinforcement is absent, resulting in the parasitoids returning to their innate response pattern (Vet 1988). The solution of associative learning is thus only present during a certain period after finding a suitable host. The infochemical detour is effective before finding the first host: it increases the host location probability of naive wasps and possibly after learned responses have waned. To understand why one parasitoid species makes use of two different solutions to the reliability-detectability problem, it is worthwile to investigate whether learning and pheromone response are used in concert or alternatively. We have recently started this and first data show that the parasitoids are quite flexible in their use of the two strategies. After an oviposition experience the parasitoids associativey learn the microhabitat odours and prefer these over other microhabitat odours or cVA, but when these learned microhabitat odours are not available, the parasitoids are attracted to cVA which brings them into another microhabitat. This demonstrates that the parasitoids rely on the infochemical detour to find hosts when their other strategy, i.e. associative learning cannot be used. The infochemical detour enables the parasitoid to track their host in new microhabitats while learning after success retains the parasitoid in this microhabitat. These data shed a new and exciting light on niche differentiation and enemy-free space theory in these species.

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The functional response of parasitoids: Probability models and sensory ecology

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A verbal derivation of a probabilistic model developed (in Journal of Animal Ecology 62: 194-204) for the functional response of a leaf-miner parasitoid, *Sympiesis sericeicornis* (Hymenoptera: Eulophidae), is given. The mechanisms producing the transition probabilities between observed behavioural states lie in the sensory ecology of host location and mine content assessment. I review the indications that *Sympiesis* uses vibrations produced by the host for both tasks. I then examine the methods used for recording and playing back vibratory signals in biotests conducted with other parasitoid species and give some of the features of a better, non-contact measurement technique, the laser vibrometer. One measurement of a moving larva highlights some of the features of the signals. Finally, I discuss the development of the laser Doppler measurement technique in research on parasitoids and the impact of the results obtained on the sensory ecology of *Sympiesis* on modeling the functional response.

Key words: functional response, host location, laser vibrometer, leaf-miner, parasitoids, *Phyllonorycter*, physiologically structured populations, *Sympiesis*, vibrations perception.

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Attempts to build models for the functional response in terms of physiology and sensory ecology are much more scarce for parasitoids than for predators. Van Batenburg et al. (1983) devised a model which attempt to predict parasitisation rate based on the behaviour of the parasitoids. It is a probabilistic model and the experiments do not allow the animals to leave the arena. Casas et al. (1993) studied the functional response of a parasitoid *Sympiesis sericeicornis* (Hymenoptera: Eulophidae) attacking the apple tentiform leaf-miner *Phyllononrycter malella* (Ger.) (Lepidoptera: Gracillariidae) at the level of a leaf harboring several hosts. The model is probabilistic, based on observed behavior and on realistic values for the parasitoid's eggload. The departure of the parasitoid from the leaf is explicitly incorporated into the model. The model was able to fit an independent set of data and provided a good explanation for an apparent paradox observed in the field.

The first aim of this paper is to explain the mechanisms of the model verbally, emphasizing some of the unique features of a probabilistic approach. The reader may refer to the original article for an exact derivation of the results. A phenomenological model based on the behaviour of the animals is sufficient for tackling many questions relating to population dynamics, but a better knowledge of the *sensory ecology* of host finding would help us both in understanding the mechanisms leading to the observed

transition probabilities between behavioural states and in assessing the validity of the model in other circumstances. The second aim of this paper is to present some of our advances in analysing the vibratoy signals produced by the host and the parasitoid.

In the second part of the paper, I review the strong indications showing that this parasitoid uses *vibratory signals* produced by the host for its location and for assessing its quality. I also review the published biotests, here defined as manipulative experiments using synthetic stimuli, with other parasitoid species. These biotests suffer from several major problems and lead me to search for alternative ways of measuring the signals. I then explain some of the measurement characteristics of a non-contact method, *the laser vibrometer*. For illustrative purposes, a single recording of a larva moving will be presented; a more thorough analysis of these results will appear in Meyhöfer, Casas & Dorn (unpubl.), Bacher et al. (1994) and Meyhöfer et al. (1994).

A PROBABILISTIC APPROACH

We first constructed a state space on the basis of an ethogram of a parasitoid's foraging sequence from landing on the leaf to departure. The state space is three dimensional with the parasitoid behaviour, the number of hosts parasitised and the eggload as dimensions. The behavioral states are: searching, hunting, ovipositing and leaving the leaf (Figure 1). The parasitoid moves from one state to the next, depending on transition probabilities as well as on the availability of both mature eggs and unparasitised hosts. We begin with a population of parasitoids having an initial distribution of mature eggs estimated from field data. The transition probabilities are estimated using one set of data. The model runs until all females have left the leaf. The output of the model is a probability distribution of the number of hosts parasitised for a given number of hosts available.

The transition probabilities of this *Markov chain* were found to be either constant (i.e., the probability to accept or reject an unparasitised host) or dependent on the number of hosts already parasitised (i.e., the leaving probability). None of the transition probabilities is dependent on the number of hosts available, except for the

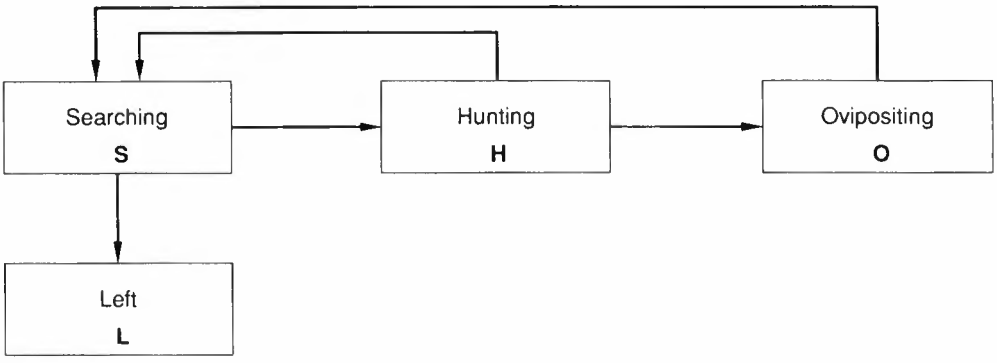

Figure 1: State space of the model and corresponding allowed transitions.

two border effects: when the female parasitised all hosts and when she ran out of eggs. Therefore, one can develop the model with an infinite number of hosts and thereby avoid the first border effect. For any number of available hosts, one can then add all the females which would have parasitised more hosts than available to those that parasitised all the hosts.

Using a highly successful method from operation research and queuing analysis, we avoided a continuous time formulation and considered only the transitions between the behavioral states (an *imbedded* Markov chain). Because our final aim is a functional response at a one-day, or even better, at a generational time scale, the information lost is not crucial; on such a scale, the visit to a leaf (ca. 10 min. per host) can be considered as a single point on the time axis. Nevertheless, developing a continuous time stochastic models was quite helpful. The simplest model, a continuous time Markov chain, gave wrong instantaneous information, as the time spent in the behavioral states is not exponentially distributed. In other words, the simple continuous time Markov chain did not correctly predict the proportion of females in state x at time t, for example. Nevertheless, setting the transition probabilities of the continuous time Markov chain equal to the transition probabilities of the Markov chain gives the same proportions of individuals ending in the state leaving, which have special properties I discuss below. In other words, the continuous Makov chain model correcly predicts the proportion of females leaving the leaf after parasitising only one host, for example. The direct relationship between both formulations implies that one can implement the continuous time Markov chain in one of the ready-to-use software packages for solving differential equations (we used SOLVER). A better – quicker and more exact – way to solve many problems in Markov chains is using linear algebra and the concept of *absorbing states*. Once the parasitoid enters the state «leaving» it will stay there forever (as shown in Figure 1, there is no transition out of that state). This is called an absorbing state. Algebraic manipulations of matrices give the proportion of females entering the different absorbing states. Although one has to do some programming in this case, new powerful software packages have most of the steps already implemented (we used MATLAB, but Mathematica, Maple, Mathcad and others would do equally well).

Looking at the functional response as a 3D probability distribution is a new and revealing approach (Figure 2). Typically, functional response data are highly variable; in their survey of functional response experiments, Trexler et al. (1988) found that the coefficient of variation was typically between 40–90%. Thus, the fit one typically obtains with the usual regression procedures does not explain much of the functional response. Worse yet, the estimates obtained have to be interpreted cautiously as the error distribution is, by definition, not normal (Casas & Hulliger 1994). Examining the three dimensional probability distribution of the functional response for *Sympiesis* reveals that the distribution is highly skewed at high numbers of hosts. Morever, the form of the distribution changes greatly at lower numbers. This kind of mechanism may explain the continuous change of variance, as a function of the host number, observed in other functional response data sets (Casas & Hulliger 1994). These statistical considerations are of prime importance when comparing the functional response between species or treatments.

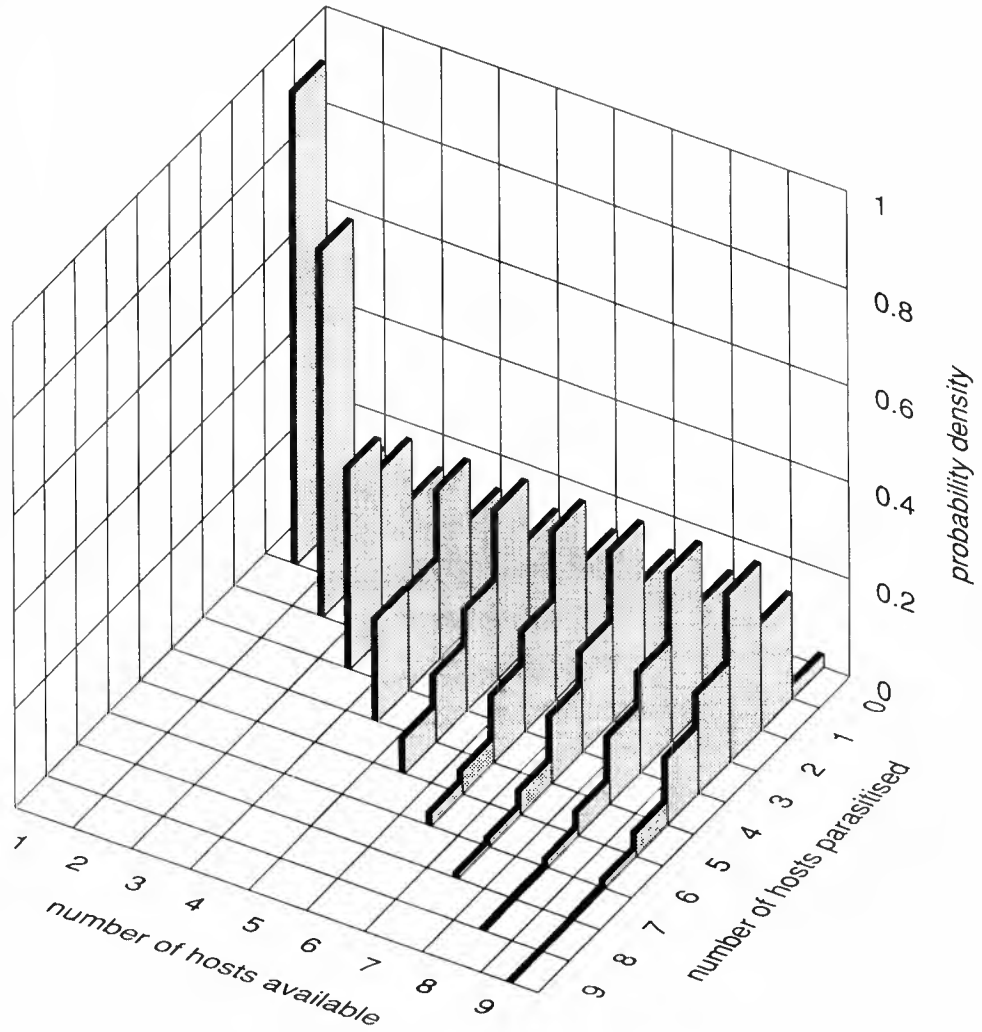

Figure 2: The functional response. The chosen viewing angles highlight the changes in the upper tail of the distribution.

THE USE OF VIBRATORY SIGNALS BY PARASITOIDS FOR HOST LOCATION

Physical stimuli play an important role for host searching and acceptance (Schmidt 1991). The use of vibrations produced by the hosts during the host location and host acceptance phases has been described on a few occasions (Schmidt 1991). For *S. sericeicornis*, six features indicate the importance of vibrations in host location and acceptance (Casas 1989):

1. The parasitoid is unable to check the host quality before landing.
2. Immediately after landing on a leaf with viable and unviable hosts, the parasitoid will attack the viable hosts first.
3. If a mine is empty, the parasitoid does not insert her ovipositor.
4. On the few occasions in which the parasitoid did insert her ovipositor into a mine without host, dissection revealed the presence of living mites or aphids.
5. The parasitoid displays the «stop and go» behaviour reported for other parasitoid species searching using vibrotaxis (Vet & van Alphen 1985).
6. The parasitoid will visit all viable hosts before departure, but typically leaves unviable hosts unvisited.

This evidence is the only evidence possible from observational studies in a field situation. A more definitive statement requires the set up of a relevant biotest.

BIOTESTS

Two types of biotests have been published so far: either scratching with a pin (Ryan & Rudinsky 1962, Lawrence 1981) or recording and replaying substrate vibrations (Sugimoto et al. 1988). In their pioneer study, Sugimoto and coworkers recorded the substrate waves produced by a leaf-miner using a pin inserted in the mine and attached to the membrane of a microphone. The waves were played back to parasitoids using a pick-up head applied on a leaf. In a two choice set-up, the parasitoids were most attracted to vibrating leaves.

There is no control on which part of the needle contacts the substrate and how it does so; the coupling may be loose, resulting in recordings which are unstable and difficult to interpret. This is a well known problem for accelerometers. The coupling of the needle to the membrane is also uncertain. Applying a pick up head to the leaf can change the dynamic properties of the leaf in unpredictable way. The only prediction is that the natural frequency (-ies) of the vibrating leaf will be higher in the real system than the observed one, as the pick up adds mass to the system.

One may therefore wonder why the insects reacted to the given stimuli, and even more so for the scratching tests, since the given signals may be unlike those produced by a host. One explanation is that the signals produced by the host are not specific at all and that parasitoids use simple rules of thumb which do not require the level of detail specified above. What, however, do the biotest signal and the real signal have in common and how they differ from other signals which trigger alternative behaviours ?. Takling this question requires the consideration of three major points:

1. There is a need to quantify the signals produced by the hosts before the appropriate stimuli can be used in a biotest. Schmitt et al. (1993) discuss in detail the insidious problems of using wrong signals in the study of vibratory communication.
2. There is a need to analyse the vibrations produced by host and parasitoid separately. Only then can we tease apart, in a recording using both insects, the vibrations produced by the host and those produced by the parasitoid. As for other tentiform leaf-miners, the host shows marked escape behaviour. Visual observations of foraging sequences reveal that the host uses, in turn, the vibratory

signals produced by the parasitoid for escape. We now have evidence that it can do so: the vibratory signals are discernible and depend on the behaviour of the parasitoid (Bacher et al. 1994). Thus, both partners are sending and receiving vibratory signals and we are currently studying the strength of the coupling between the behaviours of both partners (Meyhöfer, Casas & Dorn, unpubl.).

3. There is a need to record the vibratory signals without contacting the leaf. While the pioneering study of Sugimoto and coworkers clearly showed that it is feasible to record substrate vibrations which produce behavioural reactions of the parasitoids, their recording technique does not allow refined analysis of the time and frequency patterns of the real signals. A promising technique used in other fields for several years is the use of a laser vibrometer. I adapted this technique for our specific needs and discuss a first example below. A much more detailed and comprehensive analysis will appear in Meyhöfer, Casas & Dorn (unpubl.).

THE LASER VIBROMETER

A laser vibrometer is an optical system that measures the velocity of vibrations of solid surfaces by means of the Doppler effect. The output of the laser is proportional to the instantaneous velocity of the surface. A more detailed explanations can be found in Michelsen & Larsen (1978). Three points need to be emphasised here. First, the recording area is quite small, typically less than 100 μm^2. Second, only waves perpendicular to the surface are measured. Third, this laser enables measurements of vibrations at one point in time only; it does not scan the surface nor does it measure vibrations at different points of the surface. These features imply that the interpretation of the signals must be done with special care.

One example from a moving larvae is shown in Figure 3, while more examples and a characterization of the vibration signals will be given in Meyhöfer, Casas & Dorn (unpubl.). The record shows two salient features. First, moving behaviour produces *transient* signals (they are not stationary) which can be classified in three broad phases. In the first phase, the moving insect produces vibrations which start suddenly. The vibrations are then sustained by continuous movement in a very complex way; it depends on the time course of the force and location of the impact on the mine tissues and each change of amplitude implies the formation of overlaying transients. Finally, «free» vibrations occur when the insect stops moving. The three phases changes greatly from one recording to the next; however, we identified recurrent patterns in time and frequency domains. The transient nature of the signal, which is found in most vibrations produced by insects, has tremendous implications both for the analysis of the signals and for their subsequent use in a biotest. The analysis is best performed in the time-frequency plane, and *not* using the usual power spectrum. We use a spectrogram, which is a time-frequency analysis based on a short time Fourier transform. The idea is to consider a non-stationary signal as the concatenation of quasi-stationary segments for which stationary methods can be applied. While this is a much better approach, it still has the inherent limitation of requiring an *a priori* definition of the time window: if one wants a good frequency resolution, one must use a large window resulting in a poor time resolution, and hence, in a smoothing of short time instationarity. A good time resolution implies a short window and a poor frequency resolution. This problem can be partly alleviated

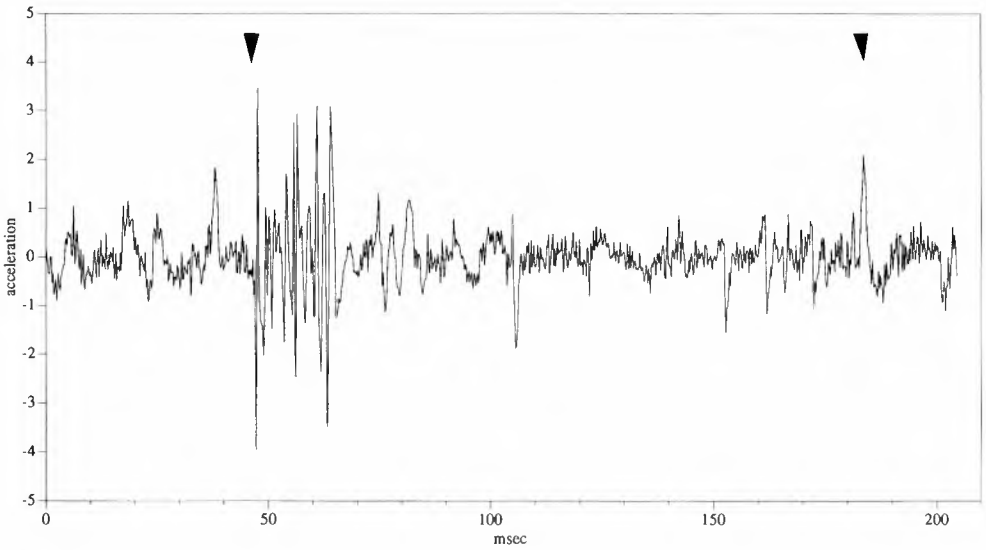

Figure 3: Record of larval movements. The acceleration is given in mm/s². The arrows mark the onset of movement behaviours, observed through binoculars. The background noise in the segments between periods of movement is the same for a leaf without a mine.

by using newer types of signal analysis (Casas, unpubl.). A proper biotest has to match both the energy content and the time course of the signal – a quite ambitious task.

The second feature shown by the recording is the difficulty of defining a «signal». While *we* classified the signals in the time and frequency domains (Meyhöfer, Casas & Dorn, unpubl.) using *our* criteria, the *parasitoid's* criteria are still unknown. Video recording concurrent with vibration measurements will tell us which movement produces which vibration patterns (part of the results are presented in Bacher et al. 1994, Meyhöfer et al. 1994). Furthermore, a combined ethogram of both host's and parasitoid's activities will show the correspondence between both partners' behaviours (Meyhöfer, Casas & Dorn, unpubl.).

OUTLOOK

Laser vibrometers and laser Doppler anemometers are widely used in bioacoustics and other engineering fields: structural analysis, material characterization and fluid dynamics, for example. Only their high cost (laser vibrometer, including vibration-free table, signal analysis software, oscilloscopes, etc. cost > $ 80,000 US) and the difficulties in handling and mastering the equipment inhibit a wider use. However, each year brings new, cheaper and better models (scanning & multi-channel lasers are now on the market) and one can foresee a bright future for these techniques in research on parasitoids. The development of the accelerometer for measuring vibrations led to dramatic advances in structural and modal analysis, both highly

quantitative fields. The same development occurred with the laser anemometer and can be predicted for the laser vibrometer. After an initial period of qualitative measurements – this phase is already over in insect neuroethology – the advances will proceed on many fronts, such as: advanced signal analysis, modeling of wave propagation through structures and materials, the biomechanics of mechanoreceptors and the neural mechanisms for directional orientation.

The results obtained on the sensory ecology of orientation behaviour will enable us to understand the mechanisms underlying the observed transition probabilities. Why does a female leave a suitable host with a probability higher than 30% (Casas et al. 1993) – a host she will readily accept shortly after (pers. obs.)? Did she perceive a stronger signal from another mine and was misled? Our model shows that females leave the leaf for other reasons than a lack of available eggs. They seldom parasitise all of them, even at low host numbers. Is then the leveling off of the functional response due to a signal jamming effect produced by the numerous hosts? Can we consider dead hosts and empty mines as non existent because the inhabitant does not send vibratory signals ? This last question is of prime importance as a foraging parasitoid is most likely to encounter leaves containing hosts of varying suitability (Casas 1989). Understanding the orientation behaviour will increase the domain of applicability of the functional response model to the very situations faced by parasitoids foraging in the field.

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Landing of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) on maize plants

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Landing behaviour of *Trichogramma brassicae* Bezdenko was studied in the greenhouse and in the field. Using insect-glue to trap parasitoids on plants is shown to be an useful technique to record landing. Landing distributions over the leaf levels of the plant are the same in the greenhouse and the field. Most wasps land on the middle and largest leaves. There is a preference for the upper part of the plant, where the number of wasps landing per unit area is higher than on the lower leaves. Most of the wasps land on the middle part of individual leaves, or the part close to the stem. The upper side of the leaves is preferred over the lower side for landing. Release height of *Trichogramma*, position of the plant in the field (in or between rows) and kairomones released by scales and eggs or sex pheromones of *Ostrinia nubilalis* have no effect on landing of *Trichogramma*.

Keywords: host-plant location, maize, *Trichogramma brassicae*

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INTRODUCTION

The parasitoid wasp *Trichogramma brassicae* Bezdenko is being used worldwide for biological control of Lepidopteran pests. Although the species has been intensively researched, very limited information is available on the behaviour of individual females in the field. Most studies concern the effect of mass releases on parasitism or the behaviour of parasitoids under laboratory circumstances. Detailed knowledge of field behaviour is important to identify the traits which have the greatest impact on parasitization efficiency. This is essential both for *Trichogramma* strain selection and for quality control in biological control projects.

Trichogramma wasps have three ways of reaching a new plant to search for host eggs: by walking, hopping or flying. In case of walking, neighbouring plants need to touch each other. This is not uncommon in full-grown maize crops. The limited visual range of *Trichogramma* (Laing 1937, Pak 1990, Wajnberg 1993) means that places where leaves touch will only be randomly encountered. When leaves are close, *Trichogramma* can jump to the other plant. In other circumstances, flight will be necessary to move to other plants.

In the case of flight, *Trichogramma* is free to choose its landing spot on the maize plant. It is presently unknown where *Trichogramma* lands on the plants, though this landing distribution is essential for understanding und evaluating host-finding behaviour. The small size of *Trichogramma* makes its impossible to follow the wasp

in flight. Spotting them on a plant is possible, but requires that the observer can move around the whole plant to scan the leaves without disturbing them. Because of this, direct observation of landing in a crop situation is not possible, and an indirect method had to be found.

Plants were sprayed with glue, causing all landing *Trichogramma* to stick to the plant on the spot where they had landed. To check whether this method gave results similar to direct observation, first a comparison was made in the greenhouse.

Ables et al. (1980) found a correlation between release height and parasitism in different leaf levels on tobacco for *Trichogramma pretiosum*. To see whether this might be found in maize too, the effect of release height was studied in the greenhouse.

It is known that several parasitoids use host cues to locate or select plants to land on (Weseloh 1980). Two experiments were conducted to see if landing by *Trichogramma* was influenced by host cues. In one experiment the host cues were concentrated in one patch, in the other the host cues were spread all over the plant.

MATERIALS AND METHODS

Materials

Plants

Greenhouse reared maize plants (*Zea mays* L.) of cultivar LG11 were used for all experiments except the last one, where the plants (cultivar Atlet) were taken from the field. Field-grown maize has a thicker stem and larger leaves. In the last experiment, plants were in the full-grown stage with ripening grains. In the field, the male flowers had been removed to keep the plants clean from pollen.

Wasps

Trichogramma brassicae Bezdenko was used for all experiments. The basic rearing material used was obtained from Antibes (strain 16), which was imported from the former Soviet Republic of Moldavia, and has been maintained on *Ostrinia nubilalis* Hübner eggs since then. (for details of rearing system see Bigler (in press)). To rear the numbers of wasps necessary for mass releases, the wasps were reared for two or three generations on eggs of *Ephestia kuehniella* Zeller, creating so-called F2 and F3 material. *Ephestia* eggs were exposed to *Trichogramma* for 24 hours. Part of the wasps emerged the day before the experiments, while the rest emerged on the day of the experiment itself.

Moths

Ostrinia nubilalis Hübner, the European Corn Borer was used in the host-cue experiments. The stock colony was taken from a Swiss field population in 1990. Individuals used in the experiment were 1-2 days old. Egg masses used in the experiments were collected from wet filtration paper on top of the rearing cages.

Glue

Glue for sticky insect traps sold in pressurized spray cans was used to make the maize plants sticky.

Methods

Observation of Trichogramma landing in the greenhouse

Maize plants of 120 cm (9–10 leaves) were used for the experiment. With a marker each leaf of two maize plants was divided in three parts of equal lenght: close to the stem, middle and point part. To calculate the leaf area, length and width of the leaves were measured and multiplied by 0.764 (Boot 1985). One plant was sprayed with glue (Stoeckler Insekten-Leim-Spray or Neudorff's Kirschfliegenfalle). Both plants were placed in a greenhouse compartment, 1.5 meter apart from each other. Two meters from the plants, a cylinder with approximately 2000 emerging and one-day old *Trichogramma* (F2) was placed on a stand at 60 cm from the floor. After opening the container, the plant without glue was scanned continuously and systematically from top to bottom by an observer, using a mirror to check the lower leaf sides. As soon as a *Trichogramma* was detected, the location was marked on a list and the wasp was removed with a wet fingertip. After two hours the plant with glue was removed from the compartment and the number of *Trichogramma* stuck to the plant counted.

During the experiments all ventilation openings were closed to avoid air currents. The temperatures varied between 25 and 35°C. The experiment was replicated ten times.

Effect of height of release of Trichogramma on landing in the greenhouse

The leaves of two potted maize plants (190cm, 12 leaves) were both divided into sectors as described for the first experiment. Both plants were sprayed with glue (Stoeckler Insekten-Leim-Spray). The plants were placed in a greenhouse compartment. One plant was placed on the floor, the other on a 1 meter pedestal. The plants were 1.5 meter apart. Two meters from both plants, a release container with 4000 one day old and hatching *Trichogramma* (F2) was placed at 1 meter height. Two hours after opening the release container, the plants were removed from the greenhouse and the number of wasps stuck to the leaves counted. The temperatures varied between 25 and 35°C. The experiment was replicated four times.

Landing of Trichogramma in the field

The fields used had normally developed maize crops, with no naturally occurring *Trichogramma*. The maize plants were sown 15 cm apart from each other within rows and 75 cm between rows. The site for the experiment was at least 7 meters from the nearest field edge.

Eight potted maize plants were sprayed with insect glue (Soveurode) the day before release, so the strongest fumes from the glue could disappear. The plants were then placed in a maize field, in a circle with a 2 meter radius. Four plants were placed within rows, and four plants between the rows. In the middle of the circle, a cylinder with about 10000 one day old and emerging *Trichogramma* (F3) was fastened to a plant at 40 cm above the ground. The cylinder in the morning between 9 and 12 a.m. After 24 hours, the plants were taken out of the field to count the number and position of wasps stuck on the plant. The sex of the recaptured wasps was also determined. The experiment was replicated three times.

Average size of maize plants was 100cm (11 leaves) in the first two replicates and 140 cm (11 leaves) in the last. The experiments were conducted in July on sunny days with temperatures between 20 and 30 °C. In the evening of the first replcation, there was a thunderstorm and some rain.

Landing of Trichogramma on leaves with localized host cues

To get a localized patch of host cues, a small cardboard cylinder (lenght 5 cm, diameter 3 cm) was fastened with one opening against the leaf of a maize plant. The other end was closed with netting. Two *Ostrinia* females were enclosed in the tube and left inside for one night so they could move over a small area of the leaf. The next morning, the tube with the moths was removed. A 10 cm piece of the leaf was cut out, with the artificial patch in the middle. An *Ostrinia* egg mass was placed in the middle of the artificial patch. One end of the leaf piece was placed in a clamp with wet paper to keep the leaf piece moistened. A clean piece of leaf of the same size was also placed in a clamp with wet paper. Both clamps were fastened to a stand with the leaf pieces about 10 cm apart. This stand was placed in the middle of a 80 x 80 x 80 cm box of plexiglass covered with white paper. One side of the box, where the observer was positioned, was left open. A release container with 1000 one day old and emerging *Trichogramma* (F3) was placed in the box, against the back. The container was opened and for 2 hours an observer noted the time and position of the wasps landing on the leaf pieces and removed each wasp after landing. The experiment was replicated eight times.

Landing of Trichogramma on plants with non-localized host cues

Six plants were used. Three plants were exposed to 10 *Ostrinia* females and 5 males in the greenhouse. Contact between *Ostrinia* and corn plants was not desired in this experiment since it was aimed to test volatile chemical kairomone cues and there should be no *Ostrinia* scales on the plant. To prevent *Ostrinia* from contacting the corn plants, plants were caged with thin netting (Monofiles Nylon netting 1/10 mm hole size, Scrynel). Previous work (Noldus 1989) had shown that a mesh size of 340 µm was able to intercept 90% of scales, so we assumed 100 µm netting was adequate for this work. Netting was kept as close to the plant as possible and a second layer of netting was put over the thin one to keep *Ostrinia* between the two layers of netting as close to the plants as possible. *Ostrinia* females and males were introduced between the two layers of netting and left 24 hours. The plants that were not exposed to *Ostrinia* were placed in another greenhouse in order to avoid any contamination with host volatiles. Afterwards, the group of treated plants and the group of untreated controls were placed 2 meters apart in a greenhouse. On each plant, three *Ostrinia* egg masses were stuck to the leaves. Approximately 10000 one day old and hatching wasps (F2) were released in the middle between the two plant groups. Two observers continuously checked one group of plants each for two hours and counted every *Trichogramma* that landed, and removed it after landing. The experiment was replicated four times.

RESULTS

Landing on plants in the greenhouse

A total of 846 wasps were observed to land on the non-glued plants, and 967 wasps were found on the plants with glue. Wasps started landing within seconds after opening the container. The number of wasps that land decreases with time. The distribution over the leaf levels is shown in Figure 1. As can be seen, most wasps land on the middle leaves. The average leaf level was 5.9 for the observed plants and 6.1

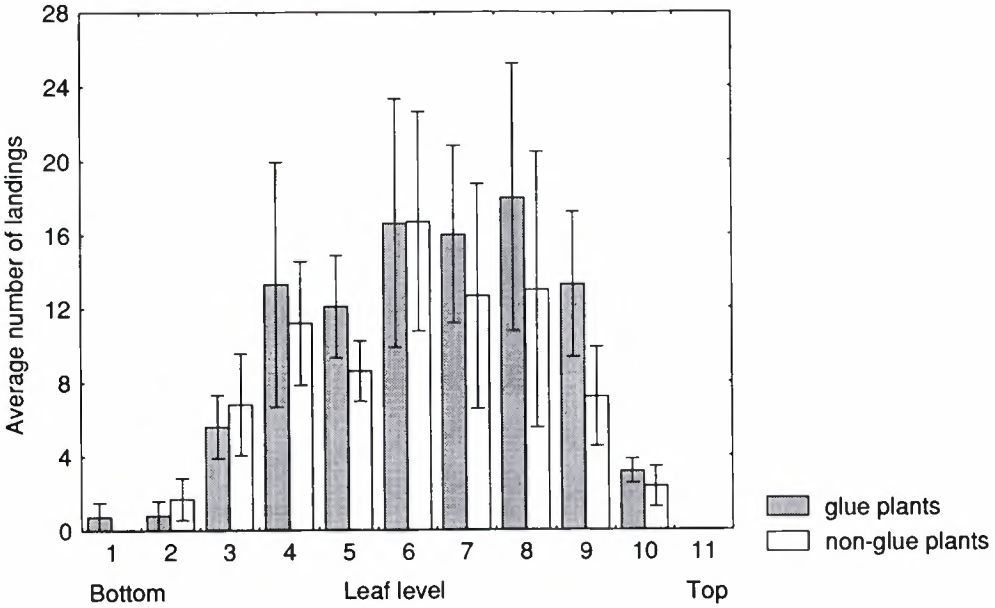

Figure 1: Distribution of landings of *Trichogramma brassicae* over different leaf levels of maize plants, determined by direct observation of landing and by using glue-sprayed plants in greenhouse experiments. The lowest leaf is level 1. (Means and s.e. of ten repetitions)

for the plants with glue. We were interested whether the number of landings is related to the surface area. Figure 2 shows that for both plants without and with glue the number of wasps per unit of leaf area is clearly higher for the upper leaves.

The distribution of landings over parts of the leaf was also studied (Table 1). A small part (3% on the plants without glue, 6% on the plants with glue) of the wasps did not land on the leaves but landed on the stem. There was a slight preference for the upper leaf side, which was somewhat more pronounced in the plants without glue. Most wasps land on the part of the leaf close to the stem or on the middle part of the leaf, and only 17% on the point of the leaf.

The correlation between the numbers that landed on the plants with glue and the numbers that landed on the clean plants each day was very high: 0.979 (Pearson product-moment correlation).

Effect of height of release of wasps

3626 wasps were found on the plants where the wasps were released at 100 cm, and 4429 on the plants where the wasps were released at ground level. The results are shown in Table 2. The distribution over the leaf levels was very much like that of the first experiment. When wasps are released at ground level the average level of landing is only 0.4 leaf lower (which is equivalent to about 8 cm) than when the wasps are released at 100 cm. The landing patterns on the low and the high plants were quite similar. There was no difference in the numbers of wasps landing in both treatments. Again, more wasps landed on higher leaf levels than can be explained just by surface area.

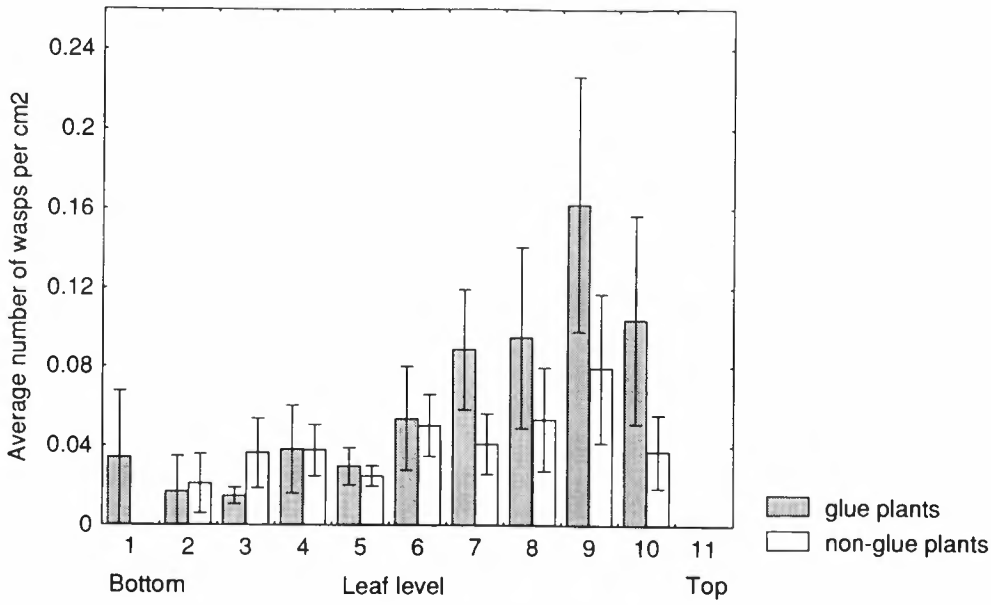

Figure 2: Number of *Trichogramma brassicae* landing per cm2 on maize plants, determined by direct observation of landing and by using glue-sprayed plants in greenhouse experiments. The lowest leaf is level 1. (Means and s.e. of ten repetitions)

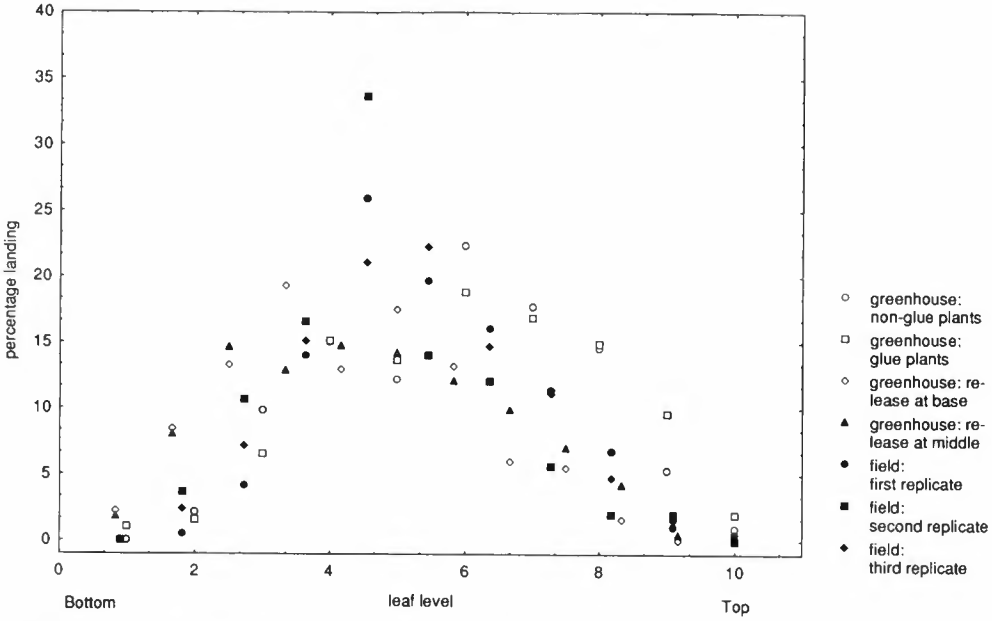

Figure 3: Relative distribution of landings of *Trichogramma brassicae* over leaf levels of maize plants. All distributions were reduced to the same x-length in order to make the distribution shapes easier to compare.

Table 1: Distribution of landing of *Trichogramma brassicae* over parts of the leaf

	plants without glue	plants with glue	p[1]
Total number landing on leaves in ten repetitions	814	906	
Number of landings	74.0±25.2[2]	90.6±30.8	*[3]
Landing			
Part of leaf close to the stem	29.4±9.8 (39.7%)	37.7±13.7 (41.7%)	n.s.[4]
Middle part of leaf	32.1±11.4 (43.4%)	37.0±13.0 (40.8%)	n.s.
Point of leaf	12.5±4.3 (17.0%)	15.9±4.2 (17.6%)	n.s.
Landing			
Upper side of leaf	48.1±18.4 (65.0%)	51.2±16.8 (56.5%)	***[5]
Lower side of leaf	25.9±7.4 (35.0%)	39.4±14.8 (43.5%)	***

[1] n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$
[2] Mean ± s.e.
[3] Wilcoxon Matched Pairs Test (CSS:Statistica, 1991)
[4] x^2-test (Siegel, 1956)
[5] x^2-test (Siegel, 1956)

Landing in the field

The distributions of landing in the field were about the same as in the greenhouse (Figure 3). Of the 10000 wasps released, 193 (1.93%), 357 (3.57%) and 252 (2.52%) were recaptured on the 8 plants. Of these, 73 % were females, while the sex ratio of the release material was 60% females, which means that less males land then expected (chi-square test, $p<0.0001$). Although the wind was quite strong in two of the replicates, this was not apparent from the distribution over the plants.

Of the 8 plants, 4 were placed in the maize rows and 4 between the rows (Table 3). Although less wasps land on the plants between the rows, the difference is not significant.

Table 2: Effect of height of release on landing of *Trichogramma brassicae* on maize plants.

	release at plant base	release at plant middle	p[1]
Total number of wasps	4429	3626	
Number landing	1107,3±191,7[2]	906,5±210,9	n.s.
Average leaf level of landing	5,15±0,29	5,56±0,28	n.s.

[1] Wilcoxon Matched Pairs Test (CSS:Statistica, 1991) n.s.: P > 0.05
[2] Mean ± s.e.

Table 3: Landing of *Trichogramma brassicae* on maize plants in and between crop rows.

	Between rows	In rows	p[1]
Number of plants	12	12	
Total number landed	334	259	
Number landing per plant	21.6±4,1[2]	27,8±4.6	n.s.

[1] two-factor ANOVA. n.s.: P > 0.05
[2] Mean ± s.e.

Table 4: Landing of *Trichogramma brassicae* on pieces of maize leaves with and without kairomones and eggs.

	clean leaves and eggs	leaves with kairomone	p
Number landing	6,1±0,3[1]	6,5±0,6	n.s.[2]
Landing in host patch		0,36±0,26	n.s.[3]

[1] Mean ± s.e.
[2] Wilcoxon Matched Pairs Test (CSS:Statistica, 1991) n.s.: P > 0.05
[3] Chi-square test comparing the number of landings in the patch with the values expected from the area

Table 5: Landing of *Trichogramma brassicae* on maize plants with and without host kairomones.

	clean plants	plants with kairomone	p
Number landing	503.75±149.17[1]	421.00±124.12	n.s.[2]

[1] Mean ± s.e.
[2] Wilcoxon Matched Pairs Test (CSS: Statistica, 1991) n.s.: P > 0.05

Effect of localized kairomones on landing

The landing of 101 wasps was observed. The number of wasps that landed on the leaf pieces with and without *Ostrinia* kairomones was almost the same (Table 4). The landing positions of the wasps on the leaf pieces were random and the number that landed within the patch with scales was no higher than that which could be expected from a random distribution.

Effect of non-localized kairomones on landing

1684 wasps were observed to land on the plants with host cues, and 2015 on the clean plants. Again, there was no effect of host cues (Table 5). In fact, the number of wasps that landed on the plants with host cues was lower than that on the clean plants, although not significantly so. Again, landing started immediately after release and decreased with time.

DISCUSSION

Landing of *Trichogramma* on plants with and without glue was similar. The distribution of landings as found indicates that all parts of the plant are visited by *Trichogramma*, although landing occurred proportionally more in the upper leaf levels. This might be a result of the positive phototaxis of *Trichogramma*.

The effect of release of height on landing positions was relatively small. When the release point is lowered one meter, the average landing position lowers by just 8 cm. Apparently, the wasps do not fly in a straight line to the plants. In biocontrol, *Trichogramma* are either released from cards hanging on the plants or from capsules lying on the ground. According to the results of these experiments, the different height of release in both methods will have little effect on the number or position of landing *Trichogramma*.

In the greenhouse experiments, it is assumed that all wasps reach the plants directly. In field situations, we estimated, based on earlier observations (Suverkropp, unpubl., Pak et al. 1985), that less than one percent reaches plants at two meters distance directly, while the other wasps make one or more landings in between. Still, the landing distributions are similar to those in the greenhouse. Apparently the plant structure itself, combined with phototaxis, influence landing strongest. Landing and flying off repeatedly will not affect the distribution over the leaf levels. The fact that relatively more wasps land on the higher leaves can explain the results of Neuffer (1987) who found that parasitization of *Ostrinia* was higher above and at the release level and above than below the release level. Whether a plant is situated within or between a row does not play an important role in the number of landings.

We could not find any effect of host cues on landing. In the experiment with the leaf pieces, the host-cues were concentrated in a small area. The patches could contain scales, excrements, sex pheromones and eggs of *Ostrinia*. Noldus & van Lenteren (1985) could not show any effect of *Mamestra brassicae* kairomones on landing, but Smits (1982) found a clear effect of *Mamestra brassicae* host cues (produced by moths on the leaf) on landing of *T. evanescens*. In his experiment, the number of wasps landing on leaves with kairomones was twice that of clean leaves. In olfactometers, *Trichogramma* often shows some attraction to eggs (Ferreira et al. 1979, Kaiser et al. 1989, Renou et al.1989, Renou et al. 1992) or sex pheromones

(Kaiser et al. 1989, Zaki 1985). In olfactometer experiments, the odour is the only stimulus available and movement is mostly by walking, so flight and landing behaviour is not well simulated.

In our experiment with whole plants, only volatile host cues could reach the plants. Fresh host-eggs were distributed on all plants, so that their odour was present as well.

Although the number of landings was not influenced by the presence of host cues, after landing they had a clear effect on behaviour. Both experiments were repeated with a similar setup to test the effect of host cues on searching efficiency. In both cases searching efficiency was significantly increased by the presence of kairomones (Suverkropp, unpubl.). Most authors (e.g. Lewis et al. 1975) are convinced that the main effect of kairomones in *Trichogramma* searching is arrestment. The results found in these experiments confirm this view.

As *Trichogramma brassicae* attacks different hosts and as the position of these hosts is not fixed in a certain zone, the pattern of landing of *Trichogramma* ensures an efficient sampling of the plants in search of other host cues.

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Defense of a sessile host against parasitoids: *Aleyrodes singularis vs. Encarsia spp.*

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Aleyrodes singularis Danzig nymphs mount their exuviae upon their backs. In addition, adults of this species cover the immatures with waxy fluff. Both these features serve as protective mechanisms against parasitism. *Encarsia transvena* is inhibited from ovipositing in *A. singularis* because of the wax, whereas *E. inaron* is less successful in parasitizing the whiteflies in the presence of the wax and exuviae than in their absence. The existence of parental care by *A. singularis* adults is suggested.

Key words: Aleyrodes, Encarsia, Host feeding, Parental care, Whiteflies.

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Defenses against natural enemies, that may involve chemical, behavioral, and morphological mechanisms, have been reported for numerous phytophagous insects (Aldrich 1988, Cornell et al. 1987, Damman 1987, Evans & Schmidt 1990, Olmstead & Denno 1992). In many cases, it has been difficult to prove the defensive value of the suggested mechanisms, and even more so, to quantify it. This is often due to the difficulty of setting up experimental conditions with appropriate controls that lack the suggested defense mechanism (Olmstead & Denno 1992).

The whitefly *Aleyrodes singularis* Danzig possesses a number of features that suggest a defensive role. The adults intermittently cover the immatures with fluffy wax, which makes their patches very conspicuous, whereas their nymphs accumulate empty exuviae upon their backs (Figure 1) (Guershon and Gerling, pers. obs.). As these characteristics can be manipulated without physically affecting the developing whitefly immature, a set-up is thus available to test their defensive role against natural enemies.

In this work, we set out to determine whether the wax secreted by the adult and the larval exuviae, have defensive roles. For this purpose, we compared the behavior of two parasitoid species upon whitefly-infested patches that had been denuded from their apparent defenses, with normal patches as controls.

Fig. 1: Side view of the exuvial «pyramid» of a IV nymphal stage of Aleyrodes singularis. The lowest, black, stage denotes the living nymph.

MATERIALS AND METHODS

1. Insect Origin. The whiteflies and the local parasitoid *Encarsia inaron* (Walker), were both field collected from *Lactuca serriola* plants and cultivated for experimental purpose. *Encarsia transvena* (Timberlake) is a polyphagous whitefly parasitoid reared by us on *Bemisia tabaci* (Gennadius) on various host plants.
2. Parasitoid time allocation on infested host patches. One parasitoid female was placed on each patch, and its behavior was followed. The following behaviors were registered using «The Observer» (Noldus 1991) event recorder: *Foraging* = walking while drumming with the antennae. *Handling* = examining the host with the antennae, and abdomen. *Drilling* = using the ovipositor to drill the host. No distinction was made if an egg had been deposited. *Host feeding* = feeding upon a wound previously inflicted upon the host by the parasitoid with the ovipositor. *Others* = preening, resting, and any other activity not previously described.

The parasitoids were released on leaves with whitefly nymphs that were either left untreated, or deprived of their wax and larval exuviae. This was done by passing a delicate air stream over the leaf followed by manual removal of exuviae with a fine brush. We used experienced parasitoid females that were kept for 24 hr prior to release, in a vial with honey in order to insure their similar ovarian condition. Exposure time was 20 min per replicate, and 15 replicates with different individuals were done for each parasitoid species. Results were expressed as percent of the relative time dedicated for each behavior, and compared using the Mann-Whitney non parametric test. The time taken for each event (means and sd) was also measured.

RESULTS

1. Encarsia inaron

1a. Behavior on untreated leaves (control)

Foraging starts as soon as the parasitoid is released upon the leaf. The female locomotes, drumming with her antennae. Walking speed diminishes when she meets a whitefly patch and mounts it.

Handling. Due to the high density of the whitefly nymphs in each patch, our observations of handling are contingent with those of foraging and searching for suitable hosts. The difficulty in separating these activities prompted us to combine them in our analyses (Figures 2 and on).

The parasitoid female performs all of her handling from above, while walking or standing on top of the wax-covered exuvial pyramid of the whitefly nymphs. The female gradually becomes covered with a white waxy cover that is particularly dense on the antennae. This does not appear to hamper the continuing examination of the hosts beneath her, through repeated ovipositor probing and antennation, which are performed without leaving the «top canopy» of the wax-covered patch. The female readily differentiates areas with nymphs from those with whitefly eggs, and does not attempt to probe the latter.

Drilling is also performed from above, forcing the female to traverse with her ovipositor the pyramid of empty pupal host exuviae on which she stands.

Host feeding was never observed.

Others. Following each drilling, the female cleaned the antennae and, in particular, the tip of the ovipositor. This was done either while standing, or walking.

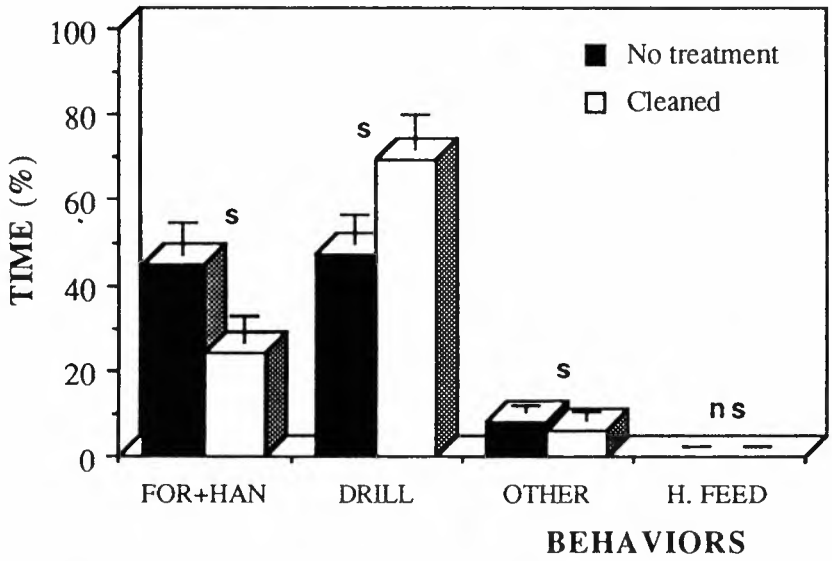

Fig. 2: Patch time allocation of *Encarsia inaron* on normal (not treated) vs cleaned patches of *Aleyrodes singularis*. For + Han = Foraging + Handling; s = significantly different (Mann-Whitney U test, $p < 0.05$); ns = not significantly different ($p > 0.05$).

1b. Behavior on cleaned hosts

As before, *foraging* and *handling* could not be clearly separated due to the extreme density of the hosts on the patch. Their joint behavioral sequences observed were identical with those of the control. However, they were significantly shorter ($p<0.05$) both when measured as a percent of all activities on the leaf (Figure 2), and in absolute duration (Figure 3). The duration of each *drilling* event was similar to those of the control (Figure 3), but the number of drillings per exposure was significantly higher ($p<0.05$, Figure 2).

2. Encarsia transvena

2a. Behavior on untreated leaves.

As soon as the wasp lands on the leaf she starts running about in an apparently aimless way, or tries to fly off. No antennal drumming was observed. Once contact with the wax is made, it sticks to her body and her attempts to clean it off are unsuccessful. The attempts to dislodge the waxy cover on her body caused her, in 27 % of the *other* behaviors category, to lose her balance, topple over, and land on her back.

2b. Behavior on cleaned hosts.

Once the wax and the larval exuviae have been removed from the nymphs, *E. transvena* females spend most of their time *drilling*. Each drilling was preceded by short *foraging* and *handling*. *Host feeding* also occurred in this observation, whereas *other* behaviors were minimal, and limited mainly to preening. The differences in percent of time allocated to the various activities by this parasitoid in the different treatments, were significant ($p<0.05$) for all the behaviors recorded (Figure 4).

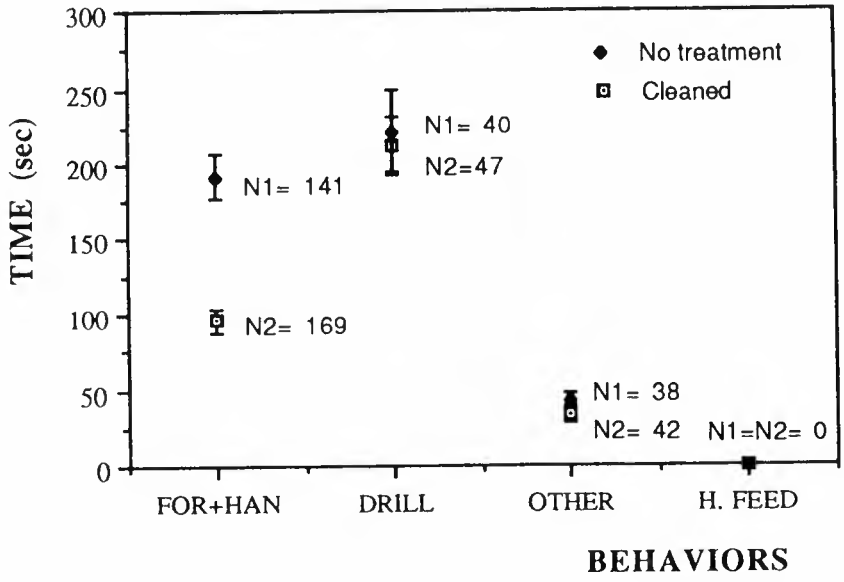

Fig. 3: Duration (mean ± SD) of each behavior of *Encarsia inaron,* on normal (not treated) vs cleaned *Aleyrodes singularis.* For + Han = Foraging + Handling; N1 = number of replicates on non-treated patches; N2 = number of replicates on treated patches.

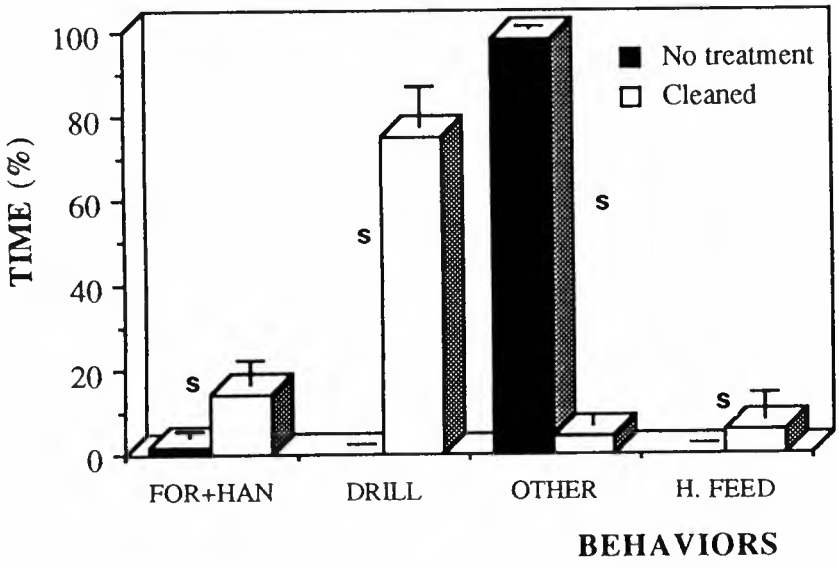

Fig. 4: Patch time allocation of *Encarsia transvena* on normal (not treated) vs cleaned patches of *Aleyrodes singularis.* For+Han = Foraging + Handling. s = significantly different (Mann-Whitney U test, $p < 0.05$).

DISCUSSION

Our observations and experiments showed that both the wax powder secreted by the whitefly adult, and the exuvial pyramid that covers the immatures in the developing whitefly colony, play a significant role in the relationships with parasitoids.

Both *E. inaron*, which is apparently adapted to parasitize whiteflies that secrete ample amounts of wax [it is also a successful parasitoid of *Siphoninus phillyreae* Haliday (Gould et al. 1992)], and *E. transvena* that is usually a parasitoid of whiteflies whose nymphs are not covered with waxy fluff, are negatively affected by the wax covering produced by *A. singularis*. However, *E. inaron* manages, nevertheless, to oviposit on that host. *E. transvena*, on the other hand, is unable to function in the presence of the wax.

The fact that parasitism by *E. inaron* is hampered by the secretions of the whiteflies, shows that they play a defensive role even in regards to this native and natural parasitoid of *A. singularis*. The wax causes entanglement of the searching parasitoid, whereas the exuvial pyramid prevents it from reaching the host directly, thereby forcing it to probe for and oviposit in the host while standing upon a pile of exuviae.

The lack of host feeding by *E. inaron* can also be regarded as a protective mechanism for the whitefly, since mortality due to this predatory activity by the adults parasitoids may be considerable (Jervis & Kidd 1986). This too can be attributed to the presence of the exuvial pyramid that prevents close contact between the parasitoid and its host. It also raises the interesting question of the mode by which *E. inaron* obtains its proteins for egg production, since it belongs to a genus whose other known members are proovigenic and do host feed (see Gerling (1990) for a review)

It is interesting to consider the possible role of the whitefly adult (usually the mother) that remains on the leaf patch and periodically deposits powdery wax upon her progeny. The fact that in the absence of such action, the waxy covers diminish (Guershon and Gerling , pers. obs.), leaving the progeny exposed to more efficient parasitism, is indicative of parental care exercised by the adult. Thus, *A. singularis* joins the whitefly *Neomaskellia bergii* that has recently been shown to guard her eggs (Kurosu et al. 1992), as well as another group of homoptera, including Aethalionidae (Brown 1976), and Membracidae (Holldobler & Wilson 1990) in which parental care has been demonstrated.

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Learned odour preferences in the parasitoid *Cotesia glomerata*

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The effect of prior experience on the foraging behaviour of the generalist parasitoid *Cotesia glomerata* was studied in windtunnel bioassays. Females were given oviposition experiences on Nasturtium leaves infested by *P. brassicae*, a plant-host-complex the parasitoid hardly attacks in natural situations, but which they prefer after an experience. Retention of the learned stimuli was tested in dual choice tests. Three days after being trained, females switched to the innately preferred Brussels sprouts – *P. brassicae* complex. The effect on the foraging behaviour of the quality of encountered host-patches and the order of encounters was measured in a second series of experiments. Four groups of females had oviposition experiences on two types of plant-host-complexes, with alternating high and low host-densities. The type of first experience and an experience with a high host density both had a significant effect on the choice for the innately preferred infested Brussels sprouts plants.

Keywords: *Cotesia glomerata*, foraging behaviour, learning, parasitoid

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Variability in responses of foraging parasitoids to environmental cues is an important phenomenon to study in order to understand differences in foraging strategies (Vet & Dicke 1992). Behavioural plasticity has been presented as an explanation for variability in responses. Changes in behaviour may be the result of learning through experience. Experience may evoke responses to stimuli or enhance the responsiveness through association of a stimulus present during the experience with a reinforcing stimulus (Vet & van Opzeeland 1984, de Jong & Kaiser 1991, Turlings et al. 1993). The impact of learning is hypothesized to be stronger on innately weak responses than on innately strong responses, since stimuli with high response potentials may be invariable or at least hardly modifiable by experience (Vet et al. 1990).

The parasitoid's need for learning has been hypothesized to be dependent on the degree of specialization on the first and second trophic level (Sheehan & Shelton 1989, Vet et al. 1990, Vet & Dicke 1992). For generalists, it would be functional to learn to concentrate on the most rewarding habitats. Learning might play a role in the habitat-sampling process, to estimate the relative availabilities of different types of plant-host combinations present. Multiple experiences may affect subsequent foraging decisions. Parasitoids may be restricted in their capacities to memorise several

different stimuli. Furthermore, learning of novel odours may interfere with the memorisation of already learned odours, such that the order of learning is determining the subsequent preferences (de Jong & Kaiser 1992, Kaiser & de Jong 1993). On the other hand, female wasps may learn to become more discriminative, and choose for the most profitable host-patch. In this paper we studied some of these aspects of learning in the generalist parasitoid species *Cotesia glomerata*, a larval endoparasitoid of several *Pieris* species. It is capable of attacking hosts on several plant species, although on some foodplants the parasitoid is hardly ever encountered, like Nasturtium (*Tropaeolum majus* L.) (Shenefelt 1972, Feltwell 1972). In an earlier study on learning in this parasitoid, naive *C. glomerata* females were found to have a strong preference for Brussels sprouts plants, but after being experienced on the alien plant-host complex Nasturtium – *P. brassicae*, the parasitoid's preference switched to the learned stimulus (Geervliet et al. 1993). For the present study we concentrated on two aspects of learning, the retention of learned information and the effects of host densities experienced during training on the preference for odours of a plant-host system.

MATERIALS AND METHODS

Insects – *C. glomerata* were reared on *P. brassicae* larvae in a greenhouse compartment at 22–26°C., 60% RH an a L:D 16:8 photoperiod. *P. brassicae* colonies were maintained on Brussels sprouts plants (*Brassica oleracea* var. *gemmifera* cv. *Titurel*) in a climate room at 20±2C., 50–70% RH and a L:D 16:8 photoperiod. Parasitoid and host colonies originated from collections from Brussels sprouts fields in the vicinity of Wageningen.

Plants – Plants or plant parts used for training and choice tests were derived from individually potted Brussels sprouts plants (S) (*Brassica oleracea* cv. *gemmifera* var. *Titurel*) and Nasturtium plants (N) (*Tropaeolum majus* L.). Infested leaves were obtained by placing foodplants in an adult *P. brassicae* cage, where female butterflies deposited their eggs on the plants during 4–6h. After hatching, larvae were allowed to feed for 16–24 h. Leaves with a standard size (35–40 cm^2) and standard number of larvae (20–25) were chosen for training and test procedures.

Training procedure – For the experiment on retention times daily 3 days old mated females of *C. glomerata* were trained individually in a petridish provided with a host-infested Nasturtium leaf. Females were allowed to oviposit in three of the *P. brassicae* larvae, after which they were transferred to a nylon gauze cage with water and honey until needed. For the experiment on the influence of experienced host-densities, 4 groups of trained females were created. Individual 3–5 days old females were offered an infested leaf of Brussels sprouts (S) followed by an infested leaf of Nasturtium (N) or the reverse. Host densities were alternated high and low over the subsequent experiences: high on S + low on N, high on N + low on S, low on S + high on N and low on N + high on S respectively. In all preflight treatments, wasps were allowed to oviposit in three of the host larvae. On leaves with high host densities wasps encountered a patch of ±20–25 1^{st} instar *P. brassicae* larvae, whereas on leaves with low host densities three 1^{st} instar larvae were offered. The amount of herbivore–damaged leaf tissue was equivalent to the number of feeding larvae (16–24 h. of feeding). After the training was completed, wasps were transferred individually

to glass vials with water and honey and kept in a climatic chamber at 15°C until needed.

Bioassays – For the choice tests a windtunnel was used as described by Blaakmeer, Geervliet, van Loon, Posthumus, van Beek & de Groot (unpubl.). For both experiments choices consisted of a Brussels sprouts leaf and a Nasturtium leaf, both equally damaged by a clutch of ±20–25 *P. brassicae* larvae. Stems of the cut leaves were put in a vial with water, that was closed with parafilm. Odour sources were placed in a T-shaped glass cilinder on a socket at the upwind end of the windtunnel. The position of the odour sources was switched left-to-right and vice-versa after every five replicates. Individual females were gently introduced into the windtunnel by allowing them to step into a glass vial at the release site. For experiment 1 daily 4–5 females were tested that were trained 1, 2, 3, 4 or 5 days before the experiment or that were naive. The latter females were 3–8 days of age. For experiment 2 four females of each training group were tested. Treatments were performed daily over several consecutive days, to control for the day–effect (Steinberg et al. 1992). After flight initiation, choices for one of the two odour sources were recorded. Landings elsewhere in the windtunnel were recorded as «no response». Each parasitoid was given one flight opportunity. Total choices for odour sources were analysed as binary data by a Chi-square test. Analysis of responsiveness in experiment 1 was done by a repeated G-test of independence. Data of experiment 2 were analyzed by GLIM (General Linear Models).

RESULTS AND DISCUSSION

Retention – Figure 1 shows that naive females that chose for one of the offered odour sources have a clear preference for the *P. brassicae* infested Brussels sprouts leaf (S). Responsiveness of naive females however is low: 53.3% of the wasps did not land on one of the two offered leaves. One day after oviposition, more females chose for the experienced plant-host-complex (Nasturtium-*P. brassicae*), although the difference was not significant (P=0.087). This was the case however, for females that did have an oviposition experience two days before testing: parasitoids significantly preferred the Nasturtium – *P. brassicae* complex. Three days after training the preference for the experienced odour source was lost and four and five days after experience, preference of *C. glomerata* switched back to the Brussels sprouts-*P. brassicae* complex. Responsiveness of females in all treatments was significantly higher than that of naive females. Our data show that the delay of reinforcement has a significant effect on the foraging behaviour of the parasitoids. The learned response waned and with time the females showed the same preference as naive females. Vet (1988) and Poolman-Simons et al. (1992) found that lack of repeated reinforcement obliterates the effect of associative learning of a substrate on subsequent choices of parasitoids. The wasps switch back to their innate preference for a certain substrate. The function of waning is unclear. Physiological constraints such as memory capacities may be involved (Poolman Simons et al. 1992). In a variable and unpredictable environment with respect to host source abundance, waning of learned responses might be adaptive. A foraging parasitoid might be able to «link» the lack of reinforcement with the absence of the source. Meanwhile, hosts have to be encountered, and new stimuli may have to be learned and memory of one learned stimulus may interfere with

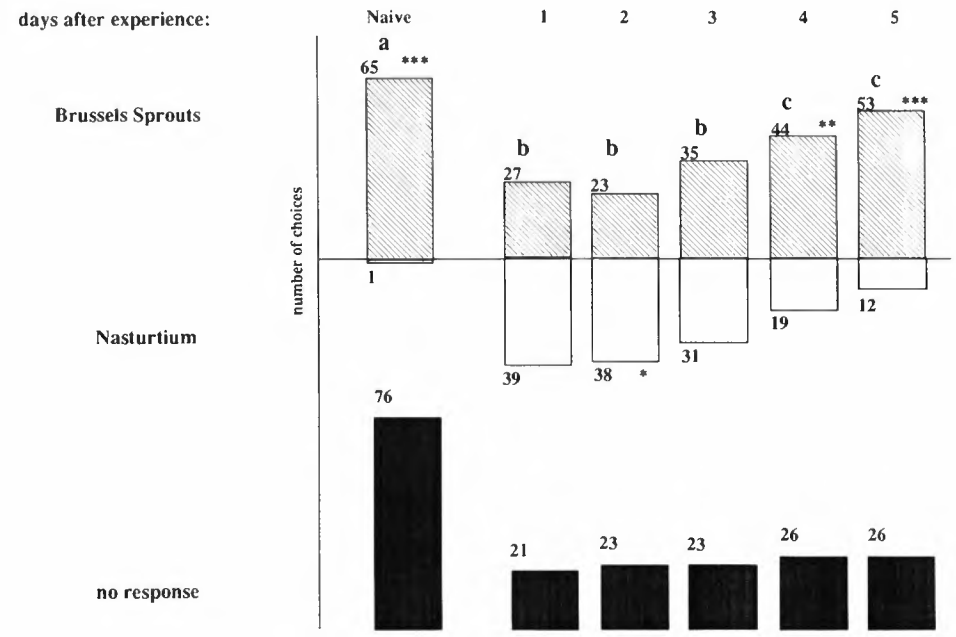

Figure 1. Influence of time after an experience on the Nasturtium-Pieris brassicae complex on the choices of Cotesia glomerata for the offered odour sources Brussel sprouts and Nasturtium, both infested by P. brassicae larvae. Asteriks indicate significant differences within treatments: * P<0.05; ** P<0.005; *** P<0.0001. Bars with same letters are not significantly different.

learning of novel stimuli (Lewis 1986, Kaiser & de Jong 1993). For a generalist species this might be true, since several potential plant-host complexes may be available and suitable. When the learned stimulus does not lead to ovipositions, a parasitoid has to switch back to the natural substrate or change to an alternative host.

In all experienced females responsiveness is very high, compared to responsiveness of naive females. It was already known, that an oviposition experience enhances the motivation of parasitoids to search for hosts (Steinberg et al. 1992, Turlings et al. 1993). Our data show that this motivation remains high. Apparently, the effect of priming did not wane, since the delay of reinforcement did not have an effect on the responsiveness *to*, but only on the *preference for* plant odours.

Experienced host densities – A first oviposition experience on a high host-density, followed by an oviposition experience on a low host-density, leads to a significant preference for the first learned odour (high on S + low on N and high on N + low on S respectively) (Figure 2). On the other hand, the first experience on a low host-density, followed by an experience on a high host-density, does not lead to a preference for either of the offered odour sources (low on S + high N and low on N + high on S). In both cases an equal distribution of choices was found for the two plant-host complexes.

Responsiveness of *C. glomerata* females that had two subsequent oviposition experiences was very high. Of all groups more than 90% of the females made a choice for one of the offered odour sources.

Apparently, the first experience that a female receives is important for subsequent choices. A high host density encountered as a first experience can not be overruled by a second experience on a low host-density, not even when the host larvae are feeding on the innately preferred Brussels sprouts plants. Furthermore, a first experience on a low host-density can not be overruled completely by a second experience on a high host density. Still 50% of the parasitoids choose for the plant-host complex on which they encountered only a low host density. These results suggest, that females do not use the encountered high host-density as the only cue to establish their preference, nor does the first experience completely fix the females' behaviour. They seem to show a strong response to the first learned plant-host complex, and a high quality of the subsequent experience might cause a change in their preference. In a previous study on *C. glomerata*, it was shown that a contact experience with host-damaged leaf tissue was already sufficient to induce a learned preference, and actual host encounters were not necessary (Geervliet et al. 1993). Probably a first experience has a high priming effect, which is not easily overruled. Results from the retention experiment suggest that learned odours wane after three days. Subsequent experiences may be learned meanwhile, but may not completely overrule the first learned odour. *C. glomerata* did memorise the last plant-host complex experienced, but this did not lead to a preference for one of the two learned odour sources. In a study on the effect of the order of learning in foraging *Drosophila*

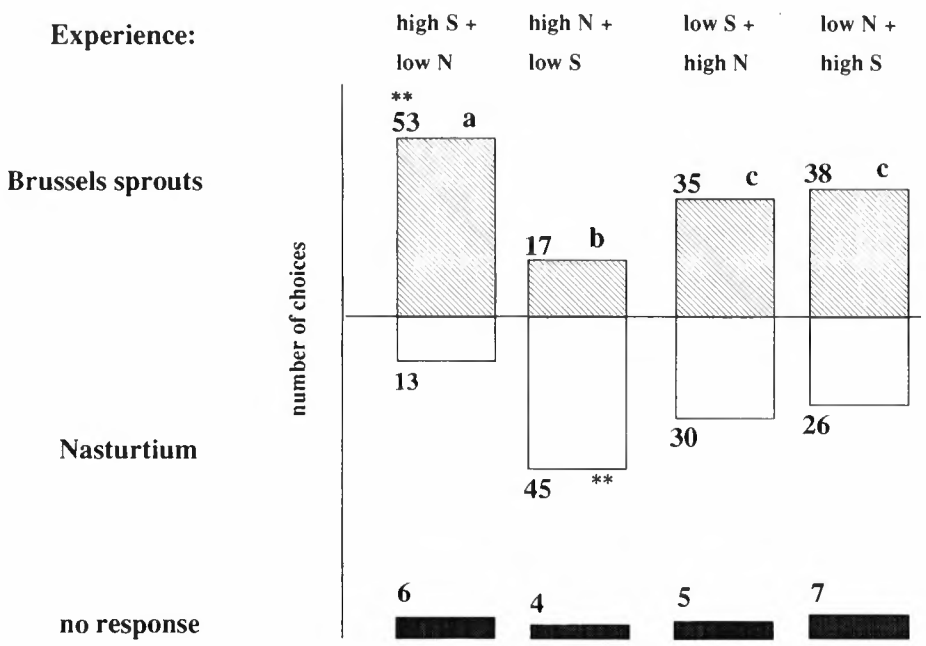

Figure 2. Effect of the order of experienced high and low host-densities on Brussels sprouts plants (S) and Nasturtium (N) on the choices of Cotesia glomerata for the different odour sources. Asteriks indicate significant differences within treatments: ** $P<0.005$. Bars with same letters are not significantly different.

parasitoids (*Leptopilina boulardi*), de Jong & Kaiser (1992) showed that the memory for a learned odour was not affected by a subsequent conditioning to another odour when wasps were tested in a no-choice situation. In choice tests, they found that parasitoids were able to memorise more than one host-associated odour. In contrast to our results, de Jong & Kaiser (1992) and Kaiser & de Jong (1993) found, that *Leptopilina boulardi* preferred the last odour they learned. Experiments are now being carried out to get more insight in the role of the order of learning and the effect of unrewarded experiences on the foraging behaviour of *C. glomerata.*

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Odour conditioning of ovipositor probing behaviour and its heritability

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We investigated the influence of different olfactory and oviposition experiences on the ovipositor probing activity of *Leptopilina boulardi*, a parasitoid of drosophila larvae, and conducted a preliminary study on the heritability of odour-conditioned probing. An original device was set up for the conditioning and observation of this behavioural activity. Systematic and persistant probing response to the odour (banana) was observed after associative conditioning, i.e. when it was delivered during an oviposition. Other treatments (unpaired occurrences of odour and oviposition, odour exposure, oviposition experience) modulated for a shorter time the probing response to the odour in a significant number of wasps. These changes corresponded to phenomena known as sensitization, backward inhibitory conditioning and habituation. Mother-daughters regression performed on the time spent probing in response to the learned odour (after associative conditioning) failed to indicate a significant heritability, possibly because of the small number of individuals observed.

Keywords: associative learning, heritability, Hymenoptera, *Leptopilina boulardi*, olfaction, ovipositor probing.

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Learning processes enable the insects to adapt their behaviour to changes in various ressources such as food, oviposition sites, throughout their life. Odours play a major role in parasitic wasps searching for hosts and the effect of odour learning on their orientation behaviour have been documented in several species (see Turlings et al. 1993 for review) and can result in higher parasitization rates (e.g. Lewis & Martin 1990). Wasps orient their displacement towards an odour perceived during a previous experience with the host (oviposition) or with host products. The simultaneity between odour delivery and experience is essential for the acquisition of persistant odour memory, as demonstrated in *Leptopilina boulardi,* a parasitoid of frugivorous *Drosophila* larvae (De Jong & Kaiser 1991). Therefore associative learning is presumably a key process for odour memorization during host searching, where the reinforcement could be defined broadly as experiencing the host. Whereas learning involved in host location at a distance has been well studied, its implication in searching for hosts once females are on the host-infested substrate has received little attention. At this step, ovipositor probing, usually triggered by the perception of host-related chemicals, is a major behaviour that leads to location of the host and to oviposition (Vet & Bakker 1985). Some studies reported that this behaviour could be released by novel stimuli after these had been associated to the presence of hosts (e.g.

Vinson et al. 1977). In the present work, we investigated whether ovipositor probing of *L. boulardi* could be triggered by an odour previously associated to an oviposition experience and established the associative nature of this conditioning by comparing females treated as above to different control groups (forward and backward unpaired presentations of odour and larvae, exposure to odour only, to larvae only, and no experience). On another hand, some studies demonstrated the heritability of different behavioural variables of parasitization (e.g. Chassain & Boulétreau 1987, Wajnberg 1989), but there were no data on learning performances. Therefore we also conducted a preliminary study on the heritability of the odour-conditioned probing response.

MATERIALS AND METHODS

Insects.
L. boulardi. Wasps used in the experiments were reared on a *Drosophila* mutant (Rosy) fed on axenic diet. This rearing was started yearly from wasps originally found in *Drosophila melanogaster* collected from Opuntia in Tunesia (Nasrallah) in 1986 and maintained in population cages on their sympatric host (*L. boulardi* G464 strain, kindly provided by Y. Carton, CNRS, F-91190 Gif-sur-Yvette). Detailed rearing procedure was given in De Jong & Kaiser (1991). All experiments were conducted on 5-7 day-old mated females, unless stated otherwise.

Odour source.
Banana extract (Haarman & Reimer, F-92000 Nanterre) placed in 2 capillary tubes (internal diameter: 1.56 mm) served as odour source in the conditioning and testing procedures.

Device for conditioning and testing ovipositor probing behaviour.
A female was introduced into a ring of pure agar-agar medium (height: 2mm, internal diameter: 6mm) placed in the cap (height: 3mm, internal diameter: 11mm) of a plastic capsule. The cap was pierced in its center (diameter: 4mm) to let the air flow over the female; this one was maintained in the agar-agar ring by a piece of nylon fabric placed underneath and a piece of acetate (perforated with pin holes) on top. We termed this whole device a caplet. It was placed on an elevated perpex support so the airflow arrived underneath, with the binocular above, mounted with a camera to observe the wasp's behaviour on a monitor. Because of the small height of the caplet, the insect was always viewed in profile to observe the ovipositor probing and the oviposition. The airflow (750ml) could be odorized by shifting half the flow into a branch containing the odour source, which rejoined the neutral branch via a Pasteur pipette, just below the caplet.

Conditioning and testing protocol.
The ovipositor probing responses to banana odour were compared between differently treated groups. For all groups, the conditioning-testing protocol included four consecutive phases (Figure 1). It began by an **initial phase** during which the female was placed in the agar-agar caplet for 3 min. Then the female underwent the **conditioning phase**, different for each group and described afterwards. Then the female was maintained in a caplet without host or odour for 12 min: this

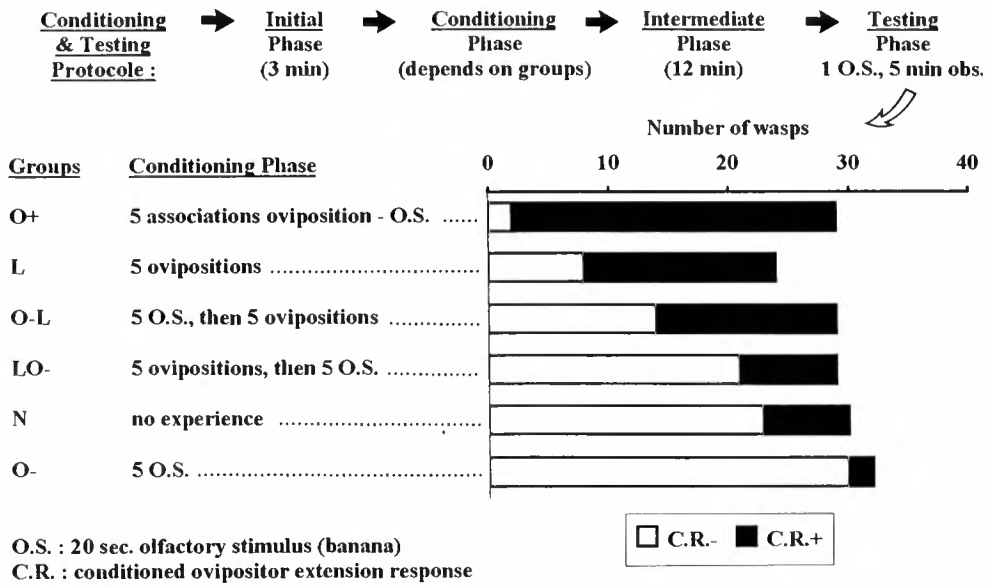

Figure 1. Conditioning and testing procedure. The upper part shows the consecutive phases of the conditioning and testing procedure underwent by individual females (see text for detailed explanation). Proportions of responses to the tested odour observed in the different groups (defined on the left part) are reported on the right part.

intermediate phase allowed for the extinction of possible probing of the agar often observed after oviposition experience (most females stopped probing before the last 3 min of this phase, the others were discarded). The **test** was then performed: the wasp received a 20 sec banana odour stimulation; the probing response was observed during the odour delivery and the following 5 min. The effects of 6 conditioning treatments were compared.

In one group, the odour was delivered only while the female was ovipositing (it was therefore placed in a caplet with 20 first and second instar *Drosophila* (Rosy) larvae). Each female underwent 5 odour-associated ovipositions; considering that the odour (O) was reinforced by the oviposition (+), this group was labelled O+. In the groups labelled O-L and LO-, females underwent unpaired presentation of odour and larvae (L) so the odour presentation was not reinforced (O-). O-L females had 5 odour stimulations first, then were transferred into a caplet with 20 larvae to perform 5 ovipositions. The reverse procedure was applied to the LO- females. In the group labelled L, females performed 5 ovipositions but never experienced the odour. O- females experienced 5 odour stimulations without any larvae. A last group was kept naive (N): instead of a conditioning phase, females were only transferred into a novel caplet without any host or odour, for 5 min. During the conditioning phase of groups O-L, LO- and O-, the 5 odour stimulations (20 sec) were separated by a 40 sec interval. This timing corresponded to the average odour stimulation received by the O+ group because mean oviposition duration was 20 sec and mean time interval between 2 ovipositions was 40 sec. During the test, ovipositor probing was quantified

by the number of wasps responding and their time spent probing. Chi-square values and Kruskal-Wallis analysis of variance based on ranks were used to compare these variables between groups.

Heritability.
The heritability of the response to the learned odour following O+ treatment was evaluated by a mother-daughters regression on the time spent probing. In order to increase the parental link between a mother and her daughters, females of the mother generation were mated with their own sons. For this purpose, they were tested unmated, then allowed to parasitize host larvae to produce parthenogenetic males. These females were kept at 15°C during the development of their sons, i.e. for ca. 17 days. They were maintained for 24 h in the presence of their sons for mating, then allowed to parasitize larvae, in order to produce the daughter generation. The storing of females at 15°C probably reduced their fertility because only 13 mothers among 45 tested produced daughters, which number varied from 1 to 10 per mother, and totalized 58. They emerged under normal conditions, i.e. in the presence of males, and were therefore mated when tested. The order in which daughters were tested was established at random to minimize possible day effects. Individuals that did not exhibit the probing response were discarded from the analysis which was then restricted to 10 families with a total number of 51 daughters. The correlation coefficient was calculated by the linear regression model performed on all the individual values of the daughters and also on the average values of sisters. In the latter case, the slope is an estimator of the daughter-mother resemblance (**sensu stricto** «heritability» in diploid systems, Falconer 1974). Its deviation from 0 was tested by Student t-test.

RESULTS AND DISCUSSION

Evidence for associative conditioning
(Figure 1). Spontaneous probing response to banana odour was exhibited by 7 females out of 30 tested in the naive group N. The proportions of responses occurring after the different treatments were compared to this spontaneous response frequency, then comparisons were made between groups which response frequencies were significantly higher. Only 2 females out of 32 tested in the O- group responded to the odour, which was almost significantly lower than group N at the 5 % level ($Chi^2 = 3.64$; $P = 0.056$). The proportion of responses produced by group LO- (8/29) was not different from group N ($Chi^2 = 0.14$; $P>0.05$). Contrastedly, responses to the odour were significantly more frequent in groups O-L, L and O+ ($Chi^2 = 5.08$, $P = 0.02$; $Chi^2 = 10.24$, $P = 0.01$; $Chi^2 = 29.4$, $P<0.001$, respectively). Almost all O+ females (27/29) responded to the odour. This proportion was significantly higher than those observed in group O-L (15/29, $Chi^2 = 12.43$, $P<0.001$) and L (16/24, $Chi^2 = 6$, $P = 0.01$). Both latter groups did not respond differently ($Chi^2 = 1.21$, $P>0.05$). Some females that had responded to the odour upon the first test were retested 1h later. The higher response in group O+ was confirmed: half the O+ wasps (6/12) still responded to the odour, whereas there was no response among the 9 retested O-L wasps and only 1/11 in group L. The time spent probing was compared between these three groups. It appeared that the probing duration of O+ females (53 sec) was also higher than in groups O-L (35 sec) and L (34 sec) (Kruskal-Wallis, $P = 0.01$).

Thus among all the treatments applied to *L. boulardi*, only the associative procedure (O+) led to an almost systematical and also persistant response to the odour. This phenomenon corresponds to Pavlovian classical conditioning where the animal learns that external events predict the occurrence of a reinforcer (Dudai 1989), although the exact nature of the reinforcer is still unclear in our case. Indeed contact with the host or host products may serve as reinforcer (e.g. Lewis & Tumlinson 1988) as well as successful oviposition. So our results show that associative learning of odours can be important in the final step of host location, based on ovipositor probing. We also found that other phenomena could modulate the probing response but in a lower proportion of individuals and over a shorter period of time. Increased odour response after oviposition experience (groups L and O-L) corresponds to sensitization

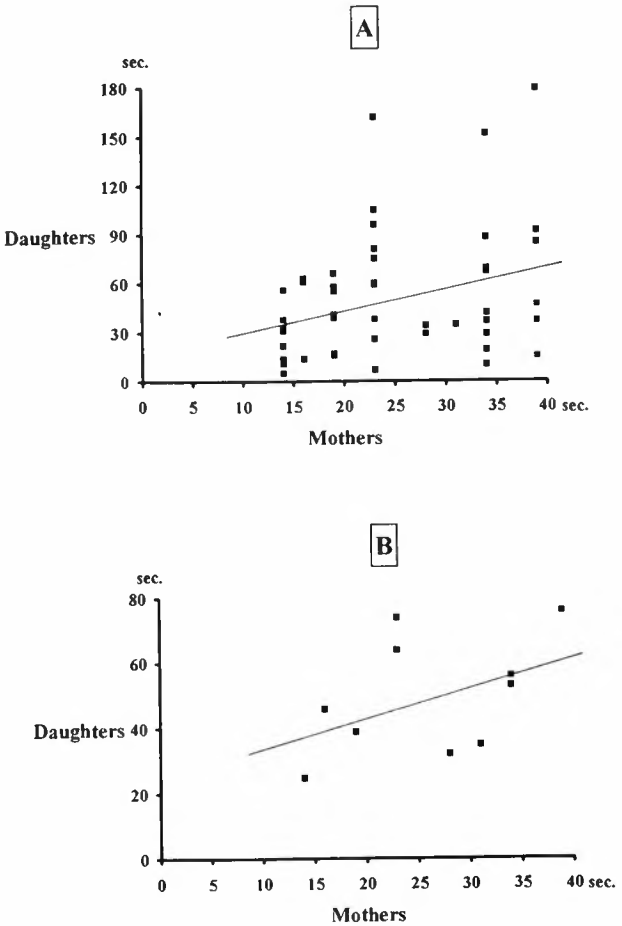

Figure 2. Mother-daughters regression on the time spent probing in response to the learned odour (following the O+ treatment). A. Each point represents the response of a mother (x-value) and one of its daughter (y-value). B. Y-values are the average responses of sisters. Values of correlation coefficient and slope of the linear regressions are given in the text.

described in honeybees after sugar intake (Erber 1981). The absence of such effect in the LO- group may reflect backward inhibitory conditioning: as the odour was delivered just after females were withdrawn from the infested substrate, it may have been associated to a negative reinforcement. This was also described in honeybees which perceived an odour shortly after food intake had stopped (Menzel et al. 1993). At last, habituation, defined as a diminution of a response to a stimulus, following repeated presentation of the same stimulus or another one (Dudai 1989), could have occurred in the O- group. Therefore our analysis revealed that all known forms of non-associative conditioning and associative learning can alter the ovipositor probing response to an odour. It implies that ovipositor searching is a highly adaptive behaviour because it can be constantly modulated by perceived chemicals and oviposition experience.

Heritability.
When taking into account all individual daughter values (Figure 2), there was a significant linear correlation between conditioned responses of mothers and daughters (slope: 1.35 ± 0.61, $P = 0.03$) but the correlation coefficient was low (0.30), due to the high intra-family variability. When performed on the average values of sisters to estimate the heritability, the analysis did not anymore show a significant linear correlation between mothers and daughters (slope: 0.91 ± 0.67, $P > 0.05$; correlation coefficient: 0.43). From the time-scale of the x- and y-axis, it appears that individuals of the daughter generation spent more time probing than the mother generation. This is due to our protocol which required to test unmated mothers, whereas daughters were mated, a state known to enhance all activities related to oviposition.

Lack of significant heritability may be due mainly to insufficient number of mothers. Its existence will be investigated using an improved protocol. The conditioning and testing procedure will be shortened in order to observe more individuals. Females of the mother generation will be mated with their brothers (isofemale lines will be created at the grand-mother generation) so they will not undergo storing at 15° and will be mated when tested. In addition to the time spent probing in response to the learned odour, it would be interesting to include other learning parameters in the analysis of genetic variability, such as caracteristics of acquisition, retention and selectivity of the learned response.

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Studies on semiochemicals affecting the Host acceptance Behaviour of *Asaphes vulgaris* Wlk. (Hymenoptera: Pteromalidae)

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Christiansen – Weniger, P. 1994. Studies on semiochemicals affecting the Host acceptance Behaviour of *Asaphes vulgaris* Wlk. (Hymenoptera: Pteromalidae). Norweigan Journal of Agricultural Sciences. Supplement 16. 277-282. ISSN 0802-1600.

Host acceptance behaviour of the aphid hyperparasitoid *Asaphes vulgaris* Wlk. was studied in order to find reasons for the divergence in attacks on *Aphidius rhopalosiphi* De Stefani Perez and *Aphelinus varipes* (Förster), which are both primary parasitoids of cereal aphids. *A. vulgaris* was not able to discriminate between *A. rhopalosiphi* and *A. varipes* internally after ovipositor contact. The response of the hyperparasitoid towards methanol extracts of a mummified aphid (*Rhopalosiphum padi* (L.), parasitized by *A. rhopalosiphi* or *A. varipes*) revealed a high attractivity of the *R. padi*/*A. rhopalosiphi* mummy in contrast to the mummy of *R. padi*/*A. varipes*. The extract of the mummy shell of *R. padi*/*A. rhopalosiphi* was still attractive to *A. vulgaris* females, suggesting that the silk, produced by the last larva of *A. rhopalosiphi*, provides kairomones, which are responsible for the host acceptance by the hyperparasitoid. However, *A. varipes* larvae do not produce silk. It is therefore supposed that the missing kairomones are one reason for the low attack rate of *A. vulgaris* on *A. varipes*.

Key Words: *Aphelinus varipes, Aphidius rhopalosiphi, Asaphes vulgaris,* host acceptance, kairomones semiochemicals

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Aphid hyperparasitoids interfere with primary parasitoids by means of parasitization, host feeding or patch displacement (Hagen & van den Bosch 1968, Keller & Sullivan 1976, Höller et al. 1991, Höller et al. 1993). *Asaphes vulgaris* Wlk. is a common hyperparasitoid of cereal aphids and known as fairly polyphagous (Sullivan 1987). The hyperparasitoid evidently evolves preferences between host species, but they appear to be variable by the behaviour of associative learning (Christiansen-Weniger 1991). However, there are primary parasitoids which remain seldom recognized hosts. The information *A. vulgaris* receives from these host species differs presumably from the information received from hosts which are frequently attacked. In this work, the origin and effects of semiochemicals on the host acceptance behaviour of *A. vulgaris* were therefore studied. Host species used in the experiments were *Aphidius rhopalosiphi* De Stefani Perez as a preferred host and *Aphelinus varipes* (F.) as a rarely attacked host of *A. vulgaris*.

MATERIALS AND METHODS

Insect cultures were maintained on oat seedlings under laboratory conditions (20± 1.5°C, 60–70% r.h., 8D : 16L). The hyperparasitoid *A. vulgaris* was reared on *Aphidius uzbekistanicus* Luzhetzki with *Sitobion avenae* F. as the host aphid. *A. rhopalosiphi* and *A. varipes* were reared on *Rhopalosiphum padi* (L.).

Two bioassays were designed in order to investigate host acceptance by *A. vulgaris*. The tests, as described below, were conducted at room temperature. Thirty replicates were performed in each experiment. The host selection behaviour of *A. vulgaris* was investigated in a 35 mm plastic Petri dish in which an experienced female was given the choice between 2 different hosts, each in 3 mummies, the two host types being arranged in an alternating position. The following hosts were offered in the choice tests:

1. *A. rhopalosiphi* and *A. varipes*;
2. *A. rhopalosiphi* treated with 1 µl extract;
3. *A. varipes* treated with 1 µl extract;
4. *A. rhopalosiphi* and *A. varipes* larvae in the wrap of the *R. padi*/*A. rhopalosiphi* mummy.

Solvent extraction: Mummy shells of *R. padi*, parasitized by *A. rhopalosiphi* or *A. varipes*, were extracted in methanol in the concentration 1:1 (mummy shell : µl solvent). Mummy shells were therefore transferred into Eppendorf reaction tubes with a 0.22 µm filter (Ultrafree MC filter, Millipore). The compounds were centrifugated for 10 min. at 12,000 rpm and the filtrate was used immediately in the test (see choice test 2. and 3.).

Exchange of larvae (see choice test 4.): The larva of *A. rhopalosiphi* was returned from the *R. padi* mummy and exchanged with the last larva of *A. rhopalosiphi* or *A. varipes*. The mummy was subsequently closed by sticking pieces of mummy shell across the hole caused by dissection. The number of attacks within one hour were counted. Parasitization of hosts was verified by dissecting the mummies after the experiment, thereby checking for the presence of eggs.

The attractivity of methanol extracts was investigated in a 35 mm plastic Petri dish containing a rubber dispenser dummy (1 mm^3, Hoechst, Germany), to which 1 µl extract (concentration 1:1, see above) or 1 µl methanol (control) had been applied. One experienced *A. vulgaris* female was introduced to the test arena and the behavioural responses, i.e. how often the female stopped in front of the dummy with aligned antennae («sightings»), how often the female traversed the dummy without arresting («traverses») and how much time the female spent examining the dummy after mounting («contact»), were recorded during one hour.

RESULTS

There was no attack on *A. varipes* when *A. vulgaris* was given the choice between *A. rhopalosiphi* and *A. varipes* (Table 1). Similarly, a low attack rate could be observed when the mummy of *R. padi*/*A. rhopalosiphi* was treated with the mummy shell extract of *R. padi*/*A. varipes*. On the other hand, *A. varipes* was significantly more

often examined by the hyperparasitoid when it was treated with the mummy shell extract of *R. padi/A. rhopalosiphi,* though no oviposition was performed. However, *A. vulgaris* females could not discriminate between the larvae of *A. rhopalosiphi* and *A. varipes* when they had been prepared in the mummy shell of *R. padi/A. rhopalosiphi* (Table 1).

The mummy extract of *R. padi/A. rhopalosiphi* led to distinct behavioural responses of *A. vulgaris* (Table 2). Divided into the parts mummy shell and larva, the mummy shell still revealed a high degree of attractivity whereas the larva extract was not in any behavioural response significantly different from the control (Table 2). The attractivity of mummy and mummy shell extracts of *R. padi/A. rhopalosiphi* was also stressed by the fact that oviposition attempts on the dummy could be observed in several females.

In contrast, the extract of *R. padi/A varipes* mummies elicited virtually no response from the hyperparasitoid (Table 3). The extract of the mummy shell differed significantly in the number of «sightings» from the control. However, no significantly higher responses were detected in the categories «traverses» and «contact». The other extracts again showed no activity (Table 3).

Compared to the attractivity of *R. padi/A. rhopalosiphi* extracts, all behavioural

Table 1. Host selection of *Asaphes vulgaris* between *Aphidius rhopalosiphi* and *Aphelinus varipes,* both reared on *Rhopalosiphum padi.* Treatment extract: 1µl extract (1 mummy shell : 1µl methanol) was applied to the test mummy; treatment larva: the last larva of *A. rhopalosiphi* was replaced by another last instar larva. For each replicate (30 replicates per selection test) a female was offered 3 mummies per host species for one hour.

offered hosts		attacked (mounted) mummies	% attack (mounting) on B	p[1])
A	B			
Aphidius rhopalosiphi (1)	*Aphelinus varipes* (2)	47[2])	0.0[2])	<0.001
		18[3])	0.0[3])	<0.001
A. rhopalosiphi + ectract of (1)	*A. rhopalosiphi* + extract of (2)	57[2])	33.3[2])	<0.05
		26[3])	30.8[3])	<0.05
Avaripes + extract of (1)	*A. varipes* + extract (2)	35[4])	31.4[4])	<0.05
A. rhopalosiphi + larva of (1)	*A. rhopalosiphi* + larva of (2)	67[2])	44.8[2])	n.s.
		31[3])	48.4[3])	n.s.

[1]) Chi-squared-goodness of fit test; expected attack on B = 0.5 (attacked (mounted) mummies

[2]) number of mummies perforated with the ovipositor

[3]) number of hosts parasitized by successful oviposition

[4]) number of hosts mounted and examined by antennation

responses of *A. vulgaris* towards the mummy extract of *R. padi/A. varipes* were significantly lower (Table 3). Responses towards the mummy shell extract of *R. padi/A. varipes* were likewise lower than the responses of the hyperparasitoid towards the comparable extracts of *R. padi/A. rhopalosiphi*, although this could only be significantly confirmed for the number of «traverses» (Table 3).

Table 2. The effects of methanol (control) or mummy extracts of *Rhopalosiphum padi/Aphidius rhopalosiphi* on the searching behaviour of *Asaphes vulgaris* females during one hour. 1µl extract (1 mummy component : 1µl methanol) was applied to a dispenser dummy.

	behavioural response categories		
Treatment	sightings n	traverses n	contact sek.
methanol	7.13[1])a[2])	3.13a	0.00a
mummy	15.50b	8.53b	108.90b
mummy shell	15.47b	8.20b	69.53ab
larva	10.20a	2.43a	13.13ab
LSD[3])	4.30	3.90	96.73

[1]) Values are means of 30 replicates
[2]) Column results with a different letter are significantly different
[3]) LSD: least significant difference ($p<0.05$)

Table 3. The effects of methanol (control) or mummy extracts of *Rhopalosiphum padi/Aphelinus varipes* on the searching behaviour of *Asaphes vulgaris* females during one hour. Exract application see table 2. The mean values of all behavioural responses of *A. vulgaris* towards the mummy –, mummy shell –, and larva – extracts were compared statistically (STUDENT t – test) with the results in table 2. Significant differences are presented as *.

	behavioural response categories		
Treatment	sightings n	traverses n	contact sek.
methanol	7.13[1])a[2])	3.13a	0.00a
mummy	9.33ab	1.87a**	7.77a*
mummy shell	13.57b	3.30a*	23.87a
larva	10.27ab	2.40a	28.23a
LSD[3])	4.29	2.54	33.44

[1]) Values are means of 30 replicates
[2]) Column results with a different letter are significantly different
[3]) LSD: least significant difference ($p<0.05$)

DISCUSSION

A. vulgaris females were strongly repelled by *A. varipes,* when the test insects were exposed to *A. rhopalosiphi* and *A. varipes* simultaneously (Table 1). The factors which led to a decision of the hyperparasitoid against *A. varipes* appeared to be located in the mummy shell and proved to be soluble in methanol (Table 1). However, *A. vulgaris* females were not able to discriminate internally after ovipositor contact between the parasitoid larva of *A. rhopalosiphi* or *A. varipes*, which again hints at the presence of chemicals in the mummy shell which modify the searching behaviour. The bioassay – active chemicals should derive from the «parasitoid – component» of the mummy shell because the influence of the «aphid – component» was excluded by rearing *A. rhopalosiphi* and *A. varipes* on the same host aphid *R. padi*. Aphid mummies formed by *Aphidius* species are composed of the aphid integument and under that the cocoon, spun by the last primary parasitoid larva. A mummy shell formed by *Aphelinus varipes* consists of a modified integument, which is sclerotized and dark coloured. No silk is produced by the parasitoid larva (Christiansen-Weniger, unpubl.).

Semiochemicals of the mummy of *R. padi/A. rhopalosiphi* emitted from the rubber dummy elicited a strong response from the test insects (Table 2). The results demonstrate that these kairomones were more detectable in the mummy shell extract than in the larva extract. Similar experiments were performed by Nutcharee Siri (1993) with the aphid hyperparasitoid *Dendrocerus carpenteri* (Curtis). She observed a high response of the hyperparasitoid towards hexane extracts of the mummy of *S. avenae/A. uzbekistanicus*. The main source of attractive chemicals was again the mummy shell. Methanol extracts of the mummy of *S. avenae/A. uzbekistanicus* and the silk, produced by the last larva of *A. uzbekistanicus*, were analysed in her work by GC – MS. In both extracts the same three compounds were identified (C25, C27, C29). The results suggest that the *A. vulgaris* females reacted in the dummy tests to compounds of the *Aphidius* cocoon.

In contrast, *A. vulgaris* females were little attracted to methanol extracts of *R. padi/A. varipes* (Table 3). The missing attack on *A. varipes* (Table 1, first choice test) may be caused by a lesser attractivity of *A. varipes* compared to the *Aphidius* host. The absence of a cocoon in *A. varipes* hosts, which is evidently important in host acceptance behaviour of the hyperparasitoid (see above), speaks for this hypothesis. On the other hand, the mummy of *R. padi/A. varipes*, treated with the attractive mummy shell extract of *R. padi/A. rhopalosiphi*, was mounted and examined by the test insects significantly more often, but no oviposition occurred. However, the mummy of *R. padi/A. rhopalosiphi*, treated with the same extract, was frequently attacked. Hence, there might be other, perhaps repellent factors playing a role in the rejection of *A. varipes* hosts, but this has still to be clarified.

SUMMARY

Host selection behaviour of the hyperparasitoid *Asaphes vulgaris* Wlk. between *Aphidius rhopalosiphi* De Stefani Perez and *Aphelinus varipes* (Förster), both primary parasitoids of cereal aphids, was studied. Host acceptance was mediated by the mummy shell (extracted in methanol) and not by the host larva. The response of the

hyperparasitoid towards various methanol extracts, applied on a rubber dummy, was high with mummy and mummy shell extracts of *Rhopalosiphum padi* (L.)/*Aphidius rhopalosiphi*. Methanol extracts of *R. padi*/*A. varipes* showed a low attractivity. The results suggest that the acceptance of *A. rhopalosiphi* is promoted by kairomones in the silk, which is produced by the last larva of *A. rhopalosiphi*. One aspect of the low parasitization of *A. varipes* by hyperparasitoids may be the low attractivity due to the absence of a cocoon in aphid mummies, which have been developed by *A. varipes*.

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Host preference of *Aleochara bilineata* and *A. bipustulata* (Coleoptera: Staphylinidae) in relation to host size and host fly species (Diptera: Anthomyiidae): a laboratory study

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Ahlström-Olsson, M. 1994. Host preference of *Aleochara bilineata* and *A. bipustulata* (Coleoptera: Staphylinidae) in relation to host size and host fly species (Diptera: Anthomyiidae): a laboratory study. Norwegian Journal of Agricultural Sciences. Supplement 16. 283-292. ISSN 0802-1600.

Host size preference and host species preference were studied for the parasitoids *Aleochara bilineata* and *A. bipustulata.* Host pupae of different species and of different weight were parasitised by *Aleochara* larvae in choice experiments. Acceptance of pupae of different ages was also studied. There was a pronounced preference for specific host sizes, *A. bilineata* preferred pupae of normal cabbage root fly (*Delia radicum*) pupal size (10-23 mg), while *A. bipustulata* preferred smaller pupae. Host species preference of *A. bipustulata* between the closely related *D. radicum*, *D. platura* and *D. antiqua* were not noticeable, but *D. radicum* was preferred to *Lonchaea* sp., another potential host. Pupae of different ages, both newly formed pupae and two months old diapausing pupae, were accepted as hosts by *Aleochara bilineata*.

Keywords: *Aleochara bilineata*, *Aleochara bipustulata*, cabbage root fly, *Delia radicum*, *Delia platura*, *Delia antiqua*, host preference, host acceptance, *Lonchaea* sp., parasitoids

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Several parasitoids of the cabbage root fly, *Delia radicum* (Linnaeus) (Diptera: Anthomyiidae), are known. Among the most important are the wasp *Trybliographa rapae* (Westwood) (Hymenoptera: Cynipoidea, Eucoilidae) and *Aleochara bilineata* Gyllenhal and *A. bipustulata* (Linnaeus), both belonging to subgenus *Coprochara* Mulsant & Rey (Coleoptera: Staphylinidae) (Wishart et al. 1957). *A. bilineata* and *A. bipustulata* can parasitise and develop in several species of Anthomyiidae, e.g. *D. radicum*, *D. platura* (Meigen), *D. florilega* (Zetterstedt), *D. antiqua* (Meigen) and *Pegomyia hyoscyami betae* Curtis (Fuldner 1960, Wishart 1957). It has been proposed that survival is lower on large pupae, because the *Aleochara* larva is then not able to eat the whole pupa, and dies when the remains of the pupa rot (Bromand 1974, Jonasson 1994, Pechke & Fuldner 1977). Survival of *A. bilineata* is lower also on very small pupae (<5 mg) where the parasitoid starvs to death in the third instar (Putnam 1957).

Aleochara eggs are laid in the soil and the first instar larva searches for a host pupa to attack. It enters the puparium (the hardened skin of the fully grown fly larva, inside which the fly pupa forms) by gnawing a hole in the wall. The larva seals the entrance hole from the inside with anal excretions (Fuldner 1960). The parasitoid completes its life cycle inside the puparium, feeding externally on the fly pupa. In the following text, the term «pupa» refers to pupa + puparium as a unit.

The larvae of an unidentified South African species of *Aleochara*, subgenus *Coprochara*, a parasitoid of *Haematobia thirouxi potans* (Bezzi) (Diptera: Muscidae) show a preference for pupae of 8 mg (Wright et al. 1989). It does not show any preference for pupal age between one and seven days (Wright & Müller 1989). *A. curtula* (Goeze) (subgenus *Aleochara* s. str.) first instar larvae do not discriminate between pupae of different ages (Pechke et al. 1987).

A. bilineata and *A. bipustulata* coexist in Northern Europe and North America (Fuldner 1960, Jonasson 1994) where both are common in brassica fields. It is interesting to study what kind of niche separation makes this coexistence possible. Phenological difference is one way by which the resources can be divided. *A. bipustulata* hibernates as adult and is active early in the spring (Heydemann 1956). *A. bilineata* hibernates as a first instar larva in the host puparium and the first generation emerges in the beginning of June (Wadsworth 1915). *A. bilineata* has two generations, and *A. bipustulata* has three generations per year (Fuldner 1960). During periods of coexistence a possible mechanism of niche separation would be different preference for host species and host sizes.

In the present investigations I have studied the host preference of *A. bilineata* and *A. bipustulata* first instar larvae, concentrating on the following questions:

Is there some preference for certain host sizes or host species? What host ages are accepted? Is there a niche separation with regard to host preference for different host sizes in the two *Aleochara* species?

MATERIALS AND METHODS

The experiments were performed at Wellesbourne (United Kingdom) in 1992 and at Alnarp (Sweden) in 1993. *A. bilineata* and *A. bipustulata*, collected in the field in 1992 (*A. bilineata* in 1991 at Wellesbourne), were held in culture according to Samsoe-Petersen et al. (1989). The beetles were kept in plastic containers in normal room conditions, except during the autumn and winter when they were moved into an environmental chamber at 20°C, 70 % rH, LD 16:8. Small clay «Leca» granules that keep water were used as a substrate. Eggs were washed out of the containers 2-3 times per week. Simultaneously the beetles were given new minced beef to eat. At Alnarp the host *Delia* flies were kept in an environmental chamber at 20°C, 70 % rH, LD 16:8. They were given water and dry Brewer's yeast, sugar and milkpowder. The maggots were reared on swedes, onions or soaked beans either in the above given conditions or at 15°C, 70 % rH and LD 12:12 for induction of diapause. In the Wellesbourne experiments the flies were kept at 24°C, 60 % rH, LD 16:8 and the maggots reared at 21°C, 75 % rH, LD 16:8 or at 13°C 69 % rH, LD 8:16 for diapause induction. Diapausing pupae were used in the following experiments.

The preference for different pupae was studied. Pupae of *D. radicum* and *D. platura* or *D. antiqua* were weighed and sorted into size classes of 1-3 mg intervals.

Host size preference (Experiment 1)
Ten *D. radicum* pupae respectively of two different size classes were used in each replicate of the experiment. The difference between the two size classes was sufficiently large (on an average about 7 mg difference) to allow differentiation with the naked eye. Different size classes were used in different replicates, and the range of the sizes are given in Table 1.

The 20 pupae were mixed and placed between two layers of moist (10 % water) vermiculite. Ten newly hatched larvae (15 eggs in the Wellesbourne experiments) of *A. bilineata* or *A. bipustulata* were placed on top of the vermiculite. The pots were covered with tight lids and after ten days the puparia were investigated for *Aleochara* entrance holes.

Host species preference (Experiment 2)
Ten pupae respectively, of the same size class, of *D. radicum* and *D. platura* or *D. radicum* and *D. antiqua* were used. Different size classes were used in different replicates, the range of these sizes are given in Table 2, but within one replicate the size of the two species were always the same. Only *A. bipustulata* larvae were available for these experiments.

At Wellesbourne it was tested if *A. bilineata* larvae would parasitise *Lonchaea* sp. (Diptera: Lonchaeidae), which occasionally occurs in brassica vegetable fields. 110 eggs were added to 70 pupae. The following year at Alnarp, *Lonchaea* pupae were collected in the field and used in a choice experiment together with *D. radicum* to test if they were less preferred as a host. Five *A. bipustulata* (the only species available) larvae were added to pots with five pupae of each species.

Acceptance of host pupae of different ages (Experiment 3)
Acceptance of host pupae of different ages was studied at Wellesboure in June 1992 after some initial unsuccessful parasitation experiments. The suspicion that diapausing pupae could be hard to penetrate aroused. An alternative explanation was that the substrate that was used for the parasitation experiments was too compact. Instead of formerly used moist fine sand the substrate was changed to moist fine vermiculite. Third instar larvae, «prepupae» that had not yet formed a free pupa inside the puparium, newly formed pupae and old diapausing pupae (about two months old) were compared.

In each replicate 30 larvae, prepupae or new pupae were placed in small glass tubes together with 40 eggs of *A. bilineata*. For a host choice experiment 50 new pupae and 50 old diapausing pupae were mixed and placed in a glass jar together with 90 eggs of *A. bilineata*.

Statistical methods
The results of Experiments 1 and 2 were analysed by paired t-test (Proc Univariate, SAS Institute 1987). The null-hypothesis of equal degree of parasitism of the two host groups was tested. The result is considered statistically significant if the two-tailed p-value is less than or equal to 0.05.

RESULTS

Host size preference (Experiment 1)
The host size preference of *A. bipustulata* was consistent in both experiments, and the smaller size class was preferred. Table 1. shows the mean difference between number of parasitised pupae of the ten pupae in the two host groups, expressed as number of parasitised pupae in the «small» host group minus the number of parasitised pupae in the «large» host group.

Table 1. Host size preference of two host groups in a choice experiment.

parasitoid species	Host size and % of pupae parasitised				n[1])	mean difference ± sd. [2])	p-value	site
	small (mg)	%	large (mg)	%				
A. bipust	9-17	26	15-23	11	24	+1.58 ±1.52	0.0001	Wel
A. bipust	4-8	57	13-17	17	3	+4.00 ±1.00	0.02	Aln
A. bilin	9-19	58	18-25	58	12	+0.00 ±2.73	1.00	Wel
A. bilin	4-8	20	11-19	53	9	-3.33 ±1.41	0.0001	Aln

[1]) number of replicates
[2]) mean difference between number of parasitised pupe of the two host groups («small» – «large») ± standard deviation

Table 2. A. bipustulata host species preference of two host grous in a choice experiment.

Host species and % of pupae parasitised		n[1])	mean difference ±sd. [2])	p-value	size classes[3])
D. radicum 43 %	D. platura 44%	13	-0.05 ±1.73	0.91	6–12 mg
D. radicum 36 %	D. radicum 34 %	10	+0.20 ±2.90	0.83	10–18 mg
D. radicum 43 %	Lonchaea sp. 17 %	6	+1.67 ±1.03	0.01	3– 7 mg

[1]) Number of replicates
[2]) Mean difference between number of parasitised pupae of the two host groups (D. radicum – other species) ± standard deviation
[3]) range of size classes used in different replicates

For *A. bilineata* there was no detectable preference for either size group in the Wellesbourne experiments, where size classes ranging from 9 to 25 mg had been used. It seemed that all these sizes were more or less of optimum size for *A. bilineata*, and therefore I decided to use smaller pupae in the Alnarp experiments to see if there is a lower limit of preference. It would have been interesting to use larger host sizes as well, but this was not possible because of the difficulty of producing larger *D. radicum* pupae. In the Alnarp experiments on *A. bilineata* with size classes of 4 to 17 mg there was a clear preference for the larger pupae.

In Figure 1 the percentage parasitism, reflecting host size preference, is plotted against host pupal size. Note that the percentage parasitism would have been different if it had been a no choice situation, or if many sizes had been offered simultaneously. The degree of parasitism reflects the preference for pupae of two compared size-classes, with a mean difference of about 7 mg. In accordance with Wright et al. (1989) quadratic regression curves have been fitted to the data. For *A. bipustulata* the regression was forced through zero, to avoid a positive intercept. It can be seen in the figure that *A. bilineata* has its maximum percentage parasitism on a higher pupal weight (maximum point of regression curve = 16.5 mg) than *A. bipustulata* (maximum point of regression curve = 4.7 mg).

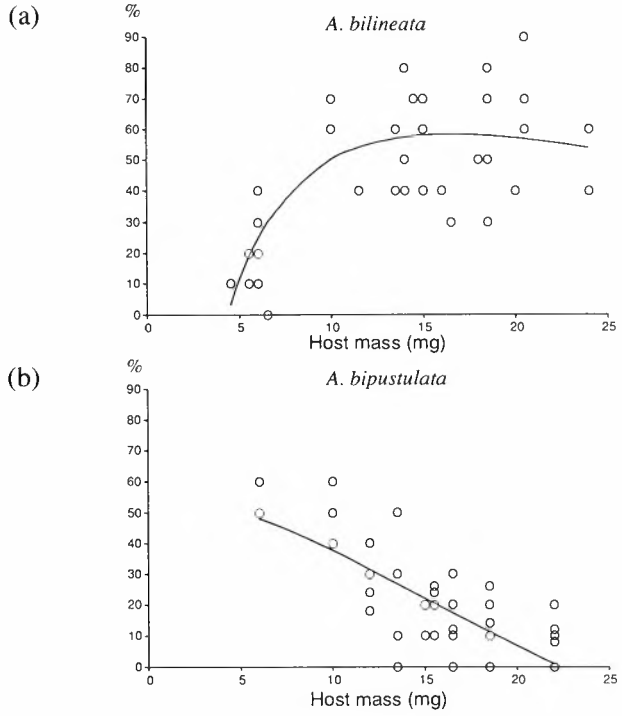

Figure 1. Percentage parasitism of (a) *A. bilineata* and (b) *A. bipustulata* versus host mass with quadratic regression curves fitted to the data. Percentage parasitism reflects the preference for pupae of two compared host sizes in choice experiments. Regression curve for *A. bilineata:* -197.8 + 420.5 ($\log_{10}$ X) -172.6 ($\log_{10}$X)^2, and for *A. bipustulata:* 0 + 145.9 ($\log_{10}$X) -108.1 ($\log_{10}$X)^2, where X is host mass.

Host species preference (Experiment 2)
The host species preference was investigated only for *A. bipustulata*. *D. radicum* and *D. platura* were equally parasitised and the null-hypothesis of equal parasitism could not be rejected. The same was true for *D. radicum* and *D. antiqua*. There was a preference for *D. radicum* to *Lonchaea* sp., which was only parasitised to a small extent in the choice experiment (Table 2). Three live *A. bipustulata* adults emerged from five parasitised *Lonchaea* pupae. In the non-choice experiment, 38 of the 70 *Lonchaea* pupae were parasitised by *A. bilineata*. Of these parasitoid larvae none survived to pupation, two larvae developed to the second and third instar respectively.

Acceptance of host pupae of different ages (Experiment 3)
All host groups offered, were parasitised (table 3.). For comments on parasitation of the larvae and prepupae, see the discussion.

In the choice experiment many larvae entered the puparia of old, diapausing pupae, as well as new pupae. There was only one replicate, so no statistical conclusions have been drawn on preference for host age.

Table 3. Acceptance of *D. radicum* juveniles at the start of the experiment and resulting number of pupae parasitised by *A. bilineata.*

experiment	developmental stage	nuber of parasitised pupae			
non-choice exp:		replicate	1	2	3
	larvae		20	18	22
	prepupae		21	21	
	new pupae		16	18	
choice exp:	new pupae		29		
	old diap. pupae		35		

DISCUSSION

The results in experiment 1 indicate that the first instar larva of *Aleochara* has a mechanism for choosing between different hosts and that *A. bipustulata* prefers a lower size range than *A. bilineata*. This implies a theoretical niche separation, even where eggs of the two species are laid in the same spot. The different results for *A. bilineata* in the Wellesbourne and the Alnarp experiments (Table 1) are assumed to be caused by the different size ranges only, and not by the fact that two different populations were used.

In experiment 3 the larvae did not seem to have any difficulty in entering old diapausing pupae. Probably the substrate was the important factor for the former problems in making pupae parasitised. Earlier the pupae were placed between two layers of dry sand, which was then moistened from above. Therefore the substrate was very compact. In a porous substrate, like vermiculite, it is easy for the larva to move

between hosts and investigate them. In the field the soil is often compact and probably hard to penetrate. The chance of finding a second host after rejecting the first one seems small unless there is an olfactory stimulus that helps the larva to find its way. On the other hand, one would expect that the ability for discriminating between different hosts, as demonstrated experimentally, should have some application under natural conditions.

Wright & Müller (1989) showed that *Aleochara* sp. (*Coprochara*) first instar larvae follow host larval tracks in the soil. In order to move between hosts they probably need to penetrate the soil without *Delia* larval tracks, perhaps between aggregates. In the present experiments the *Aleochara* larvae must have moved between host pupae even though there were no larval tracks.

According to Brooks (1951) many species attack roots that have already been damaged by other maggots, diseases or mechanical injuries. *D. platura* and *D. florilega* occur in the tunnels made by *D. radicum* or *D. floralis*. *Lonchaea flavidipennis* (Zetterstedt) is listed as a phyto/zoosaprophagous species, sometimes occurring in cabbage fields. These maggots and a few others may occur together in a brassica vegetable field, and therefore host species preference of parasitoids is of interest.

If a potential host species is less suitable for the development of the parasitoid, it should be an important property for the parasitoid to be able to avoid it when there are other hosts available. The results of experiment 2 indicate that the *A. bipustulata* larva has a mechanism for choosing between host species. *Lonchaea* sp., which has not been reported as a host species, was less preferred to *D. radicum*. Lower parasitism could also be caused by parasitoid inability of penetrating thick or hard puparium walls.

For the *Delia* hosts, *A. bipustulata* showed no host species preference. *D. radicum* and *D. platura* were equally parasitised as well as *D. radicum* and *D. antiqua* and they may all be equally suitable as hosts. Niche separation between *A. bilineata* and *A. bipustulata* in this respect can unfortunately not be discussed, since only *A. bipustulata* was available for the experiments. It need not be a neccesary factor for coexistence, since females probably will do some habitat or host selection which causes a niche separation.

Wishart (1957) found in field collections from the same field that *D. platura* was more frequently parasitised than *D. radicum* by *A. bipustulata*. This could be an effect of host size preference, since the *D. platura* pupae most likely were smaller than the *D. radicum* pupae. This would be in accordance with the present laboratory results, provided that the larvae make the major part of the host finding.

All host age groups offered in experiment 3 proved to be acceptable hosts. The host larvae and prepupae obviously had had time to form true pupae before parasitation, since host larvae can not be parasitised.

Since the females, in nature, probably do some habitat or host selection it is hard to estimate the importance of larval host preference as a factor of niche separation. Choosing between available hosts may mainly be a way to increase the survival by sorting out less suitable hosts. But if the habitat or host preference of females is very similar for *A. bilineata* and *A. bipustulata* then larval host preference could be important for their coexistence.

Data on size distribution of *A. bilineata* and *A. bipustulata* from pitfall traps in the field shows that there is a large overlap in the sizes of the two species (Jonasson

1994). This means that they to a large extent parasitise pupae of the same sizes. Judging from the size of the beetles in the field most of *A. bilineata* and *A. bipustulata* have emerged from *D. platura* or a fly species of similar size. But the part of the population which correspond to the size of *D. radicum* pupae is much larger for *A. bilineata* than for *A. bipustulata* (Jonasson 1994). This apparent niche separation caused by different preference (of larva or female) for available hosts is probably an important factor, as well as different phenology, for making the two species able to coexist.

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Regulation of *Heliothis virescens* (F.) development and hormonal metabolism by the endophagous parasitoid *Cardiochiles nigriceps* Viereck: The role of teratocytes

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Only part of the serosa of *Cardiochiles nigriceps* Viereck (Hymenoptera, Braconidae), an endophagous parasitoid of the tobacco budworm *Heliothis virescens* (F.) (Lepidoptera, Noctuidae), dissociates at the hatching, originating teratocytes. These cell are implicated in the conversion of 20-hydroxyecdysone of parasitized hosts to inactive highly polar ecdysteroids. The result is an alteration both of host pupation and of various metabolic pathways, which are under hormonal control. Several effects of parasitism observed *in vivo* can reasonably be explained by considering the functional integration of teratocytes and calyx fluid – venom of *C. nigriceps* adult females.

Keywords: ecdysteroids, host regulation, polydnavirus, serosa

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Cardiochiles nigriceps Viereck is an endophagous larval parasitoid of the tobacco budworm, *Heliothis virescens* (F.). This braconid regulates a number of host developmental patterns and metabolic pathways. Parasitized host larvae, after reaching the last instar, do not pupate and there is a substantial increase in several compounds of nutritional importance to the developing parasitoid larva (Dahlman & Vinson 1975, Pennacchio et al. 1993, in press a). These alterations in the normal developmental pattern are largely under the host's hormonal control which is regulated by parasitoid factors, both of maternal and embryonic origin.

Calyx fluid – venom of *C. nigriceps* adult females has a strong effect on the hormonal balance and development of *H. virescens* larvae, dramatically reducing the ability of pupally committed host prothoracic glands to be stimulated by PTTH, even though there is no apparent gland degeneration (Tanaka and Vinson 1991a, 1991b).

Teratocyte presence in the haemolymph of parasitized hosts, after the hatching of *C. nigriceps* larvae, and their subsequent developmental changes were first reported

by Vinson (1970). The ultrastructure of these cells has been studied (Vinson & Scott 1974), assessing also their importance in modulating the immune response of the host (Vinson 1972). We have been studying for the last few years the role of *C. nigriceps* teratocytes in determining developmental and metabolic alterations of the host, by disrupting its hormonal balance. In the present paper we report the most significant aspects of this recent study.

ORIGIN AND FATE OF SEROSAL CELLS

In embryos of parasitic Hymenoptera, the presence of an extraembryonic membrane, usually designated as the serosa, has been reported for several species (Tremblay & Caltagirone 1973). The functional role of this external cell layer is in most cases not well defined, even though resonable hypotheses, based on ultrastructural observations, suggest both a protective and a nutritional role (Ivanova-Kasas 1972, Tremblay & Caltagirone 1973, Koscielsky et al 1978, Baehrecke & Strand 1990).

A recent study (Pennacchio et al. in press b) describes in detail the morphology and the ultrastructure of *C. nigriceps* serosal cells. In this parasitoid species, the serosal membrane originates from the anterior pole of the embryo, starting from 14–15 h (rearing temperature 29±0.5°C) after parasitoid oviposition and, when completed, a continuous envelope around the developing embryo can be observed. Beginning 24–25 h after oviposition, the serosa undergoes different developmental patterns. Serosal cells in contact with the abdominal region of the parasitoid larva give rise to an anucleated syncytial layer, which persists on the body surface of the 1st instar larva, along with some distinct serosal cells around the head and thorax. In contrast, serosal cells at both terminal poles of the embryo remain as large columnar cells, with extensive microvilli along their outer edge, near the chorion. At the hatching, these cells dissociate to form teratocytes. *C. nigriceps* teratocytes are spherical cells, which do not divide, even though their size and ploidy level (Pennacchio, Vinson and Tremblay unpubl.) increase through time, as reported also for *Microplitis demolitor* Wilkinson (Strand & Wong 1991). *In vitro* rearing studies do also suggest that, before hatching, the serosa and, apparently, the ultrastructural changes described above are of nutritional importance for the developing parasitoid embryo (Pennacchio et al. in press b).

This dual ontogeny of the serosal cells observed for *C. nigriceps*, as well as the occurrence of different embryonic membranes, as in the case of *Cotesia glomerata* (L.) (Beckage pers. comm.), and the persistence of the serosa after hatching, with various modalities and functions (Grandori 1911, Hawlitzky 1972, Lawrence 1990), would suggest that this embryonic envelope is much more than a simple protective cell layer.

TERATOCYTE EFFECTS ON HOST DEVELOPMENT AND HORMONAL METABOLISM

The injection of *C. nigriceps* teratocytes, obtained from *in vitro* hatched embryos, into nonparasitized *H. virescens* last instar larvae, inhibited host pupation in a dose dependent way and according to the age of the host larvae at the time of injection

(Pennacchio et al 1992). However, these teratocyte injected larvae showed a total ecdysteroid titre unexpectedly higher than both in nonparasitized and parasitized host larvae, or in larvae injected with calyx fluid and venom, which also induced pupation inhibition (Tanaka & Vinson 1991a, 1991b).

The alteration of ecdysone metabolism in parasitized or teratocyte injected larvae has been recently studied (Pennacchio et al. in press c) and the results obtained can partly explain the apparent contrast of very different ecdysteroid titres associated with virus and venom or teratocyte injections, both leading to pupation inhibition. Purification by high performance liquid chromatography (HPLC) of haemolymph ecdysteroids, followed by their quantification by radioimmunoassay (RIA) showed that in parasitized last (5th) instar larvae of *H. virescens* the ecdysone inactivation through 26-hydroxyecdysone production and formation of highly polar ecdysteroids takes place more precociously than in nonparasitized larvae, as soon as the 20-hydroxyecdysone appeared to be produced. Furthermore, these highly polar ecdysteroids were the most abundant metabolites produced and accumulated and the 20-hydroxyecdysone was the most abundant free ecdysteroid released after their enzymatic digestion.

Injections of young teratocytes, obtained *in vitro*, into nonparasitized host last instar larvae resulted in a drastic alteration of ecdysone metabolism. Although the total ecdysteroids were about 3 times higher, the 20-hydroxyecdysone titre was 3 times lower while the ecdysone titre was up to 5 times higher than in nonparasitized control larvae.

To better understand the role of teratocytes, *in vitro* incubations of radiolabelled ecdysteroids were conducted with teratocytes explanted from *H. virescens* larvae, 5–7 days after parasitization (Pennacchio et al. in press c). When ecdysone was co-incubated with teratocytes it was recovered almost completely untransformed. In contrast, incubation of 20-hydroxyecdysone with teratocytes resulted in the recovery of 34% of the total radioactivity in the first 5 fractions, which contained ecdysteroids more polar than 20-hydroxyecdysonoic acid.

These results suggest that the teratocytes play an active role in the conversion of 20-hydroxyecdysone to polar ecdysteroids. The overall result is a low 20-hydroxyecdysone level in teratocyte-injected larvae, as well as in parasitized larvae, which show also a pronounced depression of prothoracic gland activity induced by calyx fluid and venom (Tanaka & Vinson 1991 b). The reduced titre of 20-hydroxyecdysone in teratocyte injected larvae results in pupation inhibition, even though there is a strong increase of the total ecdysteroid titer. In these larvae the increased ecdysteroid titre may be due to loss in the feedback regulation by 20-hydroxyecdysone on PTTH production, release, or on ecdysone synthesis (Steel & Davey 1985, Watson et al. 1989), due in part or totally to the rapid degradation of the 20-hydroxyecdysone to inactive metabolites, while, at the same time, prothoracic gland activity is not depressed by calyx fluid and venom.

METABOLIC ACTIVITY OF TERATOCYTES DURING DEVELOPMENT

Methanol extracts of teratocytes the same age as those used for *in vitro* incubation with radiolabelled ecdysteroids, showed the presence of ecdysteroids and the most abundant compound detected by HPLC/RIA was 20-hydroxyecdysone, along with

lower amounts of both highly polar and low polar ecdysteroids (Figure 1) (Pennacchio, Vinson & Ostuni, unpubl.). This would suggest that the enzymatic activities involved in the conversion of 20-hydroxyecdysone to inactive metabolites are probably in part or totally taking place inside the cells, after 20-hydroxyecdysone absorption.

To assess possible developmental related changes in the 20-hydroxyecdysone metabolism by teratocytes, we determined the ecdysteroid profile of cells obtained *in vitro* from *C. nigriceps* embryos explanted from parasitized host larvae 28 hrs after oviposition, when serosal differentiation has just been completed. Ecdysteroids were present in these samples and the major compounds detected were 20-hydroxyecdysone and unidentified highly polar ecdysteroids, but their concentration and ratio varied among samples (Figure 2) (Pennacchio, Vinson & Ostuni, unpubl.). The total ecdysteroid titres of Figure 2a and Figure 2b differed and appeared to reflect the host ecdysteroid titre. The polar ecdysteroids detected (Figure 2b) showed retention times coincident with those registered for polar metabolites produced *in vitro* by older teratocytes. Current investigations indicate that the ecdysteroid profile of young teratocytes, obtained *in vitro*, seems to be dependent on the ecdysteroid profile of the host haemolymph at the time of embryo dissection. This result suggests that teratocytes are able to absorb and metabolize 20-hydroxyecdysone, even before hatching, soon after their differentiation from the serosal cells.

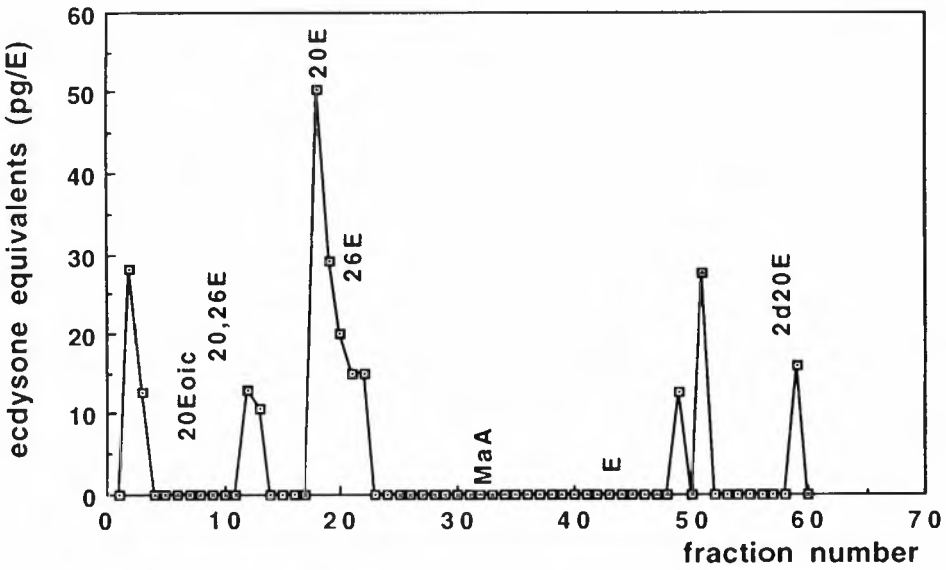

Figure 1. Titre of various ecdysteroids isolated from teratocytes of *Cardiochiles nigriceps,* obtained from parasitized *Heliothis virescens* larvae, 5–7 days after parasitoid oviposition. Methanol extraction of teratocytes was followed by solid phase extraction on a preconditioned C18 bonded silica gel disposable cartridge and ecdysteroids further purified by reversed phase – high performance liquid chromatography (RP-HPLC), both in isocratic conditions and in a gradient mode. The ecdysteroid content of 1 min fractions was assessed by radioimmunoassay (RIA) and expressed as picograms per embryo (pg/E), by considering that each embryo produces about 250 teratocytes (data from Pennacchio, Vinson & Ostuni, unpubl.).
[Ecdysteroid abbreviations : 20 Eoic = 20-hydroxyecdysonoic acid, 20, 26E = 20,26 dihydroxyecdysone, 20 E = 20-hydroxyecdysone, 26E = 26-hydroxyecdysone, MaA = Makisterone A, E = ecdysone, 2d20E = 2-deoxy-20-hydroxyecdysone].

It is also interesting to note that the ecdysteroid titre of young and old teratocytes was in most cases not very different, suggesting that considerable ecdysteroid sequestration and accumulation over time in these cells do not occur.

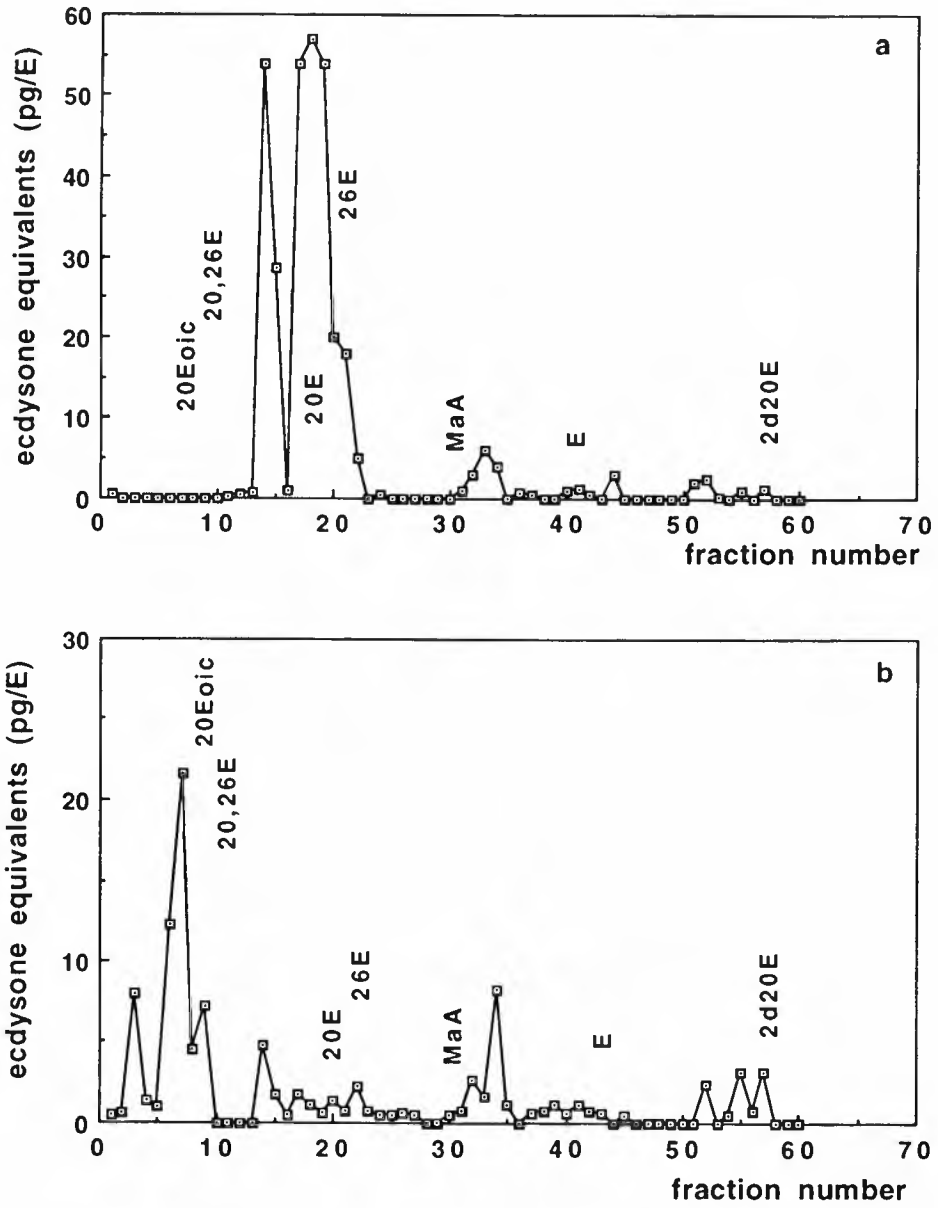

Figure 2. Titre of various ecdysteroids isolated from teratocytes of *Cardiochiles nigriceps,* obtained from 70 embryos dissected from parasitized *Heliothis virescens* larvae, 28 hr after parasitoid oviposition, and hatched in vitro on a semidefined artificial medium; (a) and (b) represent two different samples. The methodology used for ecdysteroid purification and quantification is described in the legend of Figure 1 (data from Pennacchio, Vinson & Ostuni, unpubl.).

THE INTEGRATION OF MATERNAL AND EMBRYONIC HOST REGULATION FACTORS

Although female of *C. nigriceps* parasitizes any (1st–5th) instar of its host *H. virescens*, the mature parasitoid larva emerges only from 5th instar hosts, which do not pupate and support the most demanding period of parasitoid nutrition and growth (Pennacchio et al. 1993). If teratocytes are apparently active soon after serosa differentiation, why is larval moulting not affected in parasitized insects and only pupation is inhibited? This developmental response of the host is probably due in part to calyx fluid and venom, which alter the neuroendocrine balance of last instar host larvae. In fact, Tanaka & Vinson (1991 b) clearly demonstrated that only pupally committed prothoracic glands of *H. virescens* are negatively influenced by adult female secretions of *C. nigriceps*, dramatically reducing their ability to react to PTTH, normally produced by host brain, which is apparently not impaired by parasitism. In this species, teratocytes are very likely responsible for effectively removing from the circulating haemolymph the low amount of 20-hydroxyecdysone produced in parasitized host last instar larvae. The result in parasitized hosts is the disruption both of the commitment peak and of the subsequent slow rise of 20-hydroxyecdysone (Pennacchio et al. in press c). This results in the arrestment of host development and in an increase of its nutritional suitability (Pennacchio et al. 1993, in press a). The ability of teratocytes to absorb and metabolize 20-hydroxyecdysone is observed soon after their differentiation from serosal cells. It is, however, reasonable to speculate that this metabolic activity alone does not effectively reduce the 20-hydroxyecdysone level below the critical threshold required to trigger the larva-larva moult, even because host prothoracic glands do not appear to be negatively affected by parasitism during early larval instars. Such a concept is indirectly corroborated by the fact that teratocyte injections into nonparasitized host last instar larvae show a dose dependent response and that only injections carried out before the slight increase of the ecdysteroid titre that occurs at the pupal commitment are effective in causing pupation inhibition (Pennacchio et al. 1992).

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Avoidance of Encapsulation by *Asobara tabida*, A Larval Parasitoid of *Drosophila* Species

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MONCONDUIT, H. & G. PREVOST 1994. Avoidance of encapsulation by *Asobara tabida*, a larval parasitoid of *Drosophila* species. Norwegian Journal of Agricultural Sciences. Supplement 16. 301-309. ISSN 0802-1600.

The parasitoid *Asobara tabida* which can successfully parasitize *Drosophila melanogaster* larval hosts however fails to develop in *D. simulans*, a sibling species of *D. melanogaster*. Results showed that almost half of the parasitoid eggs are encapsulated in *D. simulans* while the parasitoid usually avoids encapsulation in D. *melanogaster*. Dissections also revealed that *A. tabida* eggs fully attach to the tissue of the *D. melanogaster* hosts while attachment is never completed in *D. simulans*. It is suggested that in *D. melanogaster*, *A. tabida* evades encapsulation by embedment of its egg in the host tissue. Results are discussed regarding to the phylogenic and ecological relatedness of the two *Drosophila* host species.

Key words: *Asobara tabida, Drosophila* hosts, Evasion from encapsulation, Parasitoid.

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INTRODUCTION

Like true parasites, insect parasitoids manifest different levels of specificity to their host (Toft et al. 1991) and it is likely that specificity relates, at least in part, to the mechanism the parasitoids use to escape host defenses. Parasitoids have evolved various means of evading the defense reaction of encapsulation (Salt 1968, Vinson 1977). Some parasitoid species which elicit a depressive effect on either the hemocyte population or the phenoloxydase activity of their host unable the parasitized host to develop a normal reaction of encapsulation (Rizki & Rizki 1984, Prévost et al. 1990, Tanaka & Wago 1990, Strand & Noda 1991, Fleming 1992). Other parasitoids may prevent to be recognized by the host defense system by molecular mimicry (Salt 1968, Schmidt & Schuchmann-Feddersen 1989). Also, attachment of the host hemocytes to the parasitoid's egg may be prevented by substances injected by the parasitoid female during oviposition (Salt 1970, Schmidt & Schuchmann-Feddersen 1989).

How specificity of the host-parasitoid relationships is related to the parasitoid's mechanism to escape host defenses has been poorly explored. This question was investigated using *Asobara tabida*, a Hymenopteran Braconidae which parasites several species of the *Drosophila* genus. *A. tabida* is usually successful in a large proportion of *Drosophila melanogaster* larvae while it is known to be unsuccessful

when parasitizing *Drosophila* simulans (Mollema 1988), a sibling species of *D. melanogaster* (Eisses et al. 1979). *D. melanogaster* and *D. simulans* are cosmopolite living in the same habitat and both are present on the living area of the parasitoid *A. tabida* (Mollema 1988). Because of the phylogenic and ecological relatedness of those *Drosophila* species, it was interesting to determine how the parasitoid escapes host defenses in the susceptible host *D. melanogaster* while being unable to evade encapsulation in the sibling species *D. simulans* , the resistant host.

Study of the kinetics of *A. tabida* encapsulation in the two *Drosophila* species showed that the host *D. simulans* is not more reactive against the parasitoid but that in *D. melanogaster*, *A. tabida* eggs are totally embedded within the host tissue. Parasitoid attachment to the host tissue is supposed to permit the parasitoid to escape host defenses in *D. melanogaster*.

MATERIALS AND METHODS

Drosophila strains, *D. melanogaster* and *D. simulans*, and the parasitoid *A. tabida*, originated from Lyon, France. Insects were reared on regular *Drosophila* diet at 20°C and LC 13:11. Two experiments were carried out: in the first experiment, infested larvae were dissected several hours after oviposition by *A. tabida* females; in the second experiment, infested larvae and control non-infested ones completed their development until the emergence of adult *Drosophila* and parasitoids. Samples of *Drosophila* larvae were obtained by collecting series of 20 eggs each of either *D. melanogaster* or *D. simulans*. After *Drosophila* eggs have hatched 24 hours later, samples for the infested series were exposed for 2 hours to one *A. tabida* female. Control series were kept free from parasitism.

Dissection experiment:
Larvae which had been exposed to the parasite were dissected from 6 to 144 hours post-infestation. Host larvae containing an encapsulated parasite were separated from the ones with a non-encapsulated, developing parasite. Hosts which had mounted an encapsulation reaction were differenciated according to the amount of melanin on the capsule (unmelanised / partially melanised / melanised capsules). For host larvae containing a developing parasite, it was recorded if the parasitoid was found free in the host hemolymph or attached to the host tissue.

Development experiment:
After the insects had completed their development, the numbers of emerging adult *Drosophila* and parasitoids were recorded.

Estimated parameters:
The mean numbers of flies emerging from the non-infested series gave an estimate of *D. melanogaster* and *D. simulans* viabilities in the absence of parasitism. From the parasitized larvae in the infested series, we recorded the number of adult *Drosophila* carrying a capsule (resistant hosts) and the number of adult parasitoids (emerging from susceptible hosts). The difference between the number of flies emerging from the non-infested series and the number of insects (flies + parasitoids) emerging from the infested series gave an estimate of the number of parasitized larvae which died during development. Parameters were estimated as following:

Infestation Rate: IR % = (number of parasitized *Drosophila* / total *Drosophila* larvae) x 100 ; Encapsulation Rate: ER % = (number of *Drosophila* with capsule / number of parasitized *Drosophila*) x 100 ; Successful Parasitism Rate: SPR% = (number of *Drosophila* permitting parasitoid development / number of parasitized *Drosophila*) x 100 ; Mortality Rate (among the parasitized larvae): MR % = (estimated number of parasitized larvae which died during development) / number of parasitized *Drosophila*) x 100 . For the dissection experiment: number of parasitized *Drosophila* = number of larvae with a developing parasite + number of larvae with an encapsuled parasitoid + number of parasitized larvae which died during development ; for the experiment with *Drosophila* and parasitoids completing their development : number of parasitized *Drosophila* = number of adult parasitoids + number of adult *Drosophila* carrying a capsule + number of parasitized *Drosophila* which died during development.

Parameters (IR, ER, SPR, MR %) were estimated for each experimental set of 20 *D. melanogaster* or *D. simulans* larvae. Numbers of experimental sets of either dissected or developed larvae are reported with the results.
Statistical analysis was performed using t-test and ANOVA, after arcsin $\sqrt{\%}$ transformation of percentage datae.

RESULTS

Encapsulation in *D. melanogaster* and *D. simulans*

A. tabida eggs hatched within 120 hours post-oviposition (at 20°C). In the infested series, the mean numbers (± standard deviation) of *Drosophila* larvae alive 120 hours post-infestation (D.m.:18.0 ±1.8 ; D.s.:16.7 ±4.2) were not statistically different ($p > 0.8$) from the mean numbers of flies emerging from the control non-infested series (D.m.:16.8 ±2.7 ; D.s.:17.4 ±3.3), thus indicating that no significant mortality occurred among the parasitized hosts within the 120 hours following parasitization. The rate of encapsulation (ER1) was much higher in *D. simulans* (42.9% ±12.4) than in *D. melanogaster* (9.5 % ±13.1) when the parasitized larvae were dissected 120 hours post-infestation (Table 1). At this time of dissection, capsules surrounded parasitoid eggs only. Non-encapsulated parasitoids normally developing as 1st instar larvae (SPR1) were observed in 57.1 % (±12.4) and 90.5 % (±13.1) of the *D. simulans* and *D. melanogaster* parasitized hosts, respectively.

Results from the dissection experiment were compared to those obtained after the parasitoids had completed their development (Table 1,2). The rate of encapsulation remained unchanged in the *D. simulans* parasitized larvae (ER2 = 42.4 % ±21.9 in the development experiment versus ER1 = 42.9 % ±12.4 in the dissection experiment). Differently, the rate of encapsulation (ER2) in *D. melanogaster* reached 21.6 % (±9.7) at the end of *Drosophila* development and was significantly different from the rate of encapsulation measured in the dissection experiment (ER1 = 9.5 % ±13.1) (Table 2). It thus could be concluded that encapsulation of *A. tabida* larval stages occured in *D. melanogaster* while all encapsulations were resolved by the time the parasitoid eggs hatched in hosts of *D. simulans* species.

In both host species, the rates of successful parasitism decreased significantly when comparing the dissection experiment to the experiment of development. In the

Table 1: Mean values (± standard deviation) of the parameters estimated from the dissection (120 hours post-infestation) and the development of *D. melanogaster* and *D. simulans* larvae parasitized by A. tabida. IR%= Infestation Rate among the 20 *Drosophila* larvae of each experimental set; ER%= Encapsulation Rate, SPR%= Successful Parasitism Rate, MR%= Mortality Rate, among the parasitized larvae; n= number of sets of 20 *Drosophila* larvae; t-test: df= degree of freedom, t value, p= probability.

		D. melanogaster	D. simulans	t test
		n = 6	n= 3	
Dissection	IR1	51.0 (20.8)	51.5 (31.4)	df=7 ; t=0.033;p= 0.99
120 hours	ER1	9.5 (13.1)	42.9 (12.4)	df=7 ; t=3.04; p= 0.019
post infestation	SPR1	90.5 (13.1)	57.1 (12.4)	df=7 ; t =3.04; p=0.019
	MR1	0	0	
		n = 9	n = 9	
After	IR2	72.1 (23.0)	64.2 (23.3)	df=16; t=0.63; p=0.54
Complete	ER2	21.6 (9.7)	42.4 (21.9)	df=16; t=2.53; p=0.02
development	SPR2	51.3 (21.7)	0	df=16; t=7.54; p=0.0001
	MR2	27.1 (18.8)	57.6 (21.9)	df=16; t=3.23; p=0.005

Table 2: Compared values of the parameters estimated from dissection (ER1, SPR1) and the development (ER2, SPR2) of *D. melanogaster* and *D. simulans* larvae parasitized by *A. tabida.* ER%= Encapsulation Rate, SPR%= Successful Parasitism Rate, among the parasitized larvae; t-test : df= degree of freedom, t value, p= probability.

	Compared Values	t test
D. melanogaster	ER1 – ER2	df=10 ; t=2.66 ; p=0.019
	SPR1 – SPR2	df=13 ; t=3.84 ; p= 0.021
D. simulans	ER1 – ER2	df=10 ; t=0.094; p=0.93
	SPR1 – SPR2	df=10 ; t=22.54 ; p= 0.001

hosts of *D. melanogaster* species, the rate of *A. tabida* parasitoids wich successfully completed their development (SPR2) was decreased to 51.3 % (±21.7) comparatively to the 90.5 % (±13.1) parasitoids living 120 hours post-infestation (SPR1). Such decrease was partially explained by the encapsulations occuring around larval parasitoids. However, the decrease in successful parasitism had also to be explained by the death of 27.1 % (±18.8) of the *D. melanogaster* parasitised hosts (MR2) (Table 1, 2). In *D. simulans*, encapsulations were rarely observed later than 120 hours post-infestation, while all the larvae containing a non-encapsulated parasitoid 57.6 % (±21.9) succumbed with their parasite during their development (MR2).

Kinetics of the encapsulation reaction

In order to investigate the factors responsible for the different rates of encapsulation in *D. melanogaster* and *D. simulans* , the kinetics of the encapsulation reaction was recorded in both *Drosophila* species. Three to six samples of 20 larvae each were dissected in each *Drosophila* species for every studied time. Results are reported on Figure 1. In both host species, no encapsulation reaction started earlier than 40 hours post-oviposition by *A. tabida* females. In *D. melanogaster*, a few melanic capsules were observed 48 hours post-infestation. Most capsules were completed and melanised within 72 hours post-infestation. In *D. simulans* where some cellular capsules started to form 40 and 48 hours post infestation, a few capsules only were melanised within 96 hours post-infestation. Capsules were completed and fully melanised within 120 hours post-infestation. Therefore, the higher rate of encapsulation in *D. simulans* could not be explained by a defense reaction occuring more promptly and more efficiently in this host than in *D. melanogaster*.

Parasitoid's attachment to the host tissue

Dissection of the parasitized hosts revealed that *A. tabida* eggs tend to attach to the host tissue (Figure 2). Adhesion which first involved the larval host's fat body (Figure 2a,b) then progressed with the tissue of the host's digestive tube and trachea (Figure

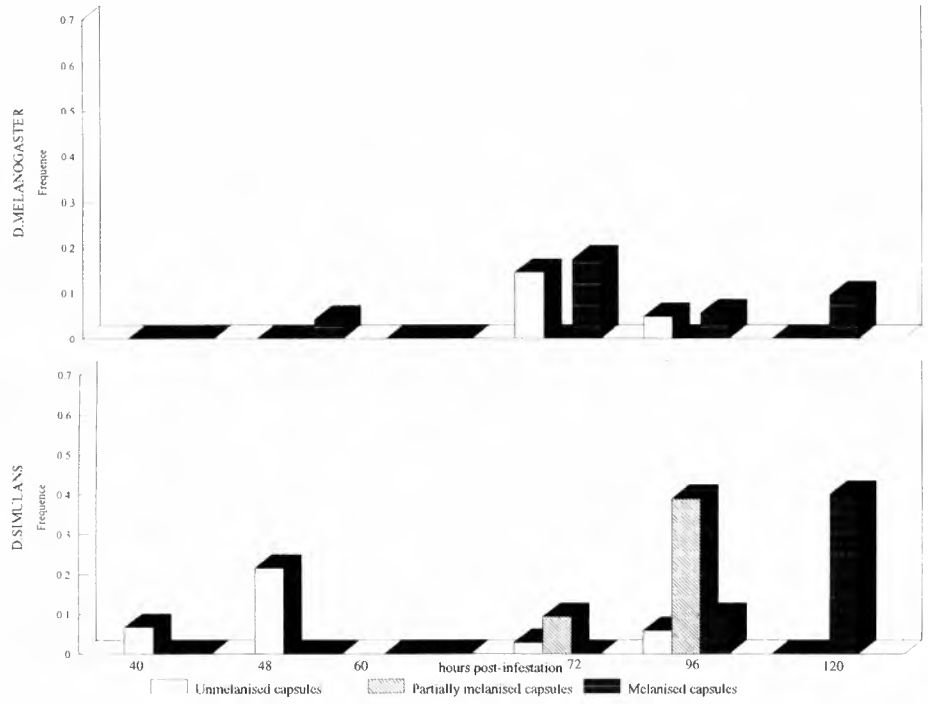

Figure 1: Kinetics of the encapsulation of *A. tabida* eggs in *D. melanogaster* and *D. simulans* larvae. Frequences are proportions among the parasitized hosts of each experimental set; 3 to 6 experimental sets of 20 larvae each were dissected in each *Drosophila* species for every studied time; for *D. melanogaster*, no dissection was made 60 hours post-infestation.

2c). The process of attachment began a few hours post-oviposition by *A. tabida* females (Figure 3). *A. tabida* eggs which were covered on 1/8 of their surface by the host fat body were observed 6 hours post-infestation in larvae of *D. simulans* species. However, adhesion to the host tissue did not progress much in this host such that a few parasitoids (12%) only were totally covered with the host digestive tube and trachea 96 hours post-infestation (Figure 3). In *D. melanogaster*, *A. tabida* eggs did not remain partially attached to the host fat body but adhesion almost immediatly involved tissue of the digestive tube and trachea. This resulted to 68.2% of the parasitoids being totally covered by the host's tissue 72 hours post-infestation, at the time when capsules formed and melanised around the eggs remaining free in the host hemolymph.

Comparing the process of capsule formation (Figure 1) to the one of parasitoid adhesion to the host tissue (Figure 3), we found that the two phenomena were inversely correlated. Few encapsulations occured in *D. melanogaster* where the parasitoid eggs were usually embedded within the host tissue, while the rate of encapsulation was high in *D. simulans* where a few parasitoid eggs were totally attached. This allowed us to consider that parasitoid attachment to the host tissue was one main factor of evading encapsulation. However, 90.5 % and 57.1 % of the *A. tabida* eggs hatched in the hemolymph of the *D. melanogaster* and *D. simulans*

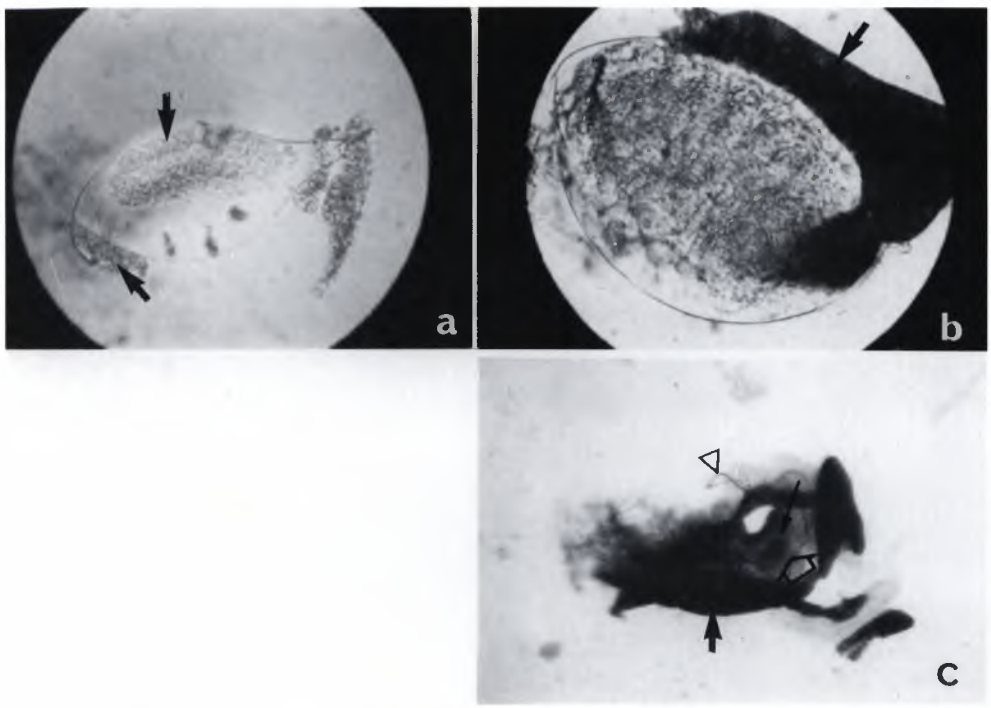

Figure 2: Attachment of *A. tabida* eggs to the tissue of *D. melanogaster* larvae. 2a: attachment to the host fat body 24 hours post-infestation; 2b: attachment to the host fat body 72 hours post-infestation; 2c: *A. tabida* egg embedded within host tissue 120 hours post-infestation, just before hatching.
→ host fat body ; ⇒ host digestive tube ; Δ trachea ; → *A. tabida* egg.

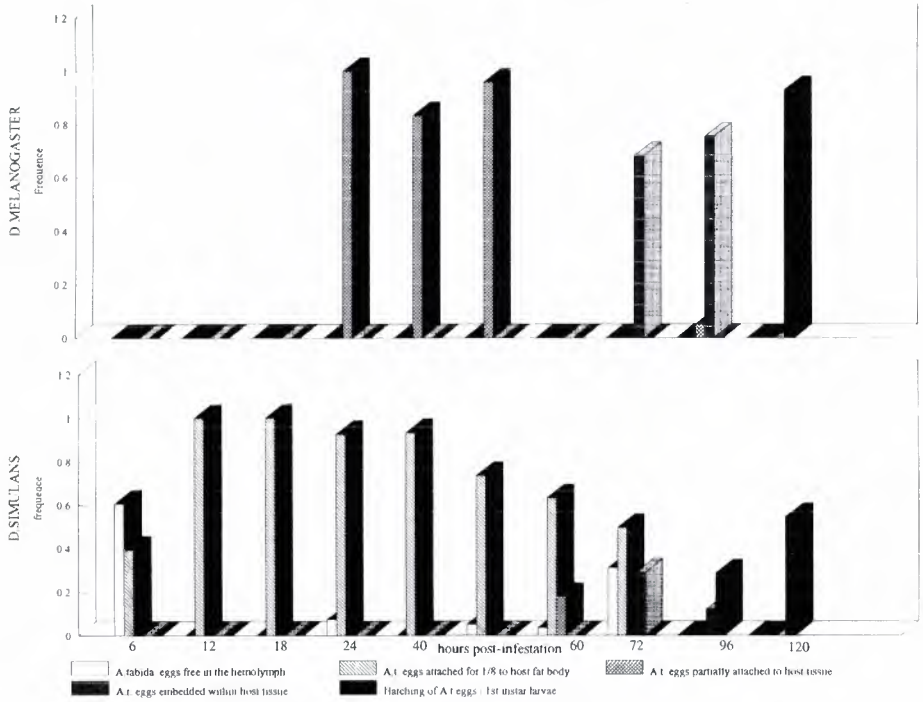

Figure 3: Kinetics of *A. tabida* eggs attachment to the tissue of the hosts *D. melanogaster* and *D. simulans*. Frequences are proportions among the parasitized hosts of each experimental set; 3 to 6 experimental sets of 20 larvae each were dissected in each *Drosophila* species for every studied time; for *D. melanogaster*, no dissection was made 6, 12, 18 and 60 hours post-infestation.

larvae, respectively. Those percentages were superior to the rates of *A. tabida* eggs totally attaching to the host tissue (75.4 % and 28.9 % in *D. melanogaster* and *D. simulans*, respectively), thus suggesting that some parasitoid larvae escaped from capsules.

DISCUSSION

A. tabida eggs were encapsulated for 21.6% in *D. melanogaster* and for 42.4% in *D. simulans*. The non-encapsulated parasitoid eggs appeared to be deeply embedded within the tissue (fat body + digestive tube + trachea) of the *D. melanogaster* larvae. Therefore, we here suggest that *A. tabida* avoid encapsulation by attaching to the host tissue in *D. melanogaster*. Rizki et al. (1990) reported that the parasitoid *Leptopilina boulardi* (Hym.; Cynipidae) which attached on a limited surface of its egg to the digestive tube of *D. melanogaster* is able to escape melanised capsules through the point of adhesion. Some parasitoid species thus may have evolved stategies to avoid encapsulation by attachment to the tissue of their hosts. There is an urgent need to determine if any physical or chemical factor of the parasitoid egg's envelope plays an

active role in such phenomenum. This question is being investigated through electronic microscopy study in the *A. tabida – Drosophila* system.

In addition, we may wonder why embedment of *A. tabida* is effective in *D. melanogaster* while it is not in the sibling species *D. simulans*. Host specific factors thus must influence the attachment of the parasitoid eggs. It was demonstrated that genetic factors govern *Drosophila* susceptibility and resistance to larval parasitoids (Carton et al. 1989, 1992, Carton & Nappi 1991, Vass et al. 1993). The question of *D. melanogaster* and *D. simulans* specific factors influencing the attachment of *A. tabida* eggs is presently explored by studying the defense reaction of hybrids from *D. melanogaster* and *D. simulans* species.

Also, our results demonstrated that encapsulation was not the only cause of death of *A. tabida* in *D. simulans*. Among the *D. simulans* parasitized larvae, 42.4 % encapsulated the parasitoid while the other 57.6 % died with their parasite during development. It is suggested that inadequation of host physiology is an important cause of failure of *A. tabida* development in *D. simulans*, and that the parasitoid *A. tabida* is unable to regulate the physiology of *D. simulans* to its own profit.

D. melanogaster and *D. simulans* are not only phylogenetically closed (Eisses et al. 1979) but exploit the same habitat, too (Mollema 1988). We may suppose that *A. tabida* females usually encounter both *D. melanogaster* and *D. simulans* larvae in the field. The possibility of ecological factors preventing *A. tabida* to lay and to loose eggs in the resistant host *D. simulans* needs to be investigated. Anyhow, physological factors appear to consitute a major limit to the add of the species *D. simulans* to the host spectrum of the parasite *A. tabida* .

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DNA content and regulation of the larval development of the tachinid parasitoid *Pseudoperichaeta nigrolineata*

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Key words: cytospectrophotometry, diapause, DNA content, *Ostrinia nubilalis*, parasitoid, ploidy, *Pseudoperichaeta nigrolineata*, Tachinidae. Norwegian Journal of Agricultural Sciences. Supplement 16. 311-312. ISSN 0802-1600.

Host-parasitoid development synchronization is a key factor in the survival of parasitoids in the nature and also conditions their efficiency as regulators of pest populations. Artificial rearing also needs a good knowledge of the dependence of the parasitoid as regards host. These phenomena have to be well known for biological control.

The tachinid parasitoid *Pseudoperichaeta nigrolineata* Walk. shows two types of larval development in *Ostrinia nubilalis* Hbn., a continuous one in non diapausing host, and a development with a quiescence during host diapause. They are synchronized with the host development, and 4 essential steps are identified, which are subject to control by the physiology of the host. The third one concerns growth resumption after arrest during instar II, at a weight near 1mg (0.7–1.5 mg), linked with the increase in the ecdysteroid level after the middle of the host's instar 5 (continuous development), or at the diapause break of the host (quiescent development). The larval growth of *P. nigrolineata*, like most of the Diptera larvae, is achieved by an increase in the cell size, correlated with a very strong level of endopolyploidy. In order to explain the phenology of events, we investigated the cellular growth, by studying the DNA content of nuclei in salivary glands and Malpighian tubules of second instar larvae. DNA content was determined, after specific staining, by cytospectrophotometric analysis or by measuring microscopically nuclei diameters. The given values of ploidy, expressed by nuclear diameters or as «n», correspond to averages.

During continuous growth, the ploidy level remains stable when the weight stays at about 1mg, in salivary glands (nuclear diameter 15–17 µm corresponding to 250 n) as well as in the Malpighian tubules (nuclear diameter 13–15 µm corresponding to 300 n). It rises after, between a weight of 2 and 4 mg with nuclear diameters from 20 to 55 µm corresponding to 350 to 2000 n in salivary glands, and from 18 to 28 µm corresponding to 450 to 2000 n in the Malpighian tubules. During the great part of the quiescence (40 to 100 days at 4°C), at a weight near 1 mg, the ploidy level is quite stable (300 n in the Malpighian tubules) and rises after, in relation to the weight and /or age increases to reach 1500-2000 n (nuclear diameter of 45 µm in salivary glands) at the end of the quiescence. For both types of development, the ploidy level could reach 1500–2000 n before II–III moulting. The larval

development phases of the parasitoid, monitored by the host physiology via hormonal balance, are characterised by different ploidy levels related to the alternation of stability and nuclear growth phases (Perru *et al.*, in press).

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Temperature affects differentially the suitabilities of the two sibling host species, *Drosophila melanogaster* and *D. simulans,* to their common larval parasitoid, *Leptopilina boulardi* (Hym.: Cynipidae)

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Boulétreau M., F. Fleury, & P. Fouillet 1994. Temperature affects differentially the suitabilities of the two sibling host species, *Drosophila melanogaster* and *D. simulans,* to their common larval parasitoid, *Leptopilina boulardi* (Hym.: Cynipidae). Norwegian Journal of Agricultural Sciences. Supplement 16. 313-319. ISSN 0802-1600.

At 25°C, *Drosophila melanogaster* larvae are better hosts for *Leptopilina boulardi* than *D. simulans* : parasitoids develop better and faster, emerged wasps have a better efficiency, and their offspring a better probability of success. At 22°C, results are quite different : parasitic larvae develop equally well in both host species (though faster in *D. melanogaster*), and wasps emerged from *melanogaster* have no superiority over those emerged from *simulans*. The physiological bases of this differential effect of temperature on both host-parasite systems are discussed, together with the ecological significance of the temperature-dependent shift in the host range of this parasitoid.

Keywords: *Drosophila*, host species, host suitability, parasitism, temperature

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INTRODUCTION

The direct effects of temperature variations on insect parasitoids are the same as for all other insects, including variations in viability, development time, and in some cases contribution to diapause induction (Claret & Carton 1980, Hertlein 1986). More interesting are the indirect consequences that arise from the primary effects on the physiology of the host itself. First, the intensity of the immune reaction elicited in the host by the eggs of parasitoids may depend on temperature (Blumberg & DeBach 1981). Second, many parasitoid species are dependent on the hormones of their host for developmental events such as moulting, diapause induction and termination (Beckage 1985, Rahmadane et al. 1987). In this case, temperature variations primarily affect the host's physiology, which in turn may have secondary consequences on the parasitoid's physiology or on the physiological host-parasitoid relationships that are involved in the so-called host suitability. Since host species can vary in their response to temperature variations, temperature may affect the comparative suitabilities of different host species to a given parasitoid.

Using larvae of *Drosophila melanogaster* and *D. simulans* (Diptera Drosophilidae) as hosts, developed either at 25°C or 22°C, we study here the interaction between host species and temperature on the development success and the adult fitness of the solitary larval endoparasitoid, *Leptopilina boulardi* (Hymenoptera: Cynipidae). Both host species can be used as hosts by *L. boulardi* in sympatric field situations (Carton et al. 1986), and furthermore they are well known for their slightly different physiological responses to temperature variations (Cohet 1980). Our results demonstrate first that the host species has a strong influence on the developmental success of the parasitoids, on their own reproductive capacity and on the fitness of their F1 offspring, and second that switching temperature from 22°C to 25°C can invert the suitabilities of both host species to the parasite.

MATERIAL AND METHODS

All the strains used in these experiments originated from Tunisia.

The overall success of the parasite depends on two traits: the probability of a host being infested, which depends on the physiology and the behaviour of the adult female, and the probability that an adult wasp will emerge from each infested host, which depends on the intensity of the cellular immune reaction and on the suitability of the host larva to the parasite.

Adult parasites were extracted from long mass rearing on *D. melanogaster* at 25°. Females were placed either at 22°, or at 25°, and individually provided with host larvae of either species. Infested hosts were kept at the same temperature up to the emergence of adult wasps. In each of these four sub-lines (*D.m.* 25°, *D.m.* 22°, *D.s.* 25°, *D.s.* 22°), emerging females were individually provided for 24 hours with larvae of either *D. melanogaster*, or *D. simulans* as hosts, and their offspring were allowed to develop at the same temperature as their mothers.

Tests were organized according to Boulétreau & Fouillet (1982): batches of 100 newly emerged host larvae were exposed each to one female wasp (one day old) for 24 hours. They were then put into vials containing a large amount of food. After development, adult flies and wasps were counted, and adult flies were dissected to look for melanotic capsules resulting from immune rejection of the parasite. Control batches of uninfested host larvae made it possible to measure preimaginal viability in both host species. Two parameters were calculated: the Degree of Infestation (D.I.), which measures the proportion of parasitized host larvae, and the Rate of Success of Parasitism (R.S.P.) which expresses the probability of an infested larva giving rise to an adult wasp (see Boulétreau & Fouillet (1982) for calculations). In the Tunisian strains used here, the frequencies of immune rejection of the parasite were zero in *D. melanogaster* larvae, and only 1.5 to 3% in *D. simulans*, and they will not be considered for the calculation of R.S.P..

RESULTS

At both temperatures, the results can be presented as a variance analysis with two controlled factors : the developmental host of the females, the final host of their offspring.

Experiment at 25°C

Results are summarized in Table 1 and Figure 1.

The species of the maternal host has strong effects : female wasps that developed on *D. simulans* infest far fewer host larvae than females that developed on *D. melanogaster*; and their offspring have a much lower developmental success.

Final hosts of both species are equally infested, but parasitic larvae develop much better in *D. melanogaster* than in *D. simulans*. Moreover their development time is shorter (-.61 days for males, -1.53 for females) and the difference is highly significant (P<.01).

Table 1 . Experiment at 25°C. Variance analysis on the Degree of Infestation (DI) and the Rate of Success of Parasitism (RSP) (see text).

	DI			RSP		
source	df	ms	p	df	ms	p
maternal host	1	4.714	1.0E-4	1	0.649	0.004
final host	1	0.149	0.28	1	1.420	1.0E-4
interaction	1	0.591	0.03	1	0.096	0.24
error	40	0.123		30	0.068	

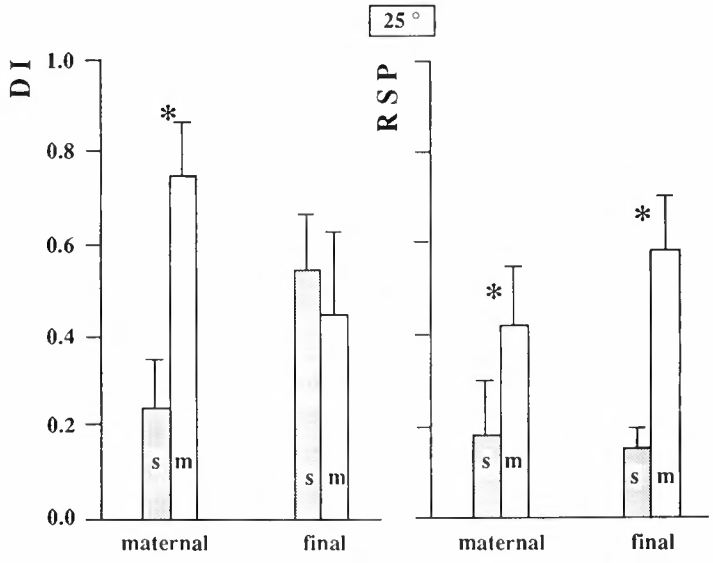

Fig. 1. Study at 25°C. Variation in the Degree of Infestation (DI) and Success of Parasitism (RSP) in response to the species of the development host of the adult female (maternal host), and the species of the final host : *Drosophila melanogaster* (m), or *D. simulans* (s). (*) : significant at the .05 level.

Concerning the degree of infestation, a significant interaction appears between the maternal host species and the final one : females emerged from *D. melanogaster* infest both host species equally, whereas those emerged from *D. simulans* infest *D. simulans* better.

Experiment at 22°C

The results are quite different (see Table 2 and Figure 2).

The maternal host has a strong effect on the features of emerging wasps, but the effect is inverted : wasps emerged from *D. simulans* infest slightly more hosts than wasps emerged from *D. melanogaster*, and their offspring develop equally well.

Concerning the final host, both species are equally infested and parasites develop better in *D. melanogaster* than in *D. simulans*. Development time is longer in *D. simulans* than in *D. melanogaster* (+1.3 days in males, +1.7 in females, P<.01).

Table 2. Experiment at 22°C. Variance analysis on the Degree of Infestation (DI) and the Rate of Success of Parasitism (RSP) (see text).

	DI			RSP		
source	df	ms	p	df	ms	p
maternal host	1	0.782	0.006	1	0.004	0.76
final host	1	0.311	0.08	1	0.216	0.02
interaction	1	0.182	0.17	1	0.003	0.79
error	56	0.095		47	0.038	

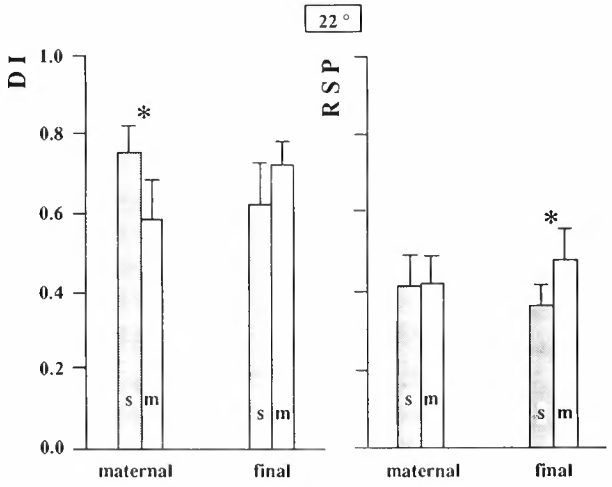

Fig. 2. Study at 22°C. Variation in the Degree of Infestation (DI) and Success of Parasitism (RSP) in response to the species of the development host of the adult female (maternal host), and the species of the final host : *Drosophila melanogaster* (m), or *D. simulans* (s).

DISCUSSION

At 25°, *D. melanogaster* is clearly a better host than *D. simulans* for *L. boulardi* : the parasite larvae develop better, consistently with Rouault (1979). This conclusion is further supported by their faster development, the better efficiency of *melanogaster* developed wasps in infesting hosts, and the better development of their offspring. However at 22°C results are different : the difference in development success of the parasitoid is significant but weak (.38 vs .49), females wasps emerged from *D. simulans* infest more hosts than those emerged from *D. melanogaster*, and their offspring develop equally well.

The influence of host species on the features of emerged wasps has been documented in a number of species, specially *Trichogramma* (Marston & Ertle 1973, Bigler et al. 1987, Hohmann et al. 1988, etc.): variations in the fecundity of wasps correlate with their size, and depend on the host's size. Here, the larvae of *D. melanogaster* and *D. simulans* differ only slightly in size, and it is likely that the differences in the fecundity of the emerged *Leptopilina* females (which are proovogenic) is accounted for by qualitative rather than by quantitative differences between host species.

The influence of the maternal host species on the development of the offspring (next generation) is not a common result. A possible interpretation of this maternal influence could be that according to the host species where they developed, *Leptopilina* females have different abilities in regulating their hosts (in the sense of Vinson & Iwantsch 1980), due to differences in the venom they inject at the time they oviposit.

The higher infestation of *D. simulans* by wasps emerged from this species, which was observed at 25°C, could result from preimaginal learning or conditioning, which is more and more documented in parasitoids (van Alphen & Vet 1986, Vet & Dicke 1992, Turlings et al. 1993).

The differential effects of temperature on the two host-parasite systems : *boulardi-melanogaster* and *boulardi-simulans* might be accounted for by the difference in the relation to temperature of both host species. Even if viability of healthy larvae of both species was about the same at 22 and 25°C, we know that the thermal optimum for development is slightly lower in *simulans* than in *melanogaster* (Cohet et al. 1980). If the ecological requirements of the parasitoid are very similar to those of *melanogaster*, increasing temperature from 22 to 25°C would keep the needed synchronization of host and parasite. On the other hand, if *boulardi* and *D. simulans* have slightly different responses to temperature changes, then increasing from 22 to 25°C might break down the tuning, and lower the success rate of the parasite larva.

Clearly, the host range of the parasitoid *L. boulardi* can vary according to temperature : larvae of *melanogaster* and *simulans* are equally suitable at 22°C, but *melanogaster* is far better at 25°C. Thus we may expect that this change of 3°C can deeply affect the ecological relationships between interacting host and parasite species, and even contribute to their geographic distribution.

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Some factors affecting host suitability for the solitary parasitoid wasp, *Venturia canescens* (Hymenoptera: Ichneumonidae)

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Development time, adult size, and mortality of the solitary ichneumonid parasitoid, *Venturia canescens* varied in response to differences in host species, host instar, host nutritional state and number of parasitoid eggs per host (superparasitism).
These results suggest that quality is not a static property of the host, but varies in response to a number of host-related and environmental factors.

Key words: *Corcyra*, development, host nutrition, parasitoid, *Plodia*, *Venturia*, superparasitism

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Host suitability is defined as the degree to which the host's physiological environment provides the conditions for immature parasitoid development through to the eclosion of fertile adult wasps (Barbosa et al. 1982). Although several workers have reviewed host suitability (Salt 1935, Doutt 1959, Vinson & Iwantsch 1980) few studies have incorporated several important host characteristics that determine the suitability of certain host stages to support parasitoid development. Much of the early work on host suitability was undertaken using idiobiont parasitoids, which attack non-growing or paralysed hosts (Askew & Shaw 1986). In these studies parasitoids attacked hosts of a fixed size, hence the amount of resources available for parasitoid development were constant (Salt 1941, Arthur & Wylie 1959, Sandlan 1979). However, many parasitoids allow their hosts to continue feeding and growing during parasitism These parasitoids, called koinobionts (Askew & Shaw 1986) are susceptible to variations in host quality during the interaction which can arise through changes in host diet (Mackauer 1986) or because of stage-specific differences in immunological response of the host to parasitism (Slansky 1986).

Although koinobionts may attack hosts varying considerably in size at oviposition, adult parasitoid size and development rate depend largely on host growth rate and potential after parasitism (Harvey et al. 1994). Thus, host suitability is often much more difficult to determine with koinobionts. The costs and benefits of the koinobiont habit are discussed by Mackauer (1986) and Gauld (1988).

In this paper, we summarize some of the factors that influence host suitability for the solitary larval endoparasitoid, *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae). *Venturia* is a koinobiont, and attacks a number of species of pyralid moths whose larvae are pests in flour mills and granaries.The successful development of *Venturia* to adult was investigated in relation to the following factors: (1) host species, (2) host instar, (3) host nutritional state, (4) superparasitism.

HOST SPECIES

Virtually all studies investigating the influence of host species on parasitoid development have used idiobiont parasitoids (eg. Salt 1941, Rotheray et al. 1984, Moratorio 1987, Corrigan & Lashomb 1990) *Venturia* can develop, with varying degrees of success, from at least 14 host species (Salt 1975). Under a given set of conditions, these hosts may vary considerably in their growth rate and final mass, so that host species may influence parasitoid development rate, adult size and degree of encapsulation (based on the assumption that larger hosts possess more potent immune responces (Slansky 1986)). *Venturia* was reared from second (L2) through fifth (L5) instars of two host species, *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae) and *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Hosts were reared with excess food throughout the experiments. *Plodia* grows more rapidly than *Corcyra*, but late fifth instar larvae are some 50% smaller than comparable stage *Corcyra* larvae.This was reflected in the size of adult *Venturia*, which were significantly larger from *Corcyra* than from *Plodia* (Figure 1a). However, the egg-to-adult development time of *Venturia* was much greater from all *Corcyra* than *Plodia* instars (Figure 1b). Mortality was also higher in wasps developing in *Corcyra,* except for L2 *Plodia* (Figure 1c). Most notably, the rate of encapsulation from L5 *Corcyra* was over 50%, as opposed to less than 10% from L5 *Plodia*. Therefore, interspecific host variations in host size and growth rate have corresponding effects on fitness-related traits of *Venturia*.

HOST INSTAR

Host size, age or stage at parasitism has been found to influence the size of emerging solitary parasitoids (Sequeira & Mackauer 1992) as well as development time (Arthur & Wylie 1959) and survivorship (Lewis & Vinson 1971). When parasitising L3-L5 instars of *Plodia*, there was little difference in the size, development time and mortality of *Venturia*. However, parasitoids developing from L2 hosts were significantly smaller, suffered higher mortality, and took much longer to develop than wasps from the other three host instars (Harvey et al. 1994). Early host instars are nutritionally unsuitable,hence *Venturia* delays its rate of development to allow the host to reach its maximum size (Harvey et al. 1994). The size of newly eclosed wasps from L2-L5 *Corcyra* was unaffected by instar, but there was a decrease in development time with instar (J.A. Harvey unpubl.).

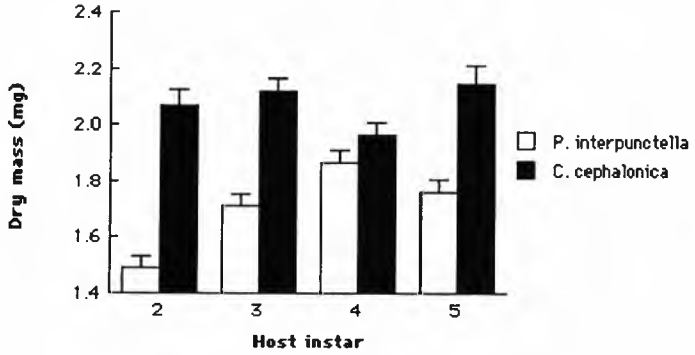

Figure. 1a. The size of newly eclosed adult *Venturia* as defined by dry mass in mg, emerging from hosts parasitized as second (L2) through fifth (L5) larval instars of *Plodia* (open bars) and *Corcyra* (shaded bars). Line bars represent standard error of the mean. Sample sizes are: *Plodia* - L2 = 37, L3 = 44, L4 = 44, L5 = 44; *Corcyra* - L2 = 38, L3 = 35, L4 = 67, L5 = 47.

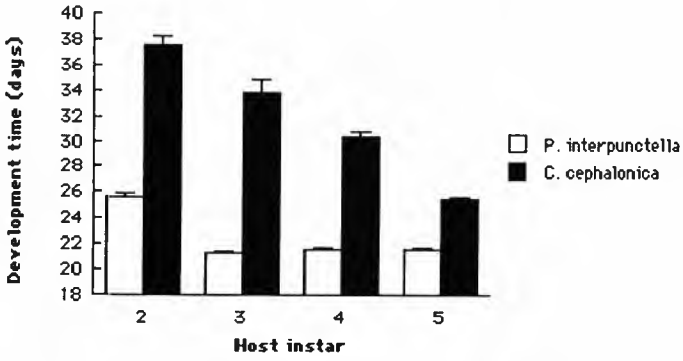

Figure. 1b. The egg-to-adult development time in days of *Venturia* emerging from hosts parasitized as L2-L5 larval *Plodia* (open bars) and *Corcyra* (shaded bars). Line bars represent standard error of the mean. Sample sizes are as in Fig. 1a.

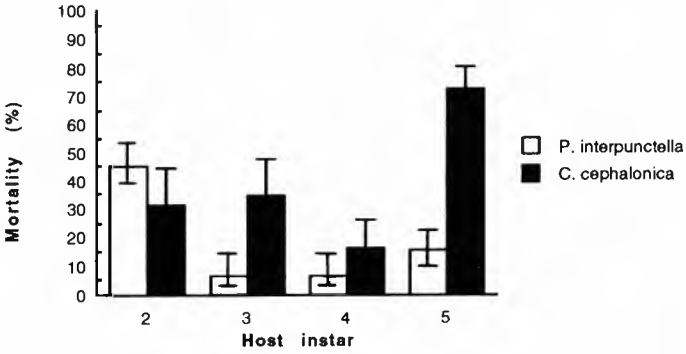

Figure. 1c. Percent mortality of *Venturia* from hosts parasitized as L2-L5 *Plodia* (open bars) and *Corcyra* (shaded bars)(+ 95% confidence intervals). Sample sizes are: *Plodia* - L2 = 67, L3 = 47, L4 = 47, L5 = 53; *Corcyra* - L2 = 58, L3 = 51, L4 = 80, L5 = 172.

HOST NUTRITIONAL STATE

Flanders (1937) suggested that host nutritional state was a possible factor influencing the development of a parasitoid within certain hosts. Some studies have shown that nutritional deficiency may prevent emergence through mortality (Beckage & Riddiford 1983) or result in the emergence of smaller parasitoids with reduced longevities and fecundities (Salt 1941). Although *Venturia* can successfully parasitize all host (*Plodia*) instars, it is unable to complete development unless the host develops past its mid-fifth instar (Harvey et al. unpubl.). *Venturia* develops more rapidly from well fed compared with poorly fed hosts. Wasps from poorly fed L3 hosts took twelve days longer to complete development than those reared from well fed L3 hosts. Once L5 hosts have ceased feeding, and entered the wandering phase, they are nutritionally suitable for parasitoid development, although differences in their size affect parasitoid size, with larger wasps emerging from larger hosts (J.A. Harvey unpubl.). Thus, host nutritional history can markedly affect the suitability of different *Plodia* instars for parasitoid development.

SUPERPARASITISM

Superparasitism, whereby a parasitoid oviposits in a previously parasitized host, has received much attention in recent years (see van Alphen & Visser (1990) for a review). Most studies have concentrated on the fitness consequences of superparasitism for the parasitoid mother, while failing to investigate any potential fitness consequences for the surviving larva (but see Bai & Mackauer (1992)).

Some studies have found that the development time of solitary parasitoids increases in superparasitized hosts (Wylie 1983), adult size is reduced (Eller et al. 1990) and mortality increases with egg number per host (Vinson & Sroda 1978), although there are exceptions in which the surviving parasitoid benefits (Bai & Mackauer 1992). We found that *Venturia* reared from L3 and L5 *Plodia* containing 1, 2 or 4 parasitoid eggs were affected differently by superparasitism. Parasitoid development time increased with egg number per host in both instars, but was more pronounced from L5 hosts. The size of wasps from L3 hosts was unaffected by superparasitism, whereas parasitoids emerging from superparasitized hosts were significantly smaller than those from singly parasitized hosts. Mortality was consistently higher in L5, as opposed to L3 hosts (Harvey et al. 1993) Thus, superparasitism potentially alters host suitability for parasitoid development.

ACKNOWLEDGEMENTS

We thank Ian Harvey, Douglas Reed, Tom Tregenza and Steve Sait for their comments on the manuscript. This work is supported by NERC Studentship GT4/90/TLS/72.

SUMMARY

Once a female wasp has selected and oviposited in a number of hosts, her fitness will depend upon the suitability of these hosts for the development of her progeny. We have shown that there are a number of factors which determine the suitability of hosts for the development of immature *Venturia*. The species of host, and its developmental stage and nutritional state affect the size of the emerging adult and the development rate and mortality of *Venturia*. Furthermore, pre-fifth instars are nutritionally inadequate unless they are allowed to to grow and exceed a certain stage or size which provides the parasitoid with sufficient resources to complete its development. The presence of conspecific wasp eggs or larvae reduces suitability by affecting the fitness of the surviving parasitoid. These effects vary with host instar at parasitism.

To date, few studies have investigated the factors which influence host suitability for koinobiont parasitoids and even in those that have, there are still a number of important factors which await investigation. Such factors include the effect of environmental conditions and pathogenic infection on host suitability. Our results with *Venturia* have illustrated the difficulty in obtaining a single measure of host suitability because it must incorporate a number of potentially diverse properties characteristic of the host, the parasitoid and the environment in which they occur.

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Pseudoparasitism: detection and ecological significance in *Epinotia tedella* (Cl.)(Tortricidae)

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Münster-Swendsen, M. 1994. Pseudoparasitism: detection and ecological significance in *Epinotia tedella* (Cl.)(Tortricidae). Norwegian Journal of Agricultural Sciences. Supplement 16. 329-335. ISSN 0802-1600.

Pseudoparasitism is caused by incomplete parasitoid attacks and may result in host sterilisation. Existing field data on *E. tedella* were analysed to show the presence and extent of pseudoparasitism. Population fertility is a key-factor in the dynamics of *E. tedella*. Fertility, yearly net increment, and average dry weight of apparently unparasitised larvae were shown to be highly correlated with previous larval parasitism. These findings suggest a high degree of pseudoparasitism, which enhances the effect of parasitoids on host regulation and dynamics considerably. A simple model of pseudoparasitism is proposed and fitted to field observations.

Keywords: *Epinotia tedella,* Parasitoids, pseudoparasitism, Tortricidae.

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INTRODUCTION

Jones et al.(1984) introduced the term «pseudoparasitism» (initially called apparent parasitism), for incomplete host attacks by an insect parasitoid. Since then, the physiology and development of pseudoparasitised hosts have been studied mainly for egg parasitoids (Jones 1985, Reed-Larsen & Brown 1990, Brown & Kainoh 1992). Usually parasitation follows the discovery of the host and includes a sequence of injections leading to the deposition of the parasitoid egg into the host body. The injections of biochemical compounds may induce various changes of the physiology and development of the host, e.g. suppression of gonad development. Since the total handling time may be long in the field, disturbance during the process of parasitation may lead to significant levels of pseudoparasitism. Natural disturbances include movement of the host larvae, a potential predator or competitor passing by, or a sudden wind burst. As a consequence, it is quite likely that, in many host-parasitoid interactions, a significant proportion of apparently healthy, unattacked hosts containing no parasitoid offsprings may have been attacked and affected by parasitoids and may be sterilised to some degree.

In such cases, the parasitoid impact on host population dynamics may be considerably higher than that estimated by traditional host dissections or rearing methods.

20 years of field data of the spruce needleminer, *Epinotia tedella* (Cl.) and its parasitoids were analysed to demonstrate the presence and extent of pseudoparasitism. A preliminary model was developed and fitted to field data.

BIOLOGY

The spruce needleminer, *Epinotia tedella* (Cl.), is univoltine. The adults emerge in early June, the larvae mine the needles of Norway spruce (*Picea abies* Karst.) from July till November, and the 5th instar larvae hibernate in a cocoon in the forest floor from December till May when pupation takes place. The 1st instar larvae are attacked by a complex of solitary, univoltine endo-parasitoids in late June and July (Münster-Swendsen 1979). During the feeding and growth phase of the host larva the parasitoid persists as a small, 1st instar larva and develop fully only just prior to host pupation.

Within the primary parasitoid complex, *Apanteles tedellae* (Nix.)(Braconidae) and *Pimplopterus dubius* (Hlmg) (Ichneumonidae) are the predominant species. Both species are monophagous on *E. tedella* in Danish spruce stands. *A. tedellae* seems to be better adapted to *E. tedella* in terms of spatial distribution and impact on host physiology (Münster-Swendsen 1980). *A. tedellae* attacks the host by penetrating the spruce needle with its ovipositor, whereas *P. dubius* forces its ovipositor through the entrance hole of the host mine. The handling time of *P. dubius* is about 1 minute (Münster-Swendsen 1979).

The physiological impact of *A. tedellae* on its host is strong and leads to small and pale 5th instar host larvae with greatly suppressed gonads, whereas the impact by *P. dubius* is much less evident (Führer 1973). These effects were found by dissections of 5th instar of the host larvae even in cases where a parasitoid egg or young larva was encapsulated and killed by host haemocytes.

Populations of *E. tedella* in Denmark oscillates with a period of 6–7 years and fertility (F), measured as density of deposited eggs divided by density of emerged females, is the key-factor (Münster-Swendsen 1991). Reduction in fertility (F_{max} minus F_{obs}) oscillates and is correlated with the degree of parasitism (r^2=0.79, n=13). Together, these two factors explain most of the dynamics of *E. tedella*, (r^2=0.90, n=13). Sublethal infection by a neogregarine (*Protozoa*) parasite account for a minor part of fertility reductions in *E. tedella*, since degree of infection never exceeded 30%, which leaves the majority of the fertility reduction unexplained. Thus, conventional host-parasitoid models explain the period but not the amplitude of host oscillations.

ANALYSIS OF FIELD DATA

The population dynamics of *E. tedella* have been studied during 20 years (1970–1989) in Grib Forest in Denmark (Münster-Swendsen 1985, 1991). In one spruce stand (stand A) all life table parameters were measured during a period of 18 years and fertility was measured in 13 years. Larval densities, parasitism, and dry weight of unparasitised larvae were measured during periods of varying length in 10 separate stands. Alltogether, data exist from 92 stand-years, but due to very low densities in some years and stands only 76 data prints were included in the analyses on population increment and parasitism.

Population net increment (R') is here defined as the change in larval density from one generation of apparently unparasitised larvae ($N_{(x)} - N_{(p)}$) to the succeeding generation of larvae ($N_{(x+1)}$). Thus, $R'=N_{(x+1)}/(N_{(x)} - N_{(p)})$, where $N_{(p)}$ denotes the density of larvae containing a parasitoid in generation (x). R' covers larval mortality during hibernation (fungal infections and predation) and adult

fertility, whereas the mortality due to succesful parasitism (hereafter called euparasitism) is excluded in this expression. Therefore, any effect of pseudoparasitism on mortality or fertility is embedded in R'. Since fertility (F) is the key-factor and responsible for the majority of density changes, R' primarily expresses the variation in F.

The aim of the following analysis is to show an influence of the parasitoids on the fertility of the apparently unparasitised population.

(a) In stand A, where fertility was measured, the reduction in fertility, expressed as a k-value ($\log F_{(max)} - \log F_{(obs)}$) showed a highly significant, positive correlation (r^2=0.79, P<0.0005, n=13) with previous larval euparasitism (Figure 1). The degrees of parasitism due to the two dominant, primary parasitoid species were not correlated (r=0.09, n=19), but both showed almost equally high correlations with reduction in fertility (*A. tedellae*: r^2=0.58, P<0.005, n=13; *P. dubius*: r^2=0.63, P<0.001, n=13).

(b) For all stands, the population net increment, R' (euparasitism subtracted), showed a highly significant, negative correlation (r^2=0.45, P<0.0001, n=76) with previous larval euparasitism (Figure 2). Separate analyses for the two parasitoids showed better correlations for *A. tedellae* (stand A: r^2=0.61, P<0.0005, n=18; all stands: r^2=0.32, P<0.0001, n=76) than for *P. dubius* (stand A: r^2=0.29, P<0.025, n=18; all stands: r^2=0.15, P<0005, n=76).

(c) Samples of larvae spinning down from the canopy prior to hibernation were sorted before dissections and males with gonads of normal size and colour isolated. These males were considered unparasitised and their individual dry weight was measured. *P. dubius* influences the developing testes in the host larva only slightly (light brown instead of dark brown testes), and therefore, the sub-samples to be weighed may have included male larvae that were pseudoparasitised by *P. dubius*. Larvae parasitised by either of the two parasitoid species have a lower dry weight than unparasitised larvae (Führer 1967). Thus, decreased dry weight in apparently unparasitised hosts may indicate the presence of pseudoparasitism.

In Figure 3 the negative correlation between average dry weight of apparently unparasitised male larvae and the degree of *P. dubius* parasitism is shown for stand A during 14 years (r^2=0.53, P<0.005, n=14). A similar relation was found when all stands and years, where dry weight had been measured, were included (r^2=0.29, P<0.0005, n=56).

A HYPOTHETICAL MODEL

A deductive model was based on (1) the probability (q) of an attack being interrupted, leading to pseudoparasitism, and (2) the attacks resulting from independent, random search and random superparasitism, the latter being justified through earlier studies (Münster-Swendsen 1979). For any frequency of hosts attacked the corresponding frequencies of multiple attacks were calculated by use of expressions of the Poisson-series. Next, the fraction attacked once was multiplied by the probability of interruption (q) to give those pseudoparasitised, and the fraction attacked twice was multiplied with q^2, the fraction attacked three times multiplied with q^3, etc.. The

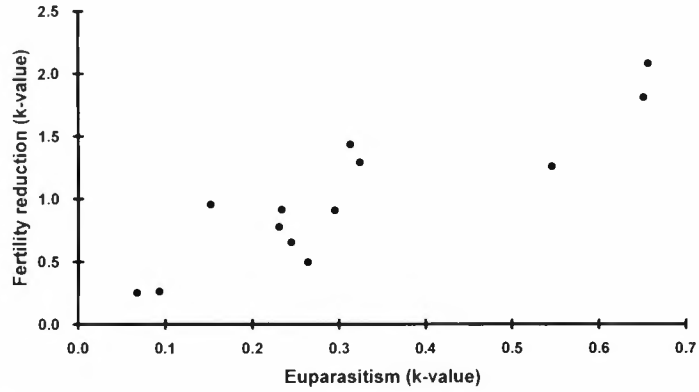

Figure 1. Fertility reduction in *E. tedella* related to previous, primary euparasitism in larvae of the same generation (r^2=0.79). Observations from 13 years in one stand are presented.

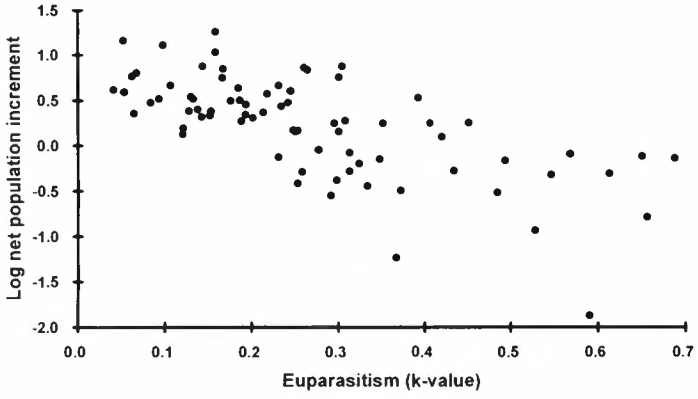

Figure 2. Log observed net population increment in *E. tedella,* euparasitism subtracted (log(N(x+1)/(N(x) – N(p))) related to previous, primary euparasitism (r^2=0.45). Observations from 76 stand-years covering 10 different spruce stands are presented.

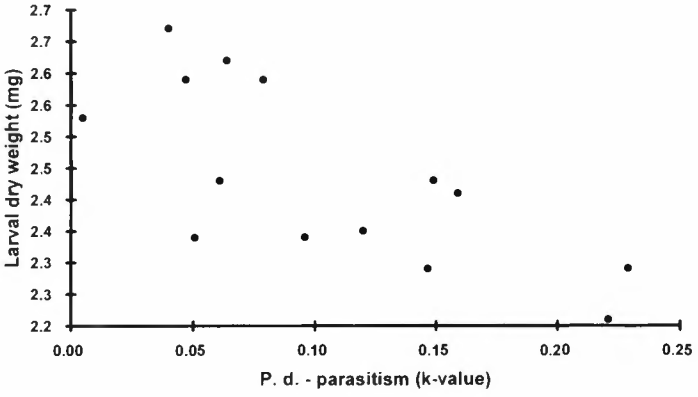

Figure 3. Dry weight of apparently unparasitised male larvae in *E. tedella* related to degree of euparasitism due to P. dubius (r^2=0.53). Observation from 14 years in one spruce stand are presented.

pseudoparasitised larvae of each Poisson-series adds up to the total frequency of pseudoparasitised larvae and this total subtracted from the frequency of attacked host provides the frequency of succesfully parasitised (euparasitised) hosts.

Such calculations was carried out under the assumptions of various probabilities (q) of interruption. Figure 4 shows the relationship between eu- and pseudoparasitism frequencies and various frequencies of hosts attacked for q=0.75.

As the hosts that are pseudoparasitised are assumed to give rise to sterile adults the fraction of sterile hosts can be estimated. Thus, the observed reduction in population (or average) fertility was compared with the observed level of euparasitism and the above estimates of sterile hosts. As shown in Figure 5, the observations of larval euparasitism and reduction of fertility within stand A was compared with the expected reduction of fertility based on observed euparasitism and the above estimates of sterility for q=0.78. Thus, if approximately 3 out of 4 attacks are interrupted before the parasitoid egg is deposited and this ultimately leads to sterile adults, then pseudoparasitism and the above model explains a large proportion of the variation in population fertility.

DISCUSSION

Euparasitism is a regulatory mortality factor of *E. tedella* (Münster-Swendsen 1985). However, population fertility shows much greater variation than do parasitism, and yet, shows a similar oscillation pattern (Münster-Swendsen 1991) and is highly correlated with observed euparasitism. The presented correlations between euparasitism and observed reduction in fertility and population net increment (R') provide strong indications of the presence of pseudoparasitism and an impact of both primary parasitoid species. The finding of a negative correlation between dry weight of apparently unparasitised male larvae and degree of parasitism due to *P. dubius* further supports the demonstration of pseudoparasitism in *E. tedella.* Moreover, the fit

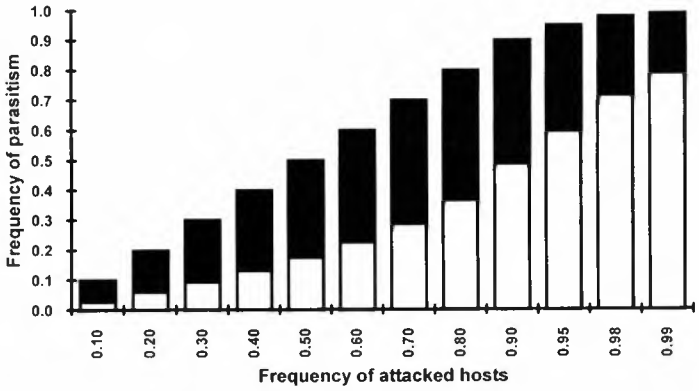

Figure 4. Frequencies of eu- and pseudoparasitism as a function of the frequency of hosts attacked by parasitoids predicted by a hypothetical model based on random, Poisson distributed attacks and a probability of interruption of an attack of q=0.75. White sections of bars denote euparasitism and black sections pseudoparasitism. Corresponding white and black sections add up to total frequency of attacked hosts.

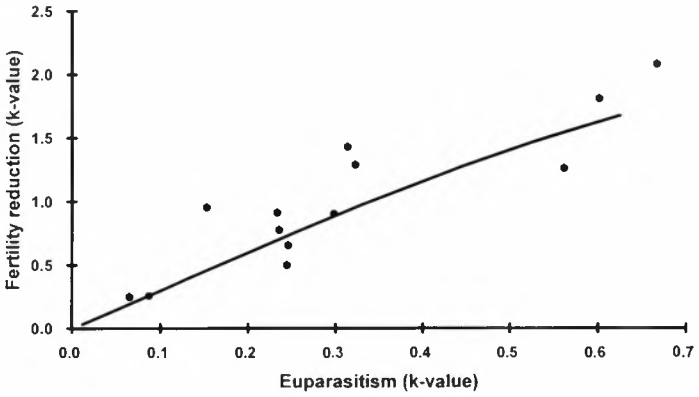

Figure 5. Observed reduction in fertility and primary euparasitism in *E. tedella* (filled circles) and reduction in fertility due to pseudoparasitism as predicted by a model based on random, Poisson distributed attacks and a probability of interruption of an attack of q=0.78 (solid line).

of a theoretical model to field observations demonstrates the effect of pseudoparasitism on host fertility.

In ongoing experiments host larvae and adults are carefully dissected to directly show the presence and frequency of pseudoparasitism caused by the two parasitoid species.

The outcome of the present model (Figure 4) shows that at a level of euparasitism of 30% another 40% may be seriously affected by pseudoparasitism, i.e. about 4 out of 7 emerging adult *E. tedella* may be sterile. Further, when an euparasitism rate of 80% is found (the highest ever recorded in *E. tedella*) only 5% of the adults may be able to reproduce resulting in a drastic reduction in host density. If the estimate of pseudoparasitism is correct, then parasitism completely dominates the dynamics of *E. tedella* through the mortality of host larvae plus a sterilisation found in the adults. Therefore, both the period and the amplitude of density oscillations observed in *E. tedella* may be explained solely by parasitism when it covers pseudoparasitism as well as euparasitism.

Unpublished simulation models of *E. tedella* show that the average density of the host is lowered considerably when pseudoparasitism is included. Thus, occurrence of pseudoparasitism may lead to improved regulation similar to that found by a decreased host fertility in a host-parasitoid model (Münster-Swendsen 1985). In this context, a very high searching efficiency combined with all the attacks leading to succesful euparasitism would result in a less stable host-parasitoid interaction compared with the situation where some attacks lead to pseudoparasitism and no parasitoid offspring.

The host searching efficiency in parasitoids is normally estimated by comparing the number of adult parasitoids with the resulting level of euparasitism. Hence, in cases where pseudoparasitism occurs the real searching efficiency and the importance of parasioids may be considerably higher than usually estimated.

The general abundance of pseudoparasitism in host-parasitoid interactions remains unknown, and ought to be studied extensively. This may greatly improve our understanding of host-parasitoid interactions and our perception of the importance of parasitoids.

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Spatial host use by *Ageniaspis fuscicollis* of patchily distributed apple ermine moths *Yponomeuta malinellus*.

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Kuhlmann, U. 1994. Spatial host use by Ageniaspis fuscicollis of patchily distributed apple ermine moths Yponomeuta malinellus. Norwegian Journal of Agricultural Sciences. Supplement 16. 337-345. ISSN 0802-1600.

Parasitism and spatial host use by *Ageniaspis fuscicollis* (Dalm.) (Hymenoptera: Encyrtidae) of *Yponomeuta malinellus* Zeller (Lepidoptera: Yponomeutidae) was studied. The current study observed low rates of parasitism in a *Y. malinellus* population at low density level. Host patches were randomly distributed within and between apple trees. The distribution of the egg-larval parasitoid *A. fuscicollis* per host patch was contagious. *Ageniaspis fuscicollis* parasitized significantly more hosts per patch in the southern side of the tree although the host was evenly distributed. In contrast, no significant differences of the percent parasitism by *A. fuscicollis* were observed in the two halves of the tree crown. The percent parasitism by *A. fuscicollis* on the level of host patches indicated an inverse density dependent relationship.

Keywords: *Ageniaspis fuscicollis,* Encyrtidae, host-parasitoid interactions, parasitism, parasitoids, patches, spatial distribution, *Yponomeuta malinellus,* Yponomeutidae.

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INTRODUCTION

The apple ermine moth (AEM), *Yponomeuta malinellus* Zeller, was considered one of the most common and destructive pests of apples in the temperate zones of the Palaeartic region (Balachowsky 1966) but is of less importance today under current pest management practices (Affolter & Carl 1986). The univoltine *Y. malinellus* feeds in characteristic tents monophagous on apple leaf clusters. The AEM was accidentally introduced into North America and was found in 1985 in apple orchards in British Columbia and Washington State, when it became a serious pest of apple trees (Parker & Schmidt 1985). *Yponomeuta malinellus* is attacked by a large number of natural enemies in central Europe (Affolter & Carl 1986). *Ageniaspis fuscicollis* is the only known parasitoid of the complex that attacks the host in the egg stage. Females of the AEM lay their eggs in batches onto branches of apple trees. Consequently, for a foraging female of *A. fuscicollis* egg batches of AEM represent host patches which might be unevenly distributed within and between apple trees.

Ageniaspis fuscicollis is oligophagous and probably restricted to the genus

Yponomeuta. It is an univoltine egg-larval parasitoid and therefore synchronised with the development of its host. The life time of the adult parasitoid is on average one week and ranged from 2 to 19 days (Junnikkala 1960). Oviposition starts immediately after the emergence of adults and continues for about two weeks (Blackman 1965). The parasitoid female lays single eggs into host eggs. Junnikkala (1960) reported that the number of eggs per female is small but according to Sitenko (1962) fed females lay 61–224 eggs and unfed females lay a maximum of 9 eggs. The eggs hibernate inside the diapausing first instar host larvae and remain dormant until the host has reached the third larval instar. Then the parasitoid egg develops polyembryony that produces up to 150 larvae which hatch from a single egg (Nenon 1974). The parasitoids developing in one embryo chain are usually of the same sex (Junnikkala 1960). This polyembryony is especially typical of some hymenopterous parasitoids, but in chalcidoids it is restricted to the family Encyrtidae (Clausen 1940). The hosts die as fifth instar larvae while the parasitoid larvae form cocoons and pupate inside the empty larvae. The body of the host is swollen and mummified. The pupal period lasts for some three weeks (Blackman 1965). All adults emerge from the mummy at the same time (Balachowsky 1966).

During a survey of natural enemies of Y. malinellus in Europe, the rate of parasitism and the spatial host use by *A. fucicollis* were studied.

MATERIAL AND METHODS

Samples of *Y. malinellus* were taken regularly from an apple orchard located near Giessen in Germany. The orchard contained 3-4 metre high unpruned trees and had a sod-annual weed ground cover.

Eggs of individual patches were counted with a stereo microscope mounted on a tripod. This method permitted to obtain accurate egg counts from each batch without destroying the egg clusters of *Y. malinellus* in the field.

A sampling method was designed to obtain parasitism data at the collection site. The sample unit most suitable in the study of populations of *Y. malinellus* and related mortality factors on apple trees is the leaf cluster. This unit of foliage is relatively stable for apple trees and on it are found all larval stages and the pupae of AEM. Ten trees selected at random were sampled. Each tree crown was divided horizontally into halves. Each half was divided into four equal quadrants according to the four cardinal points of the compass. This provided eight sampling sections within the tree crown. From each section 30 leaf clusters were collected and examined for mortality by *A. fuscicollis* and the data was subjected to a statistical analysis. Estimates of densities and mortality by the parasitoid were therefore obtained concurrently by direct sampling. For AEM, five age intervals were assessed, namely, second instar larvae until pupae. During each interval, sampling was carried out when the number in the stage was relatively stable. All the collected larvae and pupae were reared for parasitoids separated by patch, under outdoor conditions at the Institute at Delémont, Switzerland. The rate of parasitism of *A. fuscicollis* is based on mummified mature host larvae which are easily discernible. For this analysis of the spatial host use of *A. fuscicollis* all sampling intervals were summarized.

RESULTS

The host density was on average 0.66 ±0.04 SE patches per 30 leaf clusters and ranged from 0 to 4 patches (n=360). The egg numbers per host patch varied widely between 3 and 79 eggs, with a mean of 38.8 (±0.8 SE, n=405).

Additionaly, an average of 20.4 ±0.5 SE leaf clusters (n=80) per metre of branch was counted which means that an occurrence of 0.88 host patches per 2 m branch was observed.

A Chi-squared test was used to clarify whether the host patch frequencies per 30 leaf clusters follows a poisson-distribution in the field. The result of the test shows that host patches are randomly distributed (Chi-square=1.42, DF=3, P=0.299).

The Wilcoxon two sample test showed no significant differences of the number of hosts per patch between the two halves of the tree crown, top and bottom (z=-0.498, P=0.168); also no significant differences were found between the four directional quadrants (Kruskal-Wallis Test, Chi-square=0.752, DF=3, P=0.861) and, no significant differences between the northern and southern side of the tree (z=-0.397, P=0.691).

In addition, an ANOVA showed no significant differences of the number of hosts per patch between and within trees in the field (F-ratio=1.347, DF=79, P=0.229).

Frequencies of *Ageniaspis fuscicollis* per host patch were compared to the Poisson series. Table 1 illustrates that the distribution is contagious (Chi-square=717, DF=5, P<0.001).

Parasitism rates in the two halves of the tree crown and parasitism by *A. fuscicollis* in each directional quadrant are given in Table 2.

There was no significant difference of the percent parasitism by the parasitoid per patch between the two halves of the tree crown, top and bottom (Wilcoxon two sample test, z=-1.363, P=0.173).

However, significant differences of percent parasitism per patch were found between the four directional quadrants (Kruskal-Wallis Test, Chi-square=10.628, DF=3, P=0.014). All differences of the number of parasitized hosts between the particular directional quadrants and level of significance are summarized in Table 3, using the Chi-square test. Highly significant differences of the number of parasitized

Table 1. Distribution of *Ageniaspis fuscicollis* within host patches

Distribution	Frequency of host patches containing the following numbers of *Ageniaspis fuscicollis*							Chi-square
	0	1	2	3	4	5	6-15	
Observed	141	14	7	5	9	8	27	
Expected from a Poisson series	41	67	55	30	12	4	2	717 (P<0.001)

hosts were found between the northern and southern side of the tree (Chi-square=51.2, DF=1, P<0.001). The parasitoid attacked significantly more host in the southern quadrants of the tree.

Percent parasitism from patch to patch by *Ageniaspis fuscicollis* compared to the number of hosts per patch showed an inverse density dependent relationship (Figure 1). The correlation between parasitism and host density was statistically significant (r=0.5411, DF=67, P<0.001).

Table 2. Rates of parasitism by *Ageniaspis fuscicollis* in the two halves of the tree crown, top and bottom, and total parasitism in each directional quadrant in the tree crown.

Parasitism *Apeniaspis fuscicollis*	North/East	South/East	South/West	North/West
Top	5.6 % n=34	10.8 % n=72	8.6 % n=57	0.3 % n=2
Bottom	5.3 % n=47	9.5 % n=51	8.3 % n=77	7.3 % n=27
Total	5.4 % n=81	10.2 % n=123	8.4 % n=134	2.9 % n=29

Table 3. Differences between directional quadrants for parasitism by *Ageniaspis fuscicollis* using the chi-square test and levels of significance (DF=1).

Four directional quadrants	North/East	South/East	South/West	North/West
North/East				
South/East	Chi^2=22.4 P<0.001			
South/West	Chi^2=11.1 P<0.001	Chi^2=2.6 P=0.10		
North/West	Chi^2=11.0 P<0.001	Chi^2=51.3 P<0.001	Chi^2=36.5 P<0.001	

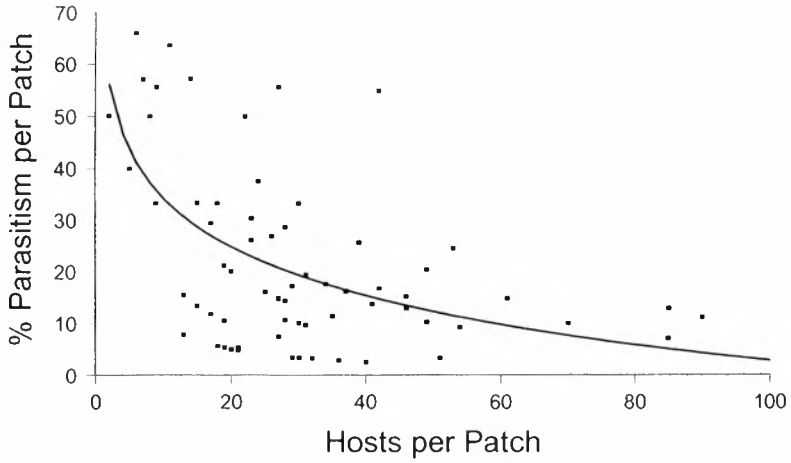

Figure 1. Relationship between percent parasitism and host density per patch for parasitoid *Ageniaspis fuscicollis* and its host *Yponomeuta malinellus,* showing inverse density dependence from patch to patch. The 69 points represent the patches. This curve is described by the equation Y=a-b logx (a=65.88, b=-13.71).

DISCUSSION

The host density at the collection site was less than one patch per 2 m branch. Chemical control is necessary when 3 or more egg-masses/ 2 m branch sampled from one tree occur (Nenon & de Meirleire 1972). The current study observed a *Y. malinellus* population on a low density level. Low rates of parasitism by *A. fuscicollis* were determined. Junnikkala (1960) reported that the numbers of this encyrtid increase rapidly during a mass occurrence of *Y. malinellus*. *Ageniaspis fuscicollis* was the most effective parasitoid in Lithuania when the *Y. malinellus* populations were high (Zayanchkauskas et al. 1979), it reduced pest abundance by 42%-70% in Georgia (Aleksidze & Abashidze 1983), by 70%-75% in Azerbaijan (Mamedov & Makhmudova-Kurbanova 1982) and by 28%-46% in France (Nenon & de Meirleire 1972).

According to Sitenko (1962) *Y. malinellus* was evenly distributed on pruned and unpruned trees. This present study confirmed this result for unpruned tress. No significant differences of the number of hosts per patch between the tree crown halves, the four directional quadrants and within and between trees were found. Parker & Schmidt (1985) reported that the AEM prefers to oviposit in the crowns of mature apple trees above 2-3 m, usually on new shoots or in axils near a bud. However, on smaller trees eggs are deposited in lower parts of the tree as well. Small trees might have more constant microclimatic conditions compared to large trees where the climatic conditions might differ between the upper and lower parts of the tree crown.

The egg-larval parasitoid *A. fuscicollis* parasitized significantly more hosts per patch in the southern directional quadrants although the host was evenly distributed. Thus it appears that adults of *A. fuscicollis* are fairly selective in their oviposition

behaviour. Also Sitenko (1962) found that parasitized larvae of *Yponomeuta* were not evenly distributed on the trees, but were found in greater numbers on the southern and western sides. With reference to the oviposition behaviour, Nenon and de Meirleire (1972) reported that eggs were laid «always in fine weather» during the warmest part of the day. It appears that the parasitoid preferred warm temperatures for oviposition. In contrast to this study, Sitenko (1962) reported greater numbers of parasitized host larvae in the upper and middle parts of the tree. The present study showed no significant differences of the percent parasitism by *A. fuscicollis* in the upper and lower half of the tree crown which might be due to the small size of trees at the collection site.

Density dependence, inverse density dependence, and density independence are relationships between parasitism and host density in field populations which were all commonly observed (Walde & Murdoch 1988). Lessells (1985) found that 66% of 43 cases examined showed some relationship between parasitism and host density. Inverse density dependence is found about one third of the time (Walde & Murdoch 1988). Interestingly most examples of inverse density dependence come from egg parasitoids (Hassell 1982). In most cases the mechanisms are unknown, but imperfect information on patch quality (Lessels 1985), egg or time limitation could be constraints on parasitoid foraging which may produce inversely density dependent parasitism (Walde & Murdoch 1988, Lessels 1985, Morrison & Strong 1981, Hassell et al. 1985).

Two well known behavioural models for the distribution of parasitoids between patches were developed. Comins & Hassell (1979) assume that parasitoids are able to detect, and only enter, those patches in which they will achieve the maximum rate of oviposition; Charnov (1976) assumes that patches are entered at random, and left when the success rate has dropped to some critical value.

Lessells (1985) suggested, that parasitoids are foraging according to the behavioural model by Charnov (1976) and will spend longer searching, or lay more eggs in higher density patches. If eggs or time are limited, there must be some critical density. Below this critical density parasitism will be density dependent. Above the critical density, eggs and time limitation may have the same effect under certain assumptions. If the parasitoid is egg limited then the same number of hosts will be parasitized in all patches and parasitism above the critical density will be inversly density dependent (Hassell 1982, Lessels 1985). If handling time or rejection time are considerable, then a smaller fraction of the time in patch will be devoted to searching in the higher density patches, and parasitism above the critical density will be inversely density dependent (Hassell 1982, Hassell et al. 1985, Lessels 1985, Morrison & Strong 1981).

From literature records three arguments for time limitation of the *A. fuscicollis* females were found. Junnikkala (1960) reported that the life time and oviposition period is rather short and Nenon & de Meirleire (1972) described that the oviposition took place only in the best weather conditions during the warmest part of the day. The synchronization of the flight period of the parasitoid with the egg stage of *Y. malinellus* could be another argument for time limitation. Sitenko (1962) reported that the peak of the parasitoid oviposition did not coincide with the peak in the host, and many eggs were laid by the host after the flight period of *A. fuscicollis* was over.

Contradictory records were extracted from the literature for arguments of egg limitation by *A. fuscicollis*. Fed females laid 61–224 eggs and unfed females laid a

maximum of 9 eggs (Sitenko 1962). Junnikkala (1960) reported that the number of eggs per female is small. *Ageniaspis fuscicollis* spread their eggs among several host patches; females lay only a small fraction of their eggs in any one patch, even if the patch contains many hosts. According to Hassell & May (1974) and Charnov (1976) one would expect parasitoids to forage longer and parasitize proportionately more hosts in patches of high host density. However, this pattern in the exploitation of host patches might not always lead to optimal foraging decisions. Spreading eggs among host patches as an adaptation to risks that vary unpredictably among patches in space, e.g. the risk of predation, certainly could result in submaximal oviposition rates (Cronin & Strong 1993). These submaximal oviposition rates by parasitoids may be a strategy that improves lifetime fitness by reducing the risk of mortality to offspring developing within a patch (Kuno 1981, Levin et al. 1984). With respect to the fact that egg batch mortality of *Y. malinellus* caused by predators reached 63.5% in Switzerland (Kuhlmann unpublished data) and in addition to the polyembryonic development of *A. fuscicollis* it seems to be reasonable for females to spread their eggs among several to many patches.

Further studies are necessary to investigate the potential and real fecundity, the oviposition behaviour including handling time and rejection time to clarify the mechanisms of the inverse density dependence by *A. fuscicollis*.

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Visual cues in food and host-foraging by hymenopterous parasitoids

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The response of food deprived parasitoids to olfactory and visual flower stimuli was tested. It was demonstrated that parasitoids can use both flower odors and colors during food foraging. The response of parasitoids to food-indicating stimuli depended on the hunger state of the individual.
It could be demonstrated that learning of olfactory and visual cues enables parasitoids to concentrate their search on plant structures that are most profitable in terms of host encounters. Parasitoids conditioned to a combination of visual and olfactory stimuli displayed a stronger preference than individuals conditioned to either sensory component alone. In additional experiments, it was shown that parasitoids can also learn to distinguish host sites on the basis of their shape or pattern.

Keywords: food foraging, host foraging, olfaction, vision

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INTRODUCTION

This report gives an overview of research on several aspects of visual orientation in hymenopterous parasitoids. The work was conducted within a collaboration between Dr. L.E.M. Vet and Prof. J.C. van Lenteren at the Wageningen Agricultural University's Department of Entomology and Dr. W.J. Lewis at the United States Department of Agriculture, Tifton, USA. In the United States, sensory orientation in *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a solitary larval parasitoid of the cotton bollworm (*Helicoverpa* spp.), was studied. The research in Wageningen concerned *Cotesia rubecula* (Hymenoptera: Braconidae), a solitary parasitoid of the small cabbage white (*Pieris rapae*).

Parasitoids have to satisfy various physiological needs (e.g. finding mates, hosts, food and shelter). Each of these drives may be reflected in aspects of their foraging behaviour. Because of the (potential) use of parasitoids as biological control agents, research on parasitoid foraging has largely concentrated on the question of how parasitoids locate their hosts. Food foraging, however, can be just as essential to the fitness of parasitoids and consequently to their effectiveness as biological control agents. This paper, therefore, will deal with sensory orientation during both foraging processes.

FOOD FORAGING

The importance of adult feeding as a fitness parameter has been described for many parasitoid species (Zoebelein 1955, Leius 1967, Jervis & Kidd 1986). Most parasitoid adults require food as an energy source, especially for flight (Elton 1966), while many synovigenic parasitoids require food for the production and maturation of eggs (Bartlett 1964, Lum 1977). On the basis of food foraging, we can distinguish two groups of parasitoids. One group consists of parasitoids that either feed directly on their hosts, on host products (honeydew), or on host associated substrates. For these parasitoids, host- and food foraging are a single process. A second group of parasitoids requires food sources that are not associated with host sites. Since the process of food foraging for these parasitoids is dissociated from host foraging, both should be considered as independent foraging processes. *Cotesia rubecula*, which parasitises the folivorous *Pieris* spp. but feeds on nectar, is an example of the latter category.

In the field, nectar-feeding parasitoids have various sugar sources available. Besides floral nectar, they can use honeydew and extra-floral nectar for feeding (Leius 1960). In numerous field studies, parasitoids have been recorded on nectar secreting structures, while laboratory feeding studies have confirmed that nectar from these various sources can increase parasitoid fitness (Jervis et al. 1993). Despite the fact that it is generally appreciated that finding food is essential to most parasitoids, the question of how parasitoids locate their food sources has not previously been elucidated.

In a preliminary study, it was investigated to what extent the fitness of *C. rubecula* is determined by sugar feeding. It could be demonstrated that provision of sugarwater both increased the parasitoid's overall foraging activity (Wäckers 1994) and prolonged the life span of males and females by a factor 9 and 14 respectively (Wäckers & Swaans 1993). This finding confirms the importance of food foraging to the fitness of parasitoids and underlines that the availability of food sources can be a crucial element in biological control.

Both olfactory and visual flower cues were studied in choice experiments. Y-tube olfactometer experiments were used to address olfactory preferences, while the innate response to visual stimuli was examined by studying free-ranging parasitoids in a flight chamber.

To study the role of olfactory and visual stimuli in food foraging, inexperienced, food-deprived parasitoids were tested in choice experiments. Since parasitoids were reared from folivorous hosts, and kept without food after eclosion, any exposure to flower odours and yellow colours could be precluded. This made it possible to determine truly innate sensory preferences with reference to these food source stimuli.

Innate olfactory responses to flower odours (floral nectar) and odours from aphid infested leaves (honeydew) were tested in a y-tube olfactometer. Parasitoids were attracted to flower odours when given a choice between flowers and the odour of undamaged leaves of the plant. This innate response could enable inexperienced parasitoids to locate floral nectar. It was demonstrated that the flower odour preference of *C. rubecula* is not restricted to Cruciferae, the plant family on which these parasitoids find their hosts (Wäckers & Swaans 1993).

Surprisingly, food-deprived parasitoids did not respond to odours from aphid

infested leaf material. This lack of response probably indicates that the parasitoids cannot perceive the presence of this food source, rather than reflecting a lack of interest. This is supported by the observation that starved *C. rubecula* readily begin feeding once honeydew has been contacted. This finding shows that food sources not only differ in their relative abundance and accessibility, but also in their detectability. The latter should therefore also be considered as a criterium when comparing potential food sources for their possible contribution to a parasitoid's diet.

To determine the parasitoid's innate responses to visual stimuli, free-ranging parasitoids were observed in a flight chamber. Four individual Brussels sprouts plants were placed in the flight chamber to create a plant patch. Two targets (2.5 x 2.5 cm) of coloured «Pantone» paper, in the basic colours «Pantone Yellow U» and «Pantone Cool Grey 2» were attached to the four cabbage plants. The Pantone colours were selected on the basis of their spectrophotometric characteristics. «Pantone Yellow U» was selected since it has a spectral maximum at 550 nm, which corresponds with one of the sensitivity maxima described for Hymenoptera (Peitsch et al. 1992). «Pantone Cool Grey 2» was selected for its uniform spectrum, while its shade matches the overall reflection of «Pantone Yellow U» (calculated over the insect's visual spectrum). To the parasitoid, both types of coloured paper should consequently be of similar brightness. Compared to the darker background of the cabbage foliage, both targets were 3.5 times brighter.

Food-deprived parasitoids made frequent landings on the yellow targets, while two of the ten individuals landed on a grey target only once. Since the percentage of landings on the yellow targets clearly exceeded random, it could be concluded that food-deprived parasitoids seek out the yellow colour. After landing on a yellow target, food-deprived parasitoids generally searched the yellow paper intensively. Searching parasitoids typically scraped their mouth parts over the target surface. This behaviour was markedly different from the less active search on plant tissue (Wäckers 1994).

The question remained whether the previously described innate preferences were fixed, or whether parasitoids possess the plasticity to adjust their innate preferences according to their state of hunger. To study the effect of the hunger state on the innate preferences of *C. rubecula*, the response of food-deprived parasitoids was compared to the response of parasitoids of the same age that had been allowed to feed on sugarwater. It could be demonstrated that the parasitoid's innate response to both odours and colours is determined by the hunger state of the individual. Given a choice in a y-tube olfactometer between flower odours and odours from host-infested leaves, food-deprived individuals chose flower odours, while sugar-fed individuals preferred host associated odours.

In the flight chamber set-up, food-deprived parasitoids sought out yellow targets and searched more actively on this colour, while sugar-fed individuals ignored the coloured targets and concentrated their search on the cabbage leaves (Wäckers 1994). This indicates that parasitoids possess different sets of innate preferences, which take priority relative to the physiological needs of the individual.

HOST FORAGING

Unlike flowers, which advertise their nectar with notable scents and visuals in order to attract pollinators, there is usually little benefit to herbivores in attracting attention to their presence. To the contrary, since predators and parasitoids could use any detectable cue to locate herbivores, the latter are subject to a strong selection pressure to minimise their apparency. Vet et al. (1991) suggested that parasitoids can use associative learning to counteract this strategy in a «cat and mouse game». Associative learning, in which highly detectable cues from the first trophic level are linked to the highly reliable (but inconspicuous) host-derived cues, allows parasitoids to employ various sources of sensory information to seek out their (hidden) hosts.

In this study, various aspects of olfactory and visual learning were investigated in the larval parasitoid *M. croceipes*, a solitary parasitoid specialised on *Helicoverpa* (Lepidoptera: Noctuidae) species. The polyphagous nature of its host, occurring on more than 200 plant species (Fitt 1989), confronts the parasitoid with a wide array of potential host habitats. These (micro)habitats are likely to differ, not only with respect to their chemical and visual characteristics, but also with respect to their appearance Gates 1980).

It was investigated whether learning of both olfactory and visual cues could enable parasitoids to differentiate between plant species as well as plant structures. Such a differentiation would be adaptive since plants can represent disparate profitabilities to the foraging parasitoid. Both frequency of infestation and host accessibility can vary greatly depending on the plant species and the infested part of the plant. *Helicoverpa* larvae can be found feeding on young shoots and flowers, where they are exposed to parasitoid attack, but also in stems, flower buds and fruiting structures (Farrar & Bradley 1985) which often renders them inaccessible to the parasitoid. Associative learning of plant stimuli could help parasitoids to concentrate their foraging on those host sites which are most profitable in terms of host encounters.

To study associative learning of chemical and visual cues, two alternative types of host sites (assemblages of hosts and associated cues) were offered to free-ranging parasitoids in a flight chamber plant patch. During training sessions, hosts were provided on only one of the types of host sites.

To study odour learning, two types of frass from *H. zea*, feeding on either cotton flowers or cotton leaves, were offered as volatile alternatives. Subsequent choice evaluations revealed that parasitoids preferred whichever frass odour had been associated with the host during training sessions. In the same manner, it could be shown that parasitoids can be conditioned to visual stimuli (Wäckers & Lewis 1994). Thus, it can be concluded that female parasitoids can learn odour cues as well as visual information to distinguish between hosts feeding on different parts of the plant.

Under natural conditions, specific olfactory and visual stimuli are usually associated. A cotton flower, for instance, combines a characteristic odour with a characteristic visual appearance. Multisensory conditioning is therefore likely to give a more accurate approximation of the impact that learning can have on parasitoid foraging. In two additional experiments, parasitoids were conditioned to a combination of an olfactory and a visual stimulus (multisensory conditioning). Firstly, it was examined whether multisensory conditioning would further increase the preference level above the level of preference achieved after conditioning the parasitoid to the individual sensory components.

Visual and olfactory learning proved to have an additive effect: parasitoids conditioned to a combination of visual and olfactory stimuli displayed a stronger preference than individuals conditioned to either sensory component alone. When conditioned to a combination of stimuli, olfactory learning was demonstrated to be dominant over visual learning (Wäckers & Lewis 1994).

Learning of the individual visual elements (colour, shape, and pattern) by the parasitoid *M. croceipes* was investigated in additional experiments. Again, two visual alternatives were offered to free ranging parasitoids, only one of which was associated with a host larva. By using alternatives that differed in either colour, shape or pattern, it was shown that parasitoids can learn to distinguish host sites on the basis of each of these visual elements. When parasitoids were conditioned to a combination of shape and colour, the latter was learned dominantly. The relative rate at which *M. croceipes* learns shape versus colour seemed to be higher than in honey bees.

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Recent cases of interspecific competition between parasitoids of the family Aphelinidae (Hymenoptera: Chalcidoidea)

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An account is given on recent cases of interspecific competition between aphelinids associated with the whiteflies *Trialeurodes vaporariorum* (Westwood), *Aleurotuba jelineki* (Frauenfeld) and *Parabemisia myricae* (Kuwana) in Campania (Southern Italy).

Keywords: Aphelinidae, interspecific competition, parasitoids

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Most aphelinids are primary parasitoids of Aphidoidea, Aleyrodoidea and Coccoidea. These minute wasps (rarely exceeding 1 mm) are known to compete intra- and interspecifically in the complex of parasitoids associated to a given host (Rosen & DeBach 1979, Viggiani 1984). The latter behaviour may lead simply to a variation of the involved parasitic activities (competition) or to a dominant parasitization of the host by a new species (competitive displacement). Both phenomena are of great interest in general ecology as in biological control.

Rarely is a single species of aphelinid associated with a given host. More commonly, even in a very limited area, there is a complex of species, which can be represented by one of the following categories of parasitoids: 1 – ectophagous species; 2 – ectophagous and endophagous species; 3 – endophagous species. In both categories 2 and 3 under «endophagous species» are also considered the *Eretmocerus* spp. developing as ecto-endophagous parasitoids.

In the first category are included several complexes of aphelinids attacking armored scale insects. The species involved belong mostly to the genus *Aphytis*.

The second category, the most numerous, includes ectophagous species as *Aphytis* and endophagous species belonging to different genera (*Encarsia, Eretmocerus, Pteroptrix*, etc.).

In the third category the complex of parasitoids can be represented by several endophagous species belonging to the same genus (i. e., *Encarsia*) or to different genera (i. e., *Encarsia* and *Eretmocerus*).

Up to now, most of the studies on the complex of aphelinids associated with a given host concern those on armored scales and whiteflies. The data available show that a complex may vary considerably in time and space. On the same worldwide pest, like *Bemisia tabaci* (Gennadius), are recorded respectively more than 20 species

of aphelinids, but in limited area (i. e., a country or part of it) they may be represented even by only one species.

In the present paper the author will give an account on recent cases of interspecific competition observed between aphelinids associated to the whiteflies *Trialeurodes vaporariorum* (Westwood), *Aleurotuba jelineki* (Frauenfeld) and *Parabemisia myricae* (Kuwana) in Campania (Southern Italy).

CASES STUDIED

Competition between parasitoids of *Trialeurodes vaporariorum* (Westwood).
The glasshouse whitefly, *T. vaporariorum*, as in many regions of the world, is a severe pest also in Italy, but in the southern part of the country, it attacks several crops even in open field. Because of the favorable ecological conditions the whitefly population can well develop all year round. Until 1976 the aphelinids associated with *T. vaporariorum* were *Encarsia tricolor* Förster, *E. partenopea* Masi and *Encarsia formosa* Gahan. The latter species, used for releases under glasshouses, in limited case is found also around them (Mazzone 1976).

At the end of 1978 *Encarsia pergandiella* Howard has been introduced into Italy from USA; the releases in the field started in 1979. The aphelinid soon colonized the new areas and spread after a few years in new sites, attacking also new hosts (Viggiani & Mazzone 1980, Mazzone & Viggiani 1985). Meanwhile, in some trials of biological control of *T. vaporariorum, E. pergandiella* appeared dominant on *E. formosa* under the experimental conditions (Mazzone et al. 1982) or the only present species (Giorgini & Viggiani 1993, unpubl.). Recently the aphelinid has been recorded from Northern Italy (Colombo & Eördegh 1992). Samplings at random in open field showed that the aphelinid is at present the dominant parasitoid of *T. vaporariorum* in Southern Italy.

The success of *E. pergandiella* is apparently based on the possibility to parasitize different young host stages in which complete the larval development, the minor thermal requirements, the possibility to use young stages of other parasitoids for the male development and other traits. The recent, accidental introduction into Italy of the aphelinids *Encarsia meritoria* Gahan (Viggiani & Laudonia 1991) and *E. transvena* (Timberlake) (Pedata & Viggiani 1993), parasitoids of some whiteflies including *T. vaporariorum*, will add interest in studying the evolution of the natural enemies associated with this pest. Under glasshouses *T. vaporariorum* is still a pest of economic importance for several crops.

Competition between parasitoids of *Aleurotuba jelineki* (Frauenfeld).
The viburnum whitefly, *A. jelineki*, was a rather common species on *Viburnum tinus* L. in Southern Italy. For several purposes the present author studied the complex of its parasitoids and described the following new species: *Amitus aleurotubae* Viggiani & Mazzone (1982) (Platygastridae), *Encarsia aleurotubae* Viggiani (1982) and *Eretmocerus longicornis* Viggiani & Battaglia (1983). Until July 1984 the dominant species was the aphelinid *Encarsia margaritiventris* (Mercet) with a degree of parasitization of about 20–30 %, but since October of the same year, *Cales noacki* Howard, a well-known parasitoid introduced also into Italy against the woolly whitefly *Aleurothrixus floccosus* (Maskell) was reared from *A. jelineki* (Viggiani &

Laudonia 1984, Laudonia & Viggiani 1984). Since then *C. noacki* became the dominant parasitoid of the viburnum whitefly and in several places a displacement of all other competitive species was observed (Guerrieri & Viggiani 1988). The dominance of *C. noacki* (degree of parasitization even of 70–80 %) may be explained considering that *A. jelineki* is univoltine as well as all associated parasitoids, except *C. noacki*. The latter species can develop at least three generations a year on the mentioned host. Moreover, the adults of *C. noacki* emerge from all young host stages (except the first one) in which the entire larval development takes place; on the contrary, all other parasitoids can oviposit in several young stages, but the full larvae are found only in the last host nymphal stage from which the new adults emerge. At present the population of *A. jelineki* in the investigated area is very low.

Competition between parasitoids of *Parabemisia myricae* (Kuwana).
The Japanese bayberry whitefly *P. myricae* was first recorded from Italy (Sicily and Calabria) in 1990 (Longo et al. 1990). In the mentioned areas the complex of parasitoids was represented by several native and introduced aphelinids. The dominant species *Encarsia meritoria* Gahan, a Nearctic species accidentally introduced (Viggiani & Laudonia 1991), was reared and released for the bio-control of *P. myricae*. In the same area, *Eretmocerus debachi* Rose and Rosen, retained native of the Nearctic region, was imported from Israel and released starting from fall 1991. This species reproduced rapidly in the field and spread into new sites, adding a risolutive contribution to the bio-control of *P. myricae* in Sicily and Calabria (Barbagallo et al. 1992).

Meanwhile, on February 1992 the present author found *P. myricae* in the Sorrento area (Italy, NA) at a very low level, but highly parasitized (holes of adult emergence, dead adults) by *Encarsia meritoria* Gahan and *Eretmocerus debachi* Rose and Rosen. Clearly the active parasitization took place at least during the fall of the previous year. These findings suggest that in this area the two parasitoids arrived indipendently from the releases carried out in Calabria and Sicily.

A successive survey on the distribution of *P. myricae* in Campania showed that the whitefly was distributed along all coastal area, but at a very low level, except in the Campi Flegrei zone, where it was at pest status. Since 1992 *P. myricae* and its natural enemies were studied in the latter area, characterized by familiar orchards. Until April 1992 no trace of parasitoids was found, but later on two species were identified: *E. debachi* and *Encarsia transvena* (Timberlake). The latter species is the senior synonym of *Encarsia bemisiae* (Ishii) from Japan, the only known parasitoid of *P. myricae* until Rose & Debach (1992).

By regular samplings, at maximum two weeks interval, data have been gathered on the density of whitefly population (young stages) and on the degree of parasitization (Viggiani, unpubl.). Preliminary results show that in the investigated area *E. meritoria* is not a parasitoid of *P. myricae*. The role of this species was taken by *E. transvena*, which coexists with *Eretmocerus debachi*, but the first aphelinid appears dominant (in average 3:1). The total degree of apparent parasitization (sensu Viggiani 1977) varies widely from 0 to 100 %, with an average of about 65 %. It appears negatively correlated to the density of the whitefly host (last young stage).

At present, 3–4 years after its accidental introduction, *P. myricae* is mantained at a very low density level by the combined action of the two aphelinids *Encarsia transvena* and *Eretmocerus debachi*.

DISCUSSION AND CONCLUSION

The interspecific competition between aphelinids associated with their main hosts, whiteflies and armored scale insects, was investigated mostly in *Aphytis* spp. (Rosen & DeBach 1979, Huffaker 1990), but some interesting cases involved also species of different genera (*Encarsia, Pteroptrix*) (Thomson et al. 1987, Podoler et al. 1988, Yu et al. 1990).

This phenomenon may cause deep variations in the involved populations, in time and space. This means that any evaluation reflects only the investigated universe. Moreover, some aspects may be lost when the study is interrupted for long time.

Actually, the competition between parasitoids does never lead to a complete displacement of one species by another, and this is why absolute ecological homologues cannot coexist. In fact a widely spread phenomenon it is not the exclusion, but the strong reduction of a parasitoid population caused by the action of another species, which became dominant. The parasitoids involved in this competition may be not necessarily retained ecological homologues.

On the other hand, in general, appears difficult to predict on the basis of laboratory studies, the dominance of one species on another in the field. The latter is a very heterogeneous and changeable system to allow a pre-selection of the dominant parasitoid in all situations. In terms of biological control the present knowledge on the competition between aphelinids shows that the best strategy still remain, when possible, the use of several parasitoids instead of the supposed best one. In this case, each of them can play a different role, when required by climatic conditions, host phenology, host population density, and so on. Several studies support this view, as those presented in this paper. Podoler et al. (1988) studied the coexistence of *Aphytis holoxanthus* DeBach and *Pteroptrix smithi* (Compere), respectively ecto- and endoparasitoids of the Florida red scale *Chrysomphalus aonidum* (L.). In the field *P. smithi* became dominant on *A. holoxanthus*, in contradiction with the clear superiority of the latter in the laboratory studies. According to the authors, among the different mechanisms that might be involved there are interspecific competition and /or differential effects of insecticides. But this does not mean that *A. holoxanthus* should be discarded in new projects (DeBach & Rosen 1991). Another interesting case concerns *Amitus hesperidum* Silvestri (Platygastridae), *Encarsia smithi* Silvestri and *E. opulenta* Silvestri, parasitoids released in Merritt Island (Florida) against the citrus blackfly, *Aleurocanthus woglumi* Ashby. The data gathered show a clear trend from initial dominance by *A. hesperidum*, to brief dominance by *E. smithi* to final dominance by *E. opulenta* (Thomson et al. 1987).

Some aspects of the competition between aphelinids are still unexplainable in several cases. The endoparasitoid, *Encarsia perniciosi* (Tower), and the ectoparasitoid, *Aphytis melinus* DeBach, both introduced into California to control the California red scale, *Aonidiella aurantii* (Mask.) coexist in Southern California. The partitioning of the scale resource by the two species explains their coexistence, but not the disappearence of *Encarsia perniciosi* in other areas (Yu et al. 1990). Unexplained is also the situation emerged in a survey on the parasitoids of the white peach scale, *Pseudaulacaspis pentagona* (Targ. Tozz.) in Italy. The dominant species *Encarsia berlesei* (Howard) coexists only in Campania with *Pteroptrix orientalis* (Silvestri) and in some localities the degree of parasitization is higher for *P. orientalis* (Garonna & Viggiani 1988, Garonna & Viggiani, unpubl.). Finally, Bennett (1993)

put the question: «Do introduced parasitoids displace native ones?». In my opinion, this is the reply: «Yes, may be, but in any case the final situation will improve the biological control of the involved pests.»

SUMMARY

Cases of interspecific competition between aphelinids associated to the whiteflies *Trialeurodes vaporariorum* (Westwood), *Aleurotuba jelineki* (Frauenfeld) and *Parabemisia myricae* (Kuwana) have been studied in Campania (Southern Italy). In the first case the dominant species became *Encarsia pergandiella* Howard, introduced from USA. The aphelinid spread from Southern to Northern Italy. The competition between parasitoids of *A. jelineki* lead to the displacement of *Encarsia margaritiventris* (Mercet) by *Cales noacki* Howard, exotic aphelinid introduced against the citrus woolly whitefly *Aleurothrixus floccosus* (Maskell). Finally, at least two aphelinids coexist on *Parabemisia myricae* (Kuwana); in some areas the dominant species is *Eretmocerus debachi* Rose & Rosen and in others *Encarsia transvena* (Timberlake).

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Parasitoids associated with different aphid species, their effectiveness and population dynamics

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An attempt was made to evaluate some plant species as a reservoir of aphids and their parasitoids (Hymenoptera: Aphidiidae). Acording to the survey *Sambucus nigra* L., *Euonymus europaea* L., *Prunus padus* L. and *Viburnum opulus* L. consisted the richest source of parasitoids. From aphids collected since early spring up to middle autumn parasitoids were reared, reaching the peak of occurence in May or June. Hyperparasitism occurring in the whole complex of aphid species significantly limited the abudance of parasitoids. During the growth season the level of parasitism oscillated in a very broad spectrum from zero up to fifty or even hundred percent, in average however being rather low on all examined plants.

Keywords: aphids, hyperparasitoids, parasitoids, population dynamics

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INTRODUCTION

Beneficial arthropods play an important role in regulating the numbers of herbivores. The parasitoids consisted one of the very important links in the chain of beneficials also in cultivated areas. The number of beneficials can be achieved to increase by creating around the cultivated land, refuges consisting of non crop plants which are attractive to predator and parasitoid insects.

The influence of a seminatural ecosystem on the potential degree of some biological agents has been discussed by many authors e.g. Stary (1970), Carrol & Hoyt (1984), Stary & Nemec (1986), Powell (1986), Keller & Duelli (1988), Hughes (1983), Chikh-Khamis & Hurej (1991). The purpose of these investigations was to evaluate the parasitoids effectiveness in reduction of different species of aphids and the influence of non crop plants on parasitoids abundance.

MATERIAL AND METHODS

The research was caried out during three years (1989–1991) in an experimental apple orchard situated in Skierniewice vicinity, Central Poland. This orchard was divided into three plots: «Chemical», «Integrated» and «Control». Whenever necessary (usually no more than 3 times per season), nonselective insecticides were applied at

the «Chemical» plot, selective insecticides – at the «Integrated» plot. The «Control» plot was never sprayed with insecticides against the pests. The orchard area was surrounded with a hedge composed of bushes of *Sambucus nigra* L., *Prunus padus L.*, *Cornus sanguinea* L., *Viburnum opulus* L., *Crataegus* sp., *Caragana* sp. and *Spiraea* sp., growing very close to one another and serving as a refuge for beneficial fauna. This area bordered upon a mixed deciduous forest (from the east), upon agricultural fields (from the north and south), and upon farm buildings (from the west).

The trees and bushes were checked usually every week from April to October and field collected aphid colonies were kept in glass containers (500 cm^3) under laboratory conditions (21–25°C, 50–80 % rh, 16L:8D). Every day all containers were checked and parasitoids were collected individually into small glass vials. The percentage parasitism for each colony was calculated on the basis of numbers of parasitoids and hyperparasitoids that emerged and the mumies without emergence holes.

RESULTS AND DISCUSSION

Depending on the year, first aphid colonies appeared at different time, but usually on the snow-ball bushes (*Viburnum opulus*), spindle (*Euonymus europaea*) and bird-cherry (*Prunus padus*) they were collected in the second part of April. On apple trees, hawthorn (*Crataegus* sp.) and elder (*Sambucus nigra*) they appeared at the beginning of May, whereas on pea bush (*Caragana* sp.) and meadowsweet (*Spiraea* sp.) during the first part of June and on dogwood (*Cornus sanguinea*) during the first ten days of August.

Twelve species of aphids have been identified (Table 1). During three years of investigations 330625 aphid individuals were collected from all examined host plants. The highest number of aphids was collected on elder whereas the lowest one on meadowsweet (Table 3).

On apple trees only two important species of aphids were found: rosy apple aphid (*Dysaphis plantaginea*) and green apple aphid (*Aphis pomi*). Detailed observations showed that usually they occurred only on a few shoots on certain trees. Some species of aphids occurred on two different species of host plant and so: *A. fabae* on snow-ball and spindle, on the other hand *A. pomi* on apple trees and in low numbers on hawthorn.

Dynamics of parasitoids population and level of aphid parasitism.

During 3 years of investigations 4950 parasitoids and hyperparasitoids were obtained from aphid specimens collected on the examined plants (Table 2), however no parasitoids were obtained from aphid colonies collected on dogwood (Table 3).

The first parasitoids were obtained as early as first aphid colonies were collected. Usually such situations occurred on spindle during the second part of April. The last hymenopteran specimens were collected during the first part of October, mostly from bird-cherry, spindle and hawthorn. The number of parasitoids collected varied from year to year but every year the peaks of their abundance usually occurred in May or June (Figure 1).

The values of parasitism oscillated in a very broad spectrum from zero up to fifty or even hundred percent of the aphids parasitised. The highest values of

Table 1. Aphid species collected on individual plant species.

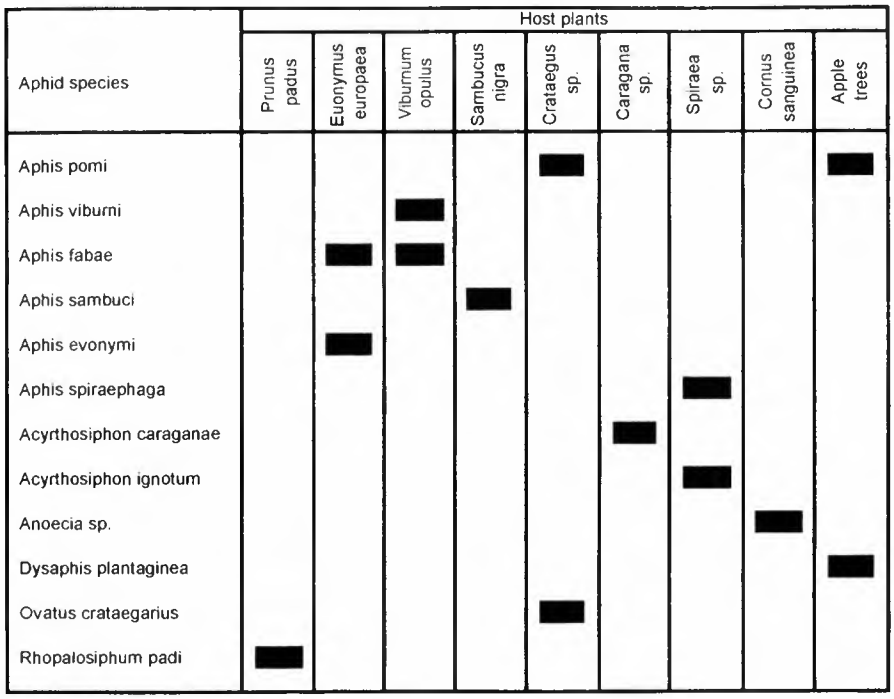

Table 2. Parasitoids collected from different hosts during the period 1989–1991.

Host plant	No of parasitoids collected			
	1989	1990	1991	Total
Prunus padus L.	49	186	439	674
Euonymus europaea L.	297	371	288	956
Viburnum opulus L.	342	92	40	474
Sambucus nigra L.	1259	430	289	1978
Crataegus sp.	47	32	15	94
Caragana sp.	171	55	22	248
Spiraea sp.	41	112	~	153
Apple trees	239	24	110	373
Total	2445	1302	1203	4950

Table 3. The percentage of parasitised aphids on different host plants.

Host plant	Year								
	1989			1990			1991		
	No. of aphids collected	Mean % of parasitization	Maximal % of parasitization	No. of aphids collected	Mean % of parasitization	Maximal % of parasitization	No. of aphids collected	Mean % of parasitization	Maximal % of parasitization
Prunus padus L	7696	0.63	50.00	20016	0.92	14.20	18222	2.40	7.58
Euonymus europaea L.	23497	1.26	46.80	13269	2.80	38.30	10181	2.83	25.27
Viburnum opulus L	12483	2.74	12.30	7938	1.15	3.90	7218	0.55	1.33
Sambucus nigra L	41493	3.05	19.40	39538	1.08	7.97	24536	1.17	3.97
Crataegus sp	2319	2.03	3.74	5147	0.62	8.00	1793	0.83	3.45
Caragana sp	4685	3.65	100.00	3163	1.74	100.00	4581	0.48	13.80
Cornus sanguinea L	13248	0.00	0.00	5631	0.00	0.00	27949	0.00	0.00
Spiraea sp	1327	3.09	26.70	2053	5.46	14.87	-	-	-
Apple trees	14885	1.6	6.65	5729	0.42	14.28	12048	0.91	12.39
Total	121633			102484			106508		

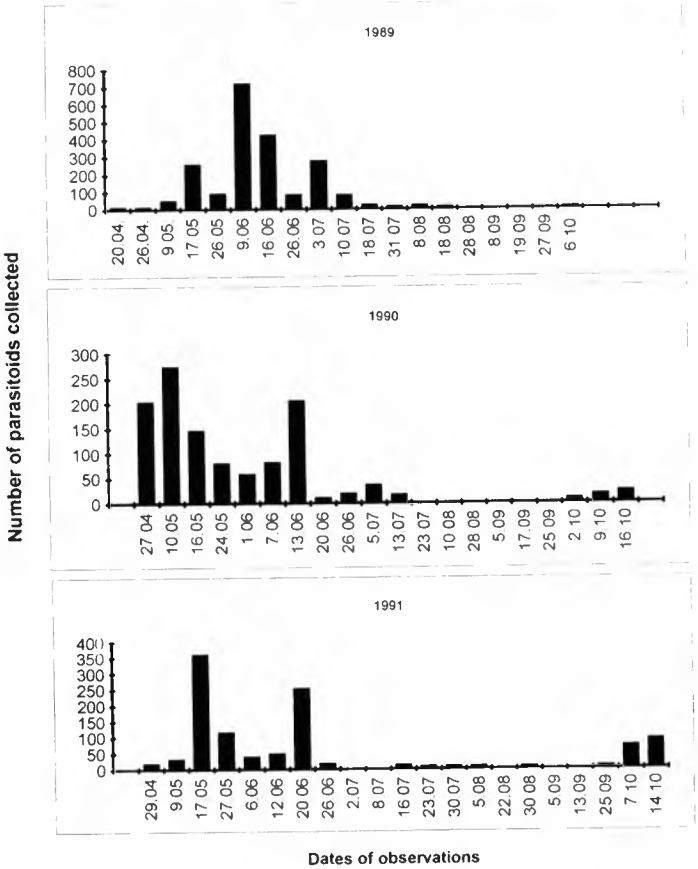

Figure 1. Population dynamics of the parasitoids and hyperparasitoids during separate growth seasons.

parasitism were observed mainly in the case of small colonies of aphids at the end of their occurrence. On the other hand the mean rates of parasitism based on all samples taken during the vegetative season from particular species of plants were rather low and did not exceed 3.65 % in 1989 (on *Caragana* sp.), 5.46 % in 1990 (on *Spiraea* sp.) and 2.83 % in 1991 (on *E. europaea*) (Table 3).

The numerical relation between parasitoids, hyperparasitoids and their hosts. Three years of investigations give plenty of informations about the abundance of parasitoids and hyperparasitoids of aphids occurring on examined plants. During this period 4950 specimens were collected, but up to now only 2174 of them were identified to the genera among which 674 to species. On the base of specimens collected during two years (1989 and 1990) one can state that primary parasitoids (Aphidiidae) constituted over 70 %. The majority of them we collected from colonies of *A. sambuci* on elder (41.7 % in 1989 and 32.8 % in 1990). A high number was also gathered on spindle and snow-ball. On these three plant species we collected over 77 % of all primary parasitoids during 1989 and over 70 % during 1990. The number of parasitoids obtained from other plant species (and of course on other aphid species) varied from 1.2 % to 16.6% (Table 4). As was confirmed on the base of specimens collected in 1989 and 1990 most of the primary parasitoids belonged to the genus *Praon* (46.1%) and *Trioxys* (41.1 %). Also the genus *Ephedrus* constituted a quite important group with the share of 11.1%. The lowest numbers were presented by the genera *Aphidius, Lipolexis* and *Diaeretiella* (Table 4).

On the base of specimens collected in 1990 seventeen species were identified, among which *Praon abjectum* made up 51.3 % and *Trioxys angelicae* – 35.1%. The other species participate only in a small amount (Table 5).

The parasitoids were significantly limited by hyperparasitoids (mainly Charipidae, Ceraphronidae, Pteromalidae) which constituted 32.2 % of all collected hymenopterans in 1989 and 24.7 % in 1990. The value of hyperparasitisation varied also depending on host and plant from 0 % on *Crataegus* sp. (1990) up to over 74 % on *Caragana* sp. (1989). High numbers of hyperparasitoids on apple trees, spindle, bird-cherry and meadowsweet were also found during individual years.

Table 4. Number of parasitoids collected from examined plants in 1989 and 1990.

Genus of parasitoids	Sambucus nigra		Viburnum opulus		Euonymus europaea		Prunus padus		Crataegus sp		Caragana sp		Spiraea sp		Apple trees		Σ		Total	%
	89	90	89	90	89	90	89	90	89	90	89	90	89	90	89	90	89	90		
Praon sp.	273	141	8	0	17	104	13	111	6	5	8	4	3	~	16	1	344	366	710	48.1
Trioxys sp.	87	80	178	47	63	102	1	0	26	0	1	1	8	~	7	33	371	263	634	41.2
Ephedrus sp.	1	0	21	1	22	0	9	1	1	10	0	0	0	~	89	16	143	28	171	11.1
Aphidius sp.										2	8	10		~			8	12	20	1.3
Lipolexis sp.										3				~				3	3	0.2
Diaeretiella												2		~				2	2	0.1
Total	361	221	207	48	102	206	23	112	33	20	17	17	11	~	112	50	866	674	1540	
%	41.7	32.8	23.9	7.1	11.8	30.6	2.7	16.6	3.8	3.0	2.0	2.5	1.3	~	12.9	7.4				
Hyperparasitoids	58	4	81	2	168	80	25	55	2	0	49	4	14	~	15	77	412	222	634	
%	13.8	1.8	28.1	4.0	62.2	27.9	52.0	32.9	5.7	0	74.2	19.0	56.0	~	11.8	60.6	[illegible]			

Table 5. Parasitoid species reared from the aphids collected during the 1990 growth season.

Parasitoid species	No.	%
Trioxys angelicae	237	35.2
Trioxys acalephae	16	2.4
Trioxys sp.	10	1.5
Praon abjectum	346	51.3
Praon bicolor	6	0.9
Praon volucrae	2	0.3
Praon sp.	12	1.8
Ephedrus persicae	17	2.5
Ephedrus lacertosus	2	0.3
Ephedrus plagiator	1	0.2
Ephedrus cerasicola	2	0.3
Ephedrus sp.	6	0.9
Aphidius ervi	3	0.4
Aphidius niger	2	0.3
Aphidius sp.	7	1.0
Lipolexis gracilis	3	0.4
Diaeretiella rape	2	0.3

On the other hand a low level of hyperparasitism occurred on aphids (*A. sambuci*) from elder both in 1989 and 1990.

The presented results suggest that the effectiveness of parasitic Hymenoptera in limiting aphid populations was dependent of host plant, year of observations, period of growth season and hyperparasitoids abundance.

Taking under consideration these conditions one can suggest that some of the examined plants could be a rich source of aphid parasitoids. For this reason biological control of aphids occurring on apple trees could be enhanced. This investigations confirmed some statements presented earlier (Olszak 1991).

The knowledge of different agents which can be helpful in pest reduction is especially important in integrated pest control as a part of the new idea spreading during the last few years and known as integrated fruit production. Also knowledge of the quantitative and qualitative relationships between different groups of parasitoids could help us appropriately evaluate their role in the agroenvironment.

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The leafminer *Chromatomyia fuscula* (Diptera:Agromyzidae) and its parasitoid complex in Norwegian barley fields

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The density of the leafminer *Chromatomyia fuscula* and the density and composition of its parasitoid complex were investigated in wheat (1990) and barley (1991–93) fields lined with forest at Ås, southern Norway. The barley was organically grown. Shoots of barley were sampled throughout the season, and the numbers of adult leafminers and parasitoids emerging from them counted. The density of *C. fuscula* seemed to be highest near the forest edge, whereas the parasitoids appeared less affected by the distance from the forest. The mortality of immature stages was high (probably due to rearing method). Each year, 6–14 species of Pteromalidae and Eulophidae (Hymenoptera: Chalcidoidea) were found. In both crops and in most years *Cyrtogaster vulgaris* was the dominant species, followed by *Diglyphus begini*.
Small samples from the adjacent grass crop and from 15 other barley fields in southern Norway revealed a similar parasitoid fauna dominated by *C. vulgaris* and *Cirrospilus vittatus*. Altogether, the parasitoid complex consisted of at least 18 chalcid species, together with at most two braconid species occurring in negligible numbers.

Key words: barley, *Chromatomyia fuscula,* distance from edge, Eulophidae, leafminer, parasitoid complex, percentage parasitization, Pteromalidae,

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INTRODUCTION

The leafminer *Chromatomyia fuscula* (Zetterstedt) attacks wild and cultivated grass species in Europe. The fly has a holarctic distribution from Northern and Central Europe across Russia, Alaska and Canada (Griffiths 1980). In Norway it is regularly present in cereals and may locally be a pest in spring barley fields (Andersen 1991). *C. fuscula* has one generation per year and seems to overwinter as adult in hitherto unknown habitats. The eggs are deposited inside the leaves of cereals and grasses in late May and in June. The larvae mine the leaves for nearly two weeks, and then pupate in the mines. The pupal stage lasts for almost three weeks, and the adults emerge in July/ early August (Andersen 1988, 1991).

The parasitoids of *C. fuscula,* and their phenology, biology and impact upon the leafminer population have been totally unknown in Norway. The present study presents data from four seasons (1990–94) on *C. fuscula* and its parasitoid complex in cereal fields from South-Eastern Norway.

MATERIAL AND METHODS

The investigation area

The experimental field («Frydenhaug») is situated at Ås, 30 km south of Oslo. From 1991 it has been managed according to the principles of organic agriculture, implying crop rotation and no use of pesticides nor artificial fertilizers. *C. fuscula* is known to completely dominate (>99% of reared individuals) the leafminer populations in barley in this area (Andersen 1991).

Frydenhaug is surrounded by conventional agricultural fields (three sides) and a mixed forest woodlot. The six course crop rotation consists of: 1) row crop (varying through the years); 2) green fodder undersown with grass/clover (i.e. timothy *Phleum pratense*, meadow fescue *Festuca pratensis*, and various clovers *Trifolium* spp); 3–5) grass ley (grass/clover); 6) spring barley (*Hordeum vulgare,* cv. «Tyra») undersown with ryegrass (*Lolium multiflorum*) and whiteclover (*Trifolium repens*). Each crop covers a rectangle of about 2.5 ha. Adjacent to the longest sides of the barley crop were green fodder in 1991, green fodder on one side and grass clover on the other in 1992, and grass/clover in 1993.

In 1990, a conventionally grown wheat (*Triticum vulgare*) field 1 km apart from Frydenhaug was studied. The wheat was undersown with grass/clover, and deciduous forest bordered two sides of the field.

To sample the geographic variability of the parasitoid assemblage, spring barley from 15 other fields in the counties of Akerhus and Østfold, southern Norway, were collected in 1993. Two of these additional fields (on the same farm) were organically grown.

Sampling methods

In all years samples consisting of 48 or 32 cereal shoots were taken once a week from plots (about 1x1 m) at several distances from the forest edge. All leaves were examined under a binocular, and number of eggs, larvae and pupae of *C. fuscula* were counted. The leaves were then sorted according to leaf position on each plant (No 1–8) and placed in glassjars with gauze at 21°C and 16 hrs photoperiod to allow flies and parasitoids to emerge. Thus each jar contained a maximum of 48 leaves which all had identical leaf number, sampling date and distance from the edge.

In addition some timothy and meadow fescue from the adjacent grass crop was collected four times for emergence of flies and parasitoids during the 1993 season. The last collection was on the day the grass was cut (20 June).

The 15 other barley fields were sampled at 25 June 1993. About 30 leaves with mines were collected near the edge in each field. A similar sample of 30 leaves was taken from Frydenhaug in order to see how much of the parasitoid fauna could be traced with this method. All leaves were individually put in glass tubes (150x19 mm), and allowed to dry under gauze for two days before closing the tubes with cork lids. They were kept at 20°C and 24 hrs of light, and the number of emerged parasitoids

and flies were recorded 2–3 times per week. All leaves were later dissected to check the number of emerged insects, and to count dead pupae.

RESULTS

Frydenhaug

The times for the earliest observations of eggs, larvae and pupae of *C. fuscula* varied little from year to year (Table 1). The same is true for the earliest samples from which parasitoids were reared.

The percentage parasitization (Table 2) calculated from adult emergence averaged 15–20 % in barley in normal years, but was higher in 1992 (41 %), which had a very dry June. Flies in wheat also had a high parasitization rate (53 %). There was a weak tendency of increased fly infestations towards the edge, whereas the parasitoids seemed more evenly distributed in the field. Unfortunately, Table 2 does not give a true picture of absolute densities, because the mortality in the collected mines was very high. The real *C. fuscula* infestation (found by counting eggs, larvae and pupae on leaves of sampled shoots, results not shown here) was about 15 individuals per shoot, that is more than ten times higher than indicated by adult emergence. Thus, table 2 gives a highly relative picture of fly infestation and parasitization, and we refrain from further statistical analysis.

The parasitoid complex consisted of chalcids of the families Eulophidae and Pteromalidae (Table 3). The pupal parasitoid *Cyrtogaster vulgaris* was the clearly dominant species in all years, both in barley and wheat. Apart from another 1–2 fairly common species (in particular the larval ectoparasitoid *Diglyphus begini*), the rest of the species occurred in rather small numbers. Among the latter is one (possibly two) unidentified species of *Miscogaster* (Pteromalidae). In addition to those in table 3, two specimens of the genus *Trichomalopsis* (Pteromalidae) were found in 1991, but it is uncertain whether they originated from *C. fuscula*. At least one braconid (*Dacnusa* sp.) is also present, but in very small numbers.

Table 1. Earliest observations of eggs, larvae and pupae of *C. fuscula* in wheat (1990) and barley (1991–93), based on plant examinations in cereals at Ås. Right column shows the first date parasitoids were registrated in the plants, based on later adult emergence.

Year	Start of sampling	Egg	Larva	Pupa	Earliest parasitoid infestation
1990	May 21	–	May 21	May 21	May 22
1991	May 22	May 22	May 30	June 6	June 10
1992	May 19	May 19	May 26	June 2	June 2
1993	May 11	May 24	June 1	June 9	June 9

Table 2. Average number of *C. fuscula* (AdF) and parasitoids (Par) emerged per shoot at different distances from the forest edge. Percentage parasitization (%) = (Par/(AdF+Par)) x 100.

Dist.	1990 Wheat			1991 Barley			1992 Barley			1993 Barley		
	AdF	Par	%	AdF	Par	%	AdF	Par	%	AdF	Par	%
5 m	0.29	0.38	57	4.2	0.4	8						
20 m	0.41	0.47	53	2.7	0.6	18	0.07	0.05	43	1.0	0.2	19
40 m	0.2	0.5	71	3.4	0.4	11	0.06	0.02	32			
80 m	0.2	0.1	40	0.8	0.6	43	0.02	0.02	50			
160 m							0.01	0.01	44	0.7	0.2	19
200 m				0.3	0.3	43						
320 m										0.8	0.3	23
Weighted mean	0.3	0.4	58	2.5	0.5	16	0.03	0.02	41	0.9	0.2	20
No. specimens	408	552		4204	787		55	38		1039	267	
No. shoot	1470			1627			1568			1300		

The picture was similar in the adjacent grass crop. A total of 37 parasitoids (6 species, Table 3) emerged from the fly-infested grass leaves (N=155) collected at four different times in 1993. The overall percentage parasitization based on emerged adults was 25. *C. vulgaris* was the most common species, followed by the larval ectoparasitoids *Cirrospilus vittatus* and *D. begini* (Table 3). The number of flies dead in the puparium was counted. Early in the season (2 June) dead pupae outnumbered the living flies by 4:1; three weeks later the relation improved to 1:1.

The 16 barley fields

The 15 (16 including Frydenhaug) barley fields sampled showed considerable variation in parasitization data (Table 4). The rearing method used (keeping the leaves individually and using cork lids) lead to an improved but still low pupal survival. Dissecting the leaves showed that no flies nor parasitoids had escaped before collection. Altogether 17 species appeared, and only two of the species found at Frydenhaug were missing (*Chrysocharis orbicularis* and *Stichtomischus* sp.). Additional species were *Closterocerus* (= *Chrysonotomyia*) *lanassa* (Walker) (Eulophidae, 1 specim.), *Callitula bicolor* Spinola (Pteromalidae, 2 specim.), and 1 indet. braconid (Opiinae, 2 specim.). A single *Trichomalopsis* sp. also emerged from a fly pupa.

The species dominating were *C. vittatus*, together with *C. vulgaris*. They were both reared from 56 % of the fields and constituted 25 and 19 % of the total number of the parasitoids emerging, respectively.

Interestingly, one of the two samples from the organic farm had the highest parasitization rate (44 % based upon adult emergence, Table 4) and the highest number of species. One of the leaves contained as many as four different species of parasitoids. Frydenhaug, which also is organically managed, was intermediate in all

Table 3. Yearly frequency distribution (%) of chalcid species on *C. fuscula* in various crops at Ås. The list is not complete (see text).

Species	1990 Wheat	1991 Barley	1992 Barley	1993 Barley	1993 Grass
Pteromalidae					
Cyrtogaster vulgaris (Walker)	75	48	14	53	43
Halticoptera circulus Walker	0.7	8	3	6	0
Miscogasterini (*Miscogaster, Stictomischus*)	0	0.3	0	0.3	0
Eulophidae					
Chrysocharis orbicularis (Nees)	0.3	2	0	0.3	0
Chrysocharis pentheus (Walker)	0.5	0.1	0	0.3	0
Chrysocharis polyzo (Walker)	0.1	2	0	0	11
Chrysocharis pubicornis (Zetterstedt)	4	3	0	2	5
Cirrospilus vittatus Walker	3	3	14	1	22
Diglyphus begini (Ashmead)	14	23	31	17	16
Diglyphus minoeus (Walker)	0	0.3	0	1	0
Hemiptarsenus unguicellus (Zetterstedt)	0.3	4	11	3	0
Neochrysocharis aratus (Walker)	1	3	28	10	3
Pediobus acantha (Walker)	0	1	0	0	0
Pnigalio soemius (Walker)	0.5	0.7	0	1	0
No. specimens	562	786	37	268	37
No. species (minimum)	11	14	6	12	6

parameters (Table 4). The four species appearing from the Frydenhaug sample were *Chrysocharis pubicornis, Neochrysocharis aratus, C. vittatus*, and *C. vulgaris*. Hence, the relatively small sample of 30 leaves included half of the species exceeding 1 % in Table 3 (1993).

The dead pupae (N = 251) were recognizable fly pupae in 57 % of the cases. Only four dead pupae were clearly parasitoids. It was not possible to identify the rest, as these died early in the pupal stage, but they were all inside fly puparia.

Table 4. Mortality of *C. fuscula* pupae and density & diversity of parasitoids in 16 barley fields, based on 30 leaves with mines from each. Results from the sample at Frydenhaug are also given separately. Numbers of emerged parasitoids (Par) and flies (AdF), and dead pupae found in fly puparia (DPu) were recorded for each location. Since parasitoids are solitary, the total number of fly pupae (TotPu) is obtained by adding Par, AdF, and DPu. The total number of individuals were 103, 550, and 247 for Par, AdF, and DPu, respectively.

	Mean (SE)	Range	Frydenhaug
Mortality due to parasitoids (Par/TotPu)	0.12 (0.02)	0.01-0.33	0.10
Mortality mainly due to method(?) (DPu/TotPu)	0.29 (0.03)	0.15-0.57	0.33
Fraction of pupae surviving to adult flies (AdF/TotPu)	0.60 (0.03)	0.33-0.77	0.57
Parasitization as given in Table 1 (Par/(Par+AdF))	0.16 (0.03)	0.02-0.44	0.15
Par/leaf	0.22 (0.05)	0.03-0.63	0.17
No of paras. species	3.38 (0.39)	1-6	4

DISCUSSION

Studies on parasitoid assemblages on phytophagous hosts indicate that the number of parasitoid species increases with host concealment up to a certain level, and then decreases as hosts becomes less available (Hawkins 1993, and references therein). Leafminers seem to represent the level of concealment having most parasitoids. Askew & Shaw (1974) found that most species of leafminers on deciduous trees were attacked by 10–20 species of parasitoids. While leafmining Lepidoptera, Hymenoptera and Coleoptera shared a number of parasitoids, leafmining Diptera had

a more distinct parasitoid fauna. Unlike most other host groups, agromyzids on herbs tend to be attacked by more parasitoid species than those feeding on trees (Hawkins 1988).

So far we have found at least 18 chalcid parasitoids on *C. fuscula* in barley. The eulophid catalogue of Boucek & Askew (1968) lists 6 species on *C.* (= *Phytomyza*) *fuscula*, 4 of these are on our list. Future studies involving larger areas will undoubtedly reveal more species. The sampling of 30 leaves from Frydenhaug indicated that a relatively small number of mined leaves is sufficient to expose major parts of the parasitoid complex, but of course larger samples are required to complete the picture. The main taxonomic difference between our and other investigations of parasitoids on agromyzids in crops (e.g. Drea et al. 1982, Darvas et al. 1984, Johnson & Hara 1987, Memmot & Godfray 1993, Schuster & Wharton 1993) is the almost complete absence of braconid parasitoids.

Both endo- and ectoparasitoids, as well as larval, larval-pupal, and pupal parasitoids are represented in the complex. All species are known to be mainly or exclusively solitary, and no cases of gregariousness was observed when examining leaves from the 16 field samples. Neither is any of the species obligate hyperparasitoids (with the possible exception of *Trichomalopsis sp.*), but *Pediobus acantha* (Boucek & Askew 1968) and *C. vulgaris* (Guppy & Meloche 1989) are reported to act as facultative secondary parasitoids.

Each year 2–3 species dominated, while the rest were present in rather small numbers. However, parasitoids rare on one host may be generalists having a great impact on the dynamics of other hosts (Memmot & Godfray 1993). The most numerous species, aptly named *C. vulgaris*, is one of the two pupal parasitoids present in any numbers (the other is *Chrysocharis pubicornis*, although its status as pupal parasitoid is somewhat uncertain, see Hansson 1985). It has been hypothesized (Hawkins et al. 1990) that competition is important among parasitoids of endophytic hosts. *C. vulgaris* is able to develop in an already parasitized host (Guppy & Meloche 1989), and it may thus prevail over competing pupal and larval-pupal parasitoids.

Host records suggest that *C. vittatus* and *Pnigalio soemius* are the most polyphagous species in this study, both attacking leafmining members of the four largest insect orders (Boucek & Askew 1968, Hansson 1987). *C. vittatus* is of particular interest here because it is one of the most common parasitoids of *Phyllocnistis labyrinthella* Bjerkander, a leafmining moth heavily attacking aspen (*Populus tremula*) in large parts of Southern Norway (Sundby 1957). *C. vittatus* reared from the moth successfully reproduce on *C. fuscula* in the laboratory (Trandem, unpubl.). The impact of large *Phyllocnistis* populations on the density of *C. vittatus* in nearby cereal fields (and vice versa) deserves closer study.

Most of the other parasitoid species are more or less specialized on agromyzids, but none is exclusively found on the genus *Chromatomyia* (Boucek & Askew 1968, Hansson 1985, 1987). Among the best known species is *D. begini*, which has been the subject of many biological control studies in the USA (see Heinz et al. 1993). Its presence in Europe has been noted (although not in the literature) since the fifties (H. v. Rosen and J. LaSalle, pers. comm.). Whether it is a native of Europe or has been introduced remains uncertain.

Trends in parasitization with increasing distance from the forest might be disguised by mortality, but it may also be that this lack of pattern is real for a cereal field of the rather small size (2.5 ha) studied. Investigating fields 10 times as big,

Landis & Haas (1992) found parasitization on first generation of the miner *Ostrinia nubilalis* to be lower in the interior than near the edge.

The substantial mortality was probably caused by disturbance (e.g. damage of pupal spiracles) and dehydration following collection. Adding water quickly leads to development of mould. Agromyzids pupating in the leaf are known as difficult to rear (Spencer 1976), and it may be that a mortality not far below 50 % is inevitable. Studies of agromyzids pupating in the soil report of mortalities above 50 % during prepupal/pupal stage (Johnson et al. 1980, Harcourt et al. 1988). Further studies are required to improve rearing methods, and to investigate how much of the mortality is inflicted by other agents, like fungi.

The scarcity of dead parasitoid pupae may imply that the parasitoids are less prone to lethal effects of collection than flies. In that case, the parasitization rate based on adult emergence overestimates the mortality caused by parasitoids. Basing the parasitization rate on pupae instead leads to a 25 % per cent lower average in the 16 field samples (Table 4). On the other hand, some parasitoid species may be rare or even absent because they were vulnerable to rearing conditions. Dead parasitoid larvae were difficult to search for and to distinguish from dead host larvae, and would not be registrated in any part of the study. Pupal parasitoids are probably less affected by leaf dehydration than are larval and larval-pupal parasitoids, as the fly puparium protects the parasitoid (as well as the host) throughout development. The relative frequences may therefore be skewed, in particular *C. vulgaris* may be overrated. Close examination of mines under development is required to clear up this problem.

Our study raises a lot of interesting questions which command further investigations, some of them already mentioned. Of particular interest is the impact of the surrounding vegetation and the management plan (*e.g.* time of tilling and cutting) upon fly and parasitoid densities. Answering these questions may simultaneously help explaining why *C. fuscula* is a pest in parts of its distribution area.

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Parasitoids of *Delia* root flies in brassica vegetable crops: Coexistence and niche separation in two *Aleochara species* (Coleoptera: Staphylinidae)

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Aleochara bipustulata and *A. bilineata* are both parasitoids of the cabbage root fly, *Delia radicum*. They are the most common members of subgenus *Coprochara* on cultivated soils in Northern Europe. Pitfall trapping of *Aleochara* spp. from May to September in a brassica vegetable crop in southern Sweden showed that the two species occurred together during most of the season. In the spring and early summer, however, *A. bilineata* was absent, while *A. bipustulata* was very abundant. Parasitoids reared from field collected fly puparia showed that *A. bilineata* utilized the whole size spectrum from small *Delia florilega/D. platura* to large *D. radicum* puparia. *A. bipustulata* developed successfully from small sized *Delia* puparia, including small sized *D. radicum* but not from large *D. radicum* hosts. The temporal separation and the host species separation of the two *Aleochara* species are only partial but obviously effective enough to permit coexistence. The possibility of utilizing both *Aleochara* species together in biological control of the cabbage root fly is discussed.

Keywords: *Aleochara*, biological control, cabbage root fly, *Brassica, Delia,* parasitoids

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INTRODUCTION

Staphylinid beetles of genus *Aleochara* Gravenhorst develop as parasitoids in puparia of higher Diptera. Thirty-five species occur in Northern Europe (Silfverberg 1992). In the cases where host associations are known, the species parasitise chiefly calypterate flies, especially Anthomyiidae, including species of economic importance (Peschke & Fuldner 1977, Dear & Smith 1978). *Aleochara* spp. parasitising *Delia* root flies of brassica vegetable crops belong almost exclusively to subgenus *Coprochara* Mulsant et Rey with five North European species. *A. bilineata* Gyllenhal and *A. bipustulata* (Linnaeus), both with a holarctic distribution, are well-known parasitoids of the cabbage root fly (CRF), *Delia radicum* (Linnaeus)(Fuldner 1960).

Newly hatched *Aleochara* larvae enter the host puparium by biting a circular hole through the puparium wall. After having consumed the host pupa and completed

three larval instars, they pupate. The members of subgenus *Coprochara* pupate inside the host puparium. Apart from being ectoparasites on fly pupae in the larval stage, the adult beetles are predators on fly eggs and larvae. These characteristics have made *A. bilineata* one of the main candidates for biological control of CRF (Bromand 1980, Finch 1993).

A. bilineata hibernates as a first instar larva inside the host puparium. It starts feeding on the pupa in the spring and the adult beetle emerges in early summer (Fuldner 1960). Emergence normally occurs a few weeks after the peak oviposition period of the first generation of CRF. Adult *A. bilineata* are therefore incapable of substantial predation on CRF eggs. A solution to this problem would be to rear *A. bilineata* under artificial conditions and to release the beetles in the field when CRF oviposition starts (Bromand 1980). An alternative would be to benefit by another prospective biological control agent: The closely related *A. bipustulata*.

A. bipustulata has a life cycle completely different from that of *A. bilineata* , since it hibernates in the adult stage (Fuldner 1960). In spite of the differences in hibernation biology, the two species often occur together in brassica vegetable fields. If two biological control agents are to be used simultaneously, it is important that any competition is minimal. The aim of this paper is to determine the host spectrum and temporal occurrence of the two *Aleochara* species and assess the risk of competition.

MATERIALS AND METHODS

The studies were performed in 1989 on a small scale ecological farm at Fulltofta, province of Skåne, southern Sweden in a 150 m^2 plot with cauliflower and white cabbage. No other brassica crops were grown on the farm. In an adjacent field, the previous year's crop was swedes. The gravel-mixed loam soil was fertilized with cow-dung compost before the brassicas were transplanted in late April. Plant distance was 0.6 x 0.6 m. During the establishment phase, the plants were protected with a light-weight crop cover («Agryl»). It was removed in mid May, when CRF oviposition started. At the same time, poultry-manure was applied as an additional fertilizer and white mustard (*Sinapis alba*) seed meal was used as a combined herbicide (Ascard & Jonasson 1991) and *Aleochara*-attracting material (Ahlström-Olsson & Jonasson 1992). The meal was applied as a 20 cm strip on the soil surface along the plant rows (ca. 20 g per row-metre in the strip). No irrigation was used.

Cylindrical (10 cm diameter x 12 cm) soil samples for pupae around the brassica roots were taken on 10 July. Pupae were washed out and identified. Individual pupae were kept separately in small plastic containers (ELISA-plates). Puparia and emerging *Aleochara* specimens were measured (see below). After the emergence of flies or parasitoids, the remaining puparia were dissected and examined. *Delia platura* (Meigen) and *D. florilega* (Zetterstedt) males were bred from the material, but puparia could not be reliably separated. These two *Delia* species, which usually occur together (Hennig 1976), are referred to as the «bean seed fly» (BSF).

The *Aleochara* activity patterns were followed by trapping from mid May to late September. Eight pitfall traps placed ca. 6 m apart over the plot area were used. Traps were 500 ml plastic cups of 12 cm diameter half-filled with water and detergent. They were emptied once a week. All *Aleochara* specimens were sorted out and glued on cardboard strips to allow genital dissection for a reliable identification.

The maximum width of pronotum was measured to the nearest 1/50 mm with an ocular micrometer. The maximum width of puparia was measured to the nearest 1/12 mm. The pupal weights indicated in tab. 2 were calculated from data from a large material of CRF pupae reared in the laboratory (cf. Ahlström-Olsson 1994).

RESULTS

Pitfall trapping yielded ca. 1000 specimens of four *Aleochara* (*Coprochara*) species during the season. *A. bipustulata* was most common (66 %), followed by *A. bilineata* (31 %), *A. binotata* Kraatz (3 %) and the coprophilous *A. verna* Say (2 specimens).

The phenology/activity curves are shown in Figure 1. For *A. bipustulata*, four peaks are indicated. The first occurred when beetles invaded the field from their

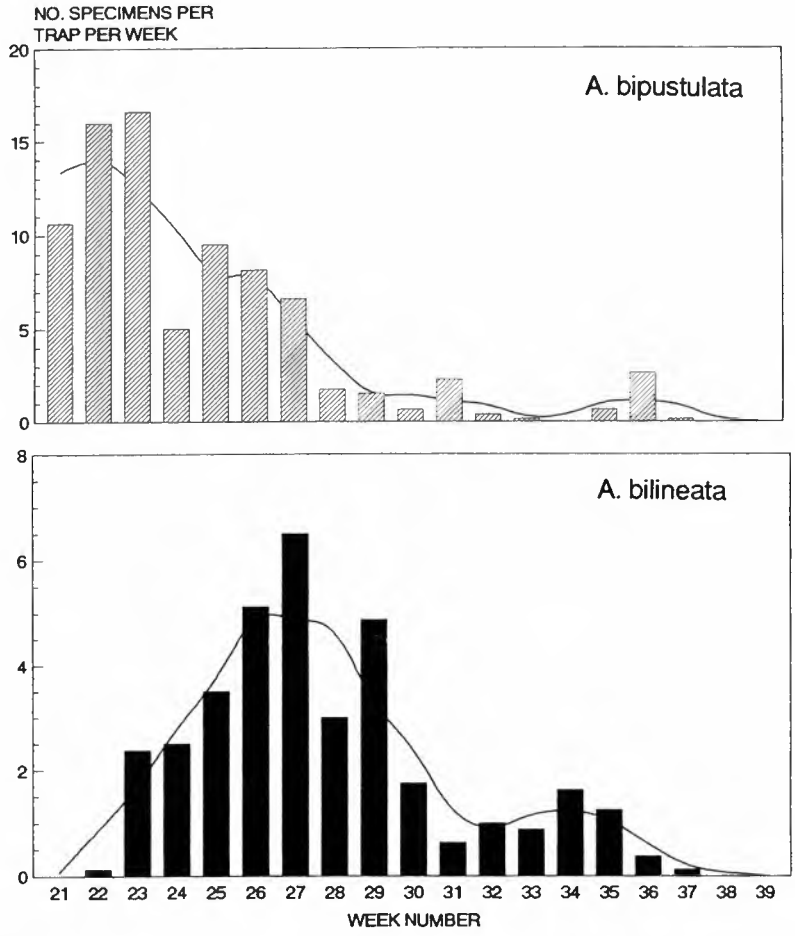

Fig. 1. Activity patterns according to pitfall trappings over the season in *A. bipustulata* and *A. bilineata*. Bars indicate the actual mean numbers of beetles caught per trap per week. The curves are based on a three-point moving average.

hibernation sites. The second peak during mid June to early July represents the first generation of the season, followed by a third peak after about five weeks (second generation) and finally, after another five weeks, a fourth peak, representing the third generation. The adults emerging during early September probably constitute the hibernating generation. The phenology curve for *A. bilineata* shows only two peaks, representing the adults developing from hibernating larvae during late June and early July and a second peak about 7 weeks later. The intervals between generation peaks in *A. bipustulata* and *A. bilineata* agree with the developmental times given by Fuldner (1960), which were 33 and 46 days respectively.

Adult *A. bipustulata* and *A. bilineata* occur simultaneously for most of the season. The only period with predominately one species active is before mid June, where *A. bipustulata* dominates (Figure 1). This period coincides with the oviposition of the first CRF generation.

Mid June to mid July is a period of special interest for the predation by *Aleochara* larvae on first generation CRF pupae. During this period the first adult generation of both *A. bipustulata* and *A. bilineata* were found in the field. The pupal period of the first CRF generation also occurs at this time. A total sample of 72 BSF puparia and 282 CRF puparia was collected from 40 plants. Between 20 and 30 % of the puparia were empty and broken at sampling or severely damaged during handling and therefore were discarded. The remaining 49 BSF and 227 CRF puparia were used for further studies. Many puparia of Agromyzidae and Drosophilidae, some of Fanniidae and a few of Muscidae were also found in the soil samples, but none of these yielded *Aleochara* specimens.

Numbers and species of parasitoids emerging from BSF and CRF puparia are listed in Table 1. Means and standard deviations of pronotal widths of emerged *A. bilineata* and *A. bipustulata* specimens are also included. *Aleochara* specimens

Table 1. Parasitoids reared from field collected *Delia* puparia. BSF = *D. platura/florilega*; CRF = *D. radicum*. Pronotal width was measured on all specimens of *A. bilineata* and *A. bipustulata* emerging.

Puparium content	Number of puparia		Pronotal width (mm, mean ±SD)	
	BSF	CRF	BSF	CRF
Aleochara bilineata	5	76	0.83 ±0.036	1.08 ±0.066
Aleochara bipustulata	7	25	0.87 ±0.053	1.03 ±0.067
Aleochara binotata	4	3		
Aleochara spp. (dead)	3	26		
Trybliographa rapae	0	15		
Trybliographa diaphana	3	0		
Unidentified mortality	4	38		
Flies (alive)	23	44		
Total	49	227		

developing in CRF pupae were significantly larger than those developing in BSF pupae. *Trybliographa rapae* (Westwood) and *T. diaphana* (Hartig) (Hymenoptera, Eucoilidae) are larval parasitoids of CRF and BSF, respectively (Kerrich & Quinlan 1960).

The distribution of *Aleochara* spp. in different pupal sizes of CRF are shown in Table 2. The distribution of *A. bilineata* did not deviate significantly from a hypothesized, random distribution over pupal size classes. *A. bipustulata*, however, showed a significant asymmetry in host size utilization. No specimens emerged from the largest host pupae. A similar asymmetry seems to occur in *A. binotata*. The pattern for dead *Aleochara* individuals is also highly asymmetric with a low proportion in small puparia and a high proportion in large puparia.

The body size distributions (pronotal widths) of beetles from the pitfall traps show that the frequency of large specimens was higher in *A. bilineata* than in *A. bipustulata* (Figure 2).

DISCUSSION

Neither of the *Aleochara* species is highly specialized to parasitise CRF, since BSF is also utilized as a host (Table 1). *A. bilineata* seems better able to cope with large CRF pupae than *A. bipustulata* (Table 2). In experiments with *A. bipustulata* parasitising *D. floralis* (Fallén), which normally is somewhat larger than CRF, Andersen (1982a) found a 90 % mortality of the parasitoid. Fuldner (1960) reported a 20 % mortality of *A. bilineata* developing in CRF pupae. If the *Aleochara* larva reaches its upper size limit and stops feeding before it has consumed the host, the remains of the host pupa may become a substrate for microorganisms (Fuldner 1960). Hence, the parasitoid may be killed, either directly by a pathogenic activity or indirectly by poisoning or suffocation. First instar *Aleochara* larvae discriminate by host size. *A. bipustulata*

Table 2. Distribution of *Aleochara* spp. in different pupal size (maximum width) classes of cabbage root fly (CRF). The size classes approximately correspond to pupal weights of 4–9, 10–14, and 15–20 mg.

Pupal size classes:	Small (1.5–1.9 mm)		Medium (2.0–2.2 mm)		Large (2.3–2.5 mm)		Test of significance	
Purarium contents	n	%	n	%	n	%	chi^2	p
(1) *A. bilineata*	22	33	42	39	12	33	0.56	0.757
(2) *A. bipustulata*	14	21	11	10	0	0	8.99	0.011
(3) *A. binotata*	3	4	0	0	0	0	–	
(4) Dead *Aleochara*	1	1	15	14	10	28	13.71	0.001
(2) + (4)	15	22	26	24	10	28	0.31	0.856
(5) Unidentified	18	27	14	13	6	17	4.33	0.115
(6)Flies	9	13	27	25	8	22	2.80	0.247
Total	67		109		36			

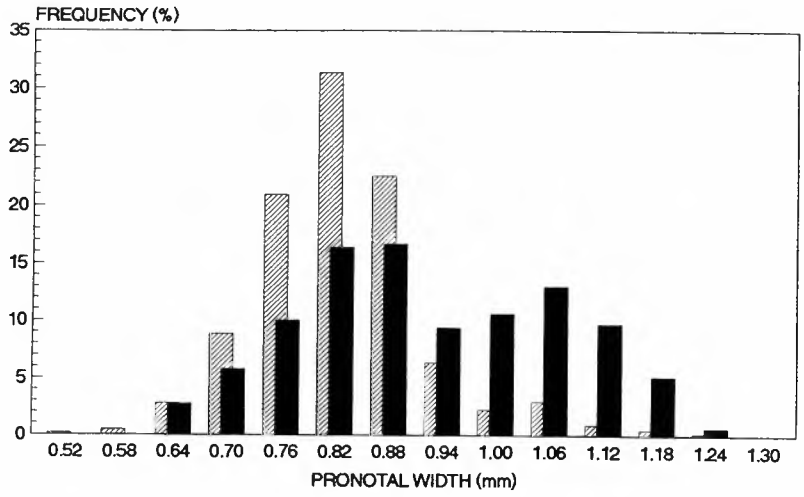

Fig. 2. Body size distribution in the total pitfall samples of *A. bipustulata* (hatched) and *A. bilineata* (black). Each bar represents 3 pooled classes differing 0.02 mm. The mid class size of each bar is indicated on the horizontal axis.

attack small, while *A. bilineata* prefers medium and large sized CRF pupae (Ahlström-Olsson 1994). In the field, the possibility of selecting a suitable host may be restricted, however. The distribution of *A. bilineata* over CRF size classes did not deviate significantly from a hypothesized, random distribution (Table 2).

The identity of the dead *Aleochara* specimens (Table 2) is unknown. The high mortality rate in large host pupae reported by Andersen (1982a) for *A. bipustulata* strongly indicates, however, that the dead specimens in the present material were mainly *A. bipustulata*. If *A. bipustulata* and the dead specimens are combined, the distribution over pupal size classes does not deviate significantly from a hypothesized, random distribution (Table 2).

The size distribution of *A. bipustulata* (Figure 2) shows a dominating peak of medium sized beetles and an additional, very small peak of large specimens. The two peaks correspond well with the mean sizes reared from field collected BSF (0.87 mm) and CRF (1.03 mm) pupae. The size distribution of *A. bilineata* (Figure 2) is clear-cut bimodal with the two peaks also corresponding to beetles reared from BSF (0.83 mm) and CRF (1.08 mm).

A prerequisite for the coexistence of the two *Aleochara* species in brassica vegetable crops is a niche separation. *A. bilineata* has a generation time of about 7 weeks and is well synchronized with the CRF generations (Fuldner 1960, Bromand 1974). *A. bipustulata* has a cycle of about 5 weeks, which seems well synchronized with that of BSF (Gratwick 1992).

The present study shows that *A. bipustulata* probably is of little importance as a parasitoid of CRF. The abundance of adult beetles during the oviposition period of the first CRF generation, however, indicates that the species may act as an egg predator (together with other staphylinids and carabids). According to Coaker (1965), *Aleochara* spp. (including *A. bipustulata*) were the most common of the beetles found around the base of brassica plants, where CRF eggs normally are laid. Dinther & Mensink (1971) and Tomlin et al. (1985) have shown that *A. bipustulata* eats fly eggs.

Further studies are needed to evaluate the role of *A. bipustulata* as an egg predator in the field. If it should prove to be efficient, one may speculate on the possibility to utilize wild *A. bipustulata* instead of artificially reared *A. bilineata* in the biological control of CRF. The niche separation in the two *Aleochara* species could perhaps be capitalized on by letting them operate in «shifts», *A. bipustulata* eating the eggs and *A.bilineata* parasitising the pupae. Adult *A. bipustulata* occur naturally in the spring and early summer in brassica crops, especially on sandy soils (Andersen 1982b). They are attracted by mustard seed meal and can therefore be manipulated to aggregate in treated fields (Jonasson 1990, Ahlström-Olsson & Jonasson 1992).

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Data about several communities of Ichneumonidae studied by use of malaise traps

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The fauna of parasitic Hymenoptera was studied in the mountain of Porta-Coeli (Sierra Calderona, Iberian System). During this project part of the Sierra Calderona burned, and a large part of the cork tree habitats was destroyed. The present study investigates the recolonization of different subfamilies of Ichneumonidae, using quantitative analysis.

Can leaf miners perceive a foraging parasitoid by its vibratory signals?

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The success of a hunting parasitoid can be highly dependent on the potential of its host to escape. Larvae and pupae of the tentiform leaf miner *Phyllonorycter malella* (Ger.) (Lepidoptera: Gracillariidae) show a very strong escape reaction when attacked by the polyphagous parasitoid *Sympiesis sericeicornis* Nees (Hymenoptera: Eulophidae). After detecting the presence of a parasitoid, the lepidopteran larvae and pupae move violently with their whole body, a behaviour denoted as «wriggling» (Meyhöfer et al., unpubl.). This behaviour can enable the host to escape the ovipositor stings; previous field observations showed that in 10% of the cases the parasitoid abandons the host without parasitizing it, sometimes after a long hide-and-seek game (Casas 1989). The escape behaviour has been observed in three different situations: after mechanical disturbance, during landing on the leaf by any kind of insect, and host location and attack behaviour by the parasitoid. These observations, as well as the presence, structure and position of typical hairs over the body of the host lead us to hypothesize that the host uses vibrations to perceive an attacking parasitoid.

As a first step in testing our hypothesis, we recorded vibrations of foraging female parasitoids with a laser vibrometer as described in Meyhöfer et al. (unpubl.). Simultaneously, activities of the females were recorded on video. The behaviour of foraging parasitoids is classified into the categories *Landing* (on the leaf), *Standing Still, Moving* and *Probing*. The transient nature of the vibrational signals as well as waves travelling several times across the leaf made it impossible to assign signals to behavioural categories defined at a finer scale. We used the maximum frequency of a signal and the highest frequency with the maximum intensity to characterise the vibrational signals belonging to each category in the frequency domain. Only frequencies that reached at least twice the intensity of the average background noise were considered as signals. In all behavioural categories with the exception of *Standing Still*, where no vibrations above the background noise were detected, the parasitoid elicited broad banded signals. The results (Table 1) show that vibrations caused by foraging females of *S. sericeicornis* can reach frequencies which are clearly higher than vibrations produced by other sources in the environment (Meyhöfer et al., unpubl.). They contain highly specific information about the female behaviour and therefore represent reliable cues by which the leaf miner could detect the presence of a parasitoid. However, as for vibrations caused by the leaf miner (Meyhöfer et al. 1994), the reported high frequencies occur as rare events and are highly dependent on leaf structures and the position of sender and receiver on the leaf. Further research will focus on the frequency distribution of signal components in time.

Table 1:
Maximum vibrational frequencies caused by a foraging female of *S. sericeicornis* during different behavioural categories.

Behavioural category	Maximum frequency	Frequency with maximum intensity
Landing	17.0 kHz	4.5 kHz
Probing	3.5 kHz	1.0 kHz
Moving	2.0 kHz	0.7 kHz

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Oviposition behaviour of the aphid parasitoid *Aphidius ervi* Haliday on its host *Acyrthosiphon pisum* (Harris)

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The oviposition behaviour of the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera, Braconidae) on its host *Acyrthosiphon pisum* (Harris) (Homoptera, Aphididae) was studied. *A. ervi* females were introduced in a Petri dish containing mixed instars of *A. pisum* and video-recorded. The quantitative analysis of parasitoid behaviour was conducted on 150 oviposition attacks, with the aid of a computer and an event-recording software (The Observer, Noldus Information Technology, Wageningen, The Netherlands). An ethometric analysis of behavioural transitions was carried out and graphically represented as a flow diagram. In most cases female parasitoids became aware of aphid presence at a distance of 1 cm or less, typically showing antennal orientation towards the host, followed by sudden attack. This behavioural step in the host selection process was apparently in part regulated by visual stimuli. Aphid dummies were prepared by flame sealing a clean glass capillary, which was filled with crushed pea aphid bodies. Two millimeters of the apical tip of the sealed capillary were exposed to virgin naive females of *A. ervi*, along with a clean empty control. Antennal examinations of capillaries filled with crushed aphids were more frequent and longer than in the case of controls. Furthermore, 21% of the 40 females tested tried to oviposit into test capillaries, while controls never stimulated such a behavioural response. When the female parasitoid comes in contact with its host, the chemical stimuli play a key role in the regulation of host recognition and acceptance behaviour. The cornicle secretion of *A. pisum* acts as a contact kairomone, stimulating a strong ovipositional response in *A. ervi* (Battaglia et al. 1993), independently from the size and shape of the treated aphid dummies. Glass beads, ranging in size from 2 up to 6 mm in diameter, as well as flat surfaces, were all accepted by *A. ervi* females when coated with *A. pisum* cornicle wax. Naive females of the parasitoid showed a reaction rate, to all types of aphid dummies, significantly higher than females with oviposition experience.

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Isolation and identification of kairomones utilized by southern pine beetle parasitoids

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When bark beetle larvaes reach the last instar and pupate, parasitoids are attracted to their host trees. We are currently studing the volatile compounds that help the parasitoids to locate host trees, and identify larvaes and pupaes under the bark.

Bolts of parasitoid attractive or not-attrative beetle infested pine trees, were cut and brought to the laboratory, were they were put in 55 gal. barrels. Dried and charcoal filtered air was passed over the bolts, and the released volatiles were collected on Porapak Q columns (15 cm x 2 cm I.D.; Q = 2-5 L/min.). The columns were extracted with diethyl ether and the extracts were analysed using combined gas chromatography and mass spectrometry (GC-MS; Hewlett-Packard 5890-5970). The chromatograms from different pine trees (*i.e.* shortleaf and Virginia; attractive and non-attractive) were analysed qualitatively and semi-quantitatively. The amount of each compound was calculated on the basis of arbitrary area units in the chromatograms, aeration time, and extract volumes.

Hundreds of compounds in the different collections were identified or described, most of which were present in all samples. The main difference between bolts, attractive or non-att-ractive to parasitoids, are the different amounts of oxygenated compounds. For both shortleaf and Virginia pines the releases of oxygenated monoterpenes, such as camphor, fenchone, isopino-camphone, pinocamphone, *trans*-pinocarveol, α-terpineol, and *trans*-verbenol, are much higher from parasitoid attractive pine trees than from non-attractive ones. The release of 4-allyl anisole and bornyl acetate from attractive shortleaf pines is much higher than from non-attractive ones, while the release of these compounds are very low from all Virginia pines.

Several of the above mentioned oxygenated compounds have been tested in field bio-assayes.

Our hypothesis is that general compounds, such as oxygenated monoterpenes, guide the parasitoid females to an attractive bark beetle infested tree. After the females have landed on the tree, they actively search on the surface for late instar larvaes and pupaes in the bark. As a second step in the hostfinding, specific volatiles emmanating through the bark from the pupal chambers help the parasitiods to locate the larvae and the pupae.

Learning affects how *Anaphes* nsp. discriminates (Hymenoptera: Mymaridae)

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Learning affects how several species of parasitoids select habitat and host. Although it has been shown that host discrimination, the ability to distinguish parasitized from non-parasitized hosts, does not need to be learnt, experience in previously visited patches influences the willingness of a parasitoid to superparasitize and therefore changes its reaction to the stimuli perceived during host discrimination (Visser et al. 1992). The process of host discrimination could also be influenced by learning because increased efficiency would result in time saving.

To evaluate if experience modified behavioral sequences in host discrimination, *Anaphes* nsp. (Mymaridae), a solitary egg parasitoid of *Listronotus oregonensis* (Curculionidae), was used. Individual mated *Anaphes* females, aged one day and experienced once in a unparasitized egg, were placed in contact with a patch consisting of 20 eggs, half of them parasitized one hour before the experiment either by the same female (self-discrimination), by a conspecific (conspecific discrimination) and by a closely related species *Anaphes sordidatus* (Girault) (interspecific discrimination). Unparasitized hosts were always accepted and eggs punctured were replaced by eggs of the same category. Ten replicates were performed and for each female the 10 first rejections were classified as antennal rejection if only antennal drumming was observed or as sting rejection if it occured after penetration of the ovipositor.

Learning was demonstrated in all cases. While sting rejections were more numerous in the first few encounters, antennal rejection gradually increased until it represented the majority of rejections at the end of the experiment. Differences in learning were found between the three experiments. In self-discrimination, sting rejections represented 75% of the cases at the first encounter but it fell rapidly to 13% after 6 encounters and at 7% after 10 encounters. Learning was slower in both conspecific and interspecific discriminations where sting rejections still represented the majority of cases until the 5^{th} or 6^{th} encounter but, for both cases, it represented only 8% at the end of 10 encounters.

Upon encountering a parasitized host, a female *Anaphes* touches the surface of the egg with its antennae for about 3 sec before rejecting it, but a sting rejection takes about 42 sec. The advantage of recognizing external marking with antennal drumming is obvious but it appears that the females need to learn those marks, probably by

association with the stimuli present in the egg. The odor left by the same female is learned more rapidly than the odor left by conspecific or by *A. sordidatus*. We hypothesize that internal marking indicates to the female that the host is parasitized but external marking indicates the identity of the first female that has oviposited. Such an information would be more variable and therefore adaptive to learn.

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The composition and phenology of the genus Dichrogaster doumerc in central and south-eastern Spain (Hym., Ichneumonidae)

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Dichrogaster Doumerc, 1855 is a moderate sized genus of *Gelina* (Cryptinae, Phygadeuontini), of almost worldwide distribution. The species parasitize cocoons of Chrysopidae.

In the Iberian Peninsula only one species was referred by Antiga & Bofill (1904), *D. aestivalis* (Gravenhorst, 1829), but the revision of material shows that the specimen does not belong to *Dichrogaster*, so this study reports the first data about the species composition and phenology of this genus in Spain.

In two different habitats, 245 individuals were collected in two Malaise traps during one year. The studied localities were El Ventorrillo (near Madrid) a mountainous area at a height of 1480 m. and Moncada (Valencia) a Mediterranean area near the sea.

The results show that three species occur in El Ventorrillo: *D. modesta* (Gravenhorst, 1829), *D. longicaudata* (Thomson, 1884) and one undescribed species. In this area, *D. modesta* is the predominant species, followed by the new species. The flight activity of them has clear peaks in summer. *D. longicaudata* seems to be very rare (only seven specimens were collected from July to August).

In Moncada only *D. longicaudata* and the new species are present. The former is much more abundant than the latter, and has peaks of flight activity in spring and at the end of autumn, and distinct minima both at the end of summer and winter.

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In vitro rearing of *Brachymeria intermedia* (Nees) (Hymenoptera Chalcididae) on veal homogenate-based diets

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Brachymeria intermedia is a solitary pupal endoparasitoid of the gipsy moth *Lymantria dispar* (L.) as well as of several other lepidopterans. Complete *in vitro* culture of this parasitoid was achieved on two oligidic diets containing 20% extract of *Galleria mellonella* L. pupae and 80% commercial baby food (Plasmon^{R}), i.e. veal homogenate for babies at the beginning of weaning (a) or well on in weaning (b). Both diets were set in 1.2% agar and supplemented with gentamycin sulphate. Five replicates were carried out, each comprising 30-35 eggs per diet. No significant differences were found between the two diets in the percent of hatched eggs. On diet (a) 89.7% larvae reached maturity, 41.8% of which became pupae, while on diet (b) the percent yields of mature larvae and pupae were 70.6 and 7.6 respectively. Completion of larval development on both diets took approximately 2-3 days more than the time usually required in the host. The percent yields of adults, calculated on the number of hatched eggs were 27.4% and 2.7% in diet (a) and (b), respectively. Adults were fecund. Diet (a), therefore, proved to be more suitable than diet (b) for *B. intermedia*. This was probably due to the fact that the level of proteins, carbohydrates and lipids as well as of the calories of the a-homogenate is higher than that of the b-homogenate.

The a-homogenate is no salt added, whereas the b-homogenate contains 0.25% sodium chloride. The former, moreover, has a smoother texture than the latter. These factors, however, did not apparently affect the development of *B. intermedia*: in a subsequent test, performed using a diet containing 80% commercial sodium chloride-added veal homogenate with coarser texture (c), intended for babies starting to chew, the percentage of adult yields was as high as 33%. The level of proteins, carbohydrates and lipids of the c-homogenate is only slightly different from that in the a-homogenate.

It may therefore be concluded that commercial a- and c- veal homogenates for babies are potentially effective ingredients in artificial diets for *B. intermedia*.

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Population dynamics of braconid wasps in East Spain (Hymenoptera: Braconidae)

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The phenology of a braconid population in East Spain has been investigated. Material was collected by Dr. M.J. Verdu at Montcada (province of Valencia) by means of a white Malaise trap for one year, from March 1992 to March 1993. The trap was placed in a *Pinus halepensis* habitat surrounded by orange grove.

The samples were separated to subfamily level. The collected specimens belonged to 17 subfamilies of Braconidae. The group of greatest abundance was Aphidiinae, with 71.4 % of the total of specimens; followed by Alysiinae and Microgastrinae, but with about 7 %. Miracinae was collected with 3.4 % of relative abundance. Braconinae, Euphorinae, Homolobinae, Macrocentrinae and Opiinae were subfamilies ranging from 1 % to 1.9 %. Adeliinae, Agathidinae, Blacinae, Cheloninae, Helconinae, Neoneurinae, Orgilinae and Rogadinae had a relative abundance of less than 0.6 %.

The abundance of Braconidae showed two peaks: one from middle May to middle July, the other from beginning of October till the end of December. The first period had a smaller number of specimens but the diversity of subfamilies was the highest. The second peak consisted almost exclusively of Aphidiinae.

Venom gland and reservoir morphology in Opiinae wasps (Hymenoptera, Braconidae)

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Venom reservoir and venom gland morphology have been investigated in Opiinae wasps, looking for some differences between several groups of species of this subfamily.

The metasomas of dry museum specimens were removed and soaked for 15 minutes in NaOH 1% at 100°C. The metasomas were then transferred to 50% alchohol, removing the venom apparatus; these apparatus were stained with 1% chlorazole black, dehydrated and mounted on microscope slides using entellan.

All the Opiinae species have a type I venom apparatus, which is characterized by a reservoir with a thick muscular wall and spiral thickenings on its surface. The study revealed some differences in the reservoir morphology, primary duct morphology and number of gland filaments.

Studies on short range host location of *Rhopalicus tutela* (Walk.) (Hymenoptera: Pteromalidae) A common larval parasitoid on *Ips typographus L.* (Coleoptera: Scolytidae)

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Parasitoids on bark beetles must be able to locate host larvae under the bark of spruce logs. Field observations has indicated that female parasitoids use their antennae to detect and locate potential hosts for oviposition. During active search the parasitoids bend down the tip of their antennae to the substrate. These are then sweeped over the substrate until the exact position of the host larvae is located.

Scanning electron microscopy studies of the antennae from *Rhopalicus tutela* has revealed that the outermost distal segments (flaggelar segments) of the antennae has an unusual high concentration of, at least, three types of sensilla. Knowledge of antennae morphology is of great help for the purpose of making electrode insertion easier when single-cell recordings are conducted (see below).

Our hypothesis is that the parasitoids, when locating host larvae, use their antennae to detect chemical cues emitted from bark beetle larvae or associated organisms. The object of this study is to isolate and identify these chemical cues.

The searching behaviour of an actively searching female is followed. Just as the ovipositor is inserted she is taken away and volatiles are collected on Porapack® Q plugs (50 mm x 3 mm I.D.). As controls we use air entrainments from places where the female just has passed without eliciting the oviposition behaviour.

The plugs were extracted with diethyl ether and the extracts where analysed using combined gas chromatography and mass spectrometry (GC-MS).

Several hundreds of different components in the air entrainment were detected by GC-MS. Only a few of these are bioactive. In order to get information of retention times of components with biological activity we use combined gas chromatography - electroantennogramdetector (GC-EAD) technique.

A living parasitoid is fixed in a specially constructed holder. The left antenna is fixed in dental wax. The indifferent electrode is inserted in the frons. The recording electrode is put into the tip of the antenna. Capillary glass electrodes with Ringer solution are used.

A short video recording of the searching behaviour is included in the poster.

Variation in host use in *Encarsia formosa*

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By exploiting the genetic variation in ecologically important traits that must exist within a parasitoid species, we may be able to better utilize that species for biological control. We tested the hypothesis that different populations of the parasitic wasp *Encarsia formosa* varied in their use of different host species, *Bemisia tabaci* and *Trialeurodes vaporariorum*. The wasp populations tested were chosen because they had been reared for many generations on either of these two hosts, and we made the assumption that laboratory rearing on a particular host was a selective force for performance on that host. The experiment was conducted in such a way that we could distinguish heritable differences between populations from physiological differences due to the immediate host from which an individual wasp eclosed. For the bioassay we caged individuals wasps with whitefly larvae for a period of five hours; performance was measured by the percentage of whiteflies that tuned into darkened wasp pupae. The three wasp populations tested did vary in their performance on *Bemisia tabaci*, with one of the *B. tabaci* reared populations performing considerably better than the others. There was no significant variation between populations in their use of their preferred host, *Trialeurodes vaporariorum*, however. The physiological effect of rearing was also significant; *B. tabaci* produced wasps with lowered performance, although again this did not vary when tested on *T. vaporariorum*. In summary, it appears that populations of *E. formosa* do differ in their performance on different host species, thus the use of populations differentially adapted to a particular host may improve the performance of this biological control agent. As *E. formosa* is an asexual wasp, this system provides a conservative test of the likelihood of heritable variation in traits of such ecological importance as host use in a parasitoid.

Morphology of the venom apparatus in the *Euphorinae* (Hymenoptera: Braconidae)

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The subfamily Euphorinae comprises 34 genera and over 550 species of parasitic wasps. Euphorinae has the most diverse host associations of any braconid subfamily. The euphorines have been associated with Orthoptera, Hemiptera, Psocoptera, Neuroptera, Coleoptera, Lepidoptera and Hymenoptera.

The euphorine females present a venom apparatus type 2. Typically, the type 2 venom apparatus consists of a thin-walled reservoir surrounded by relatively few muscles, two gland filaments one cell in thickness surrounding a central lumen, and a venom duct which extends from the base of the reservoir into the ovipositor.

Detailed venom reservoir and venom gland intima morphology have been investigated in representative genera of Euphorinae.

Chemically mediated host searching behaviour in a parasitoid of *Phyllonorycter blancardella* F. (Lepidoptera, Gracillariidae) on apple

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The larvae of the Gracillariid moth *Phyllonorycter blancardella* F. build mines in the leaves of apple plants. By reducing photosynthesis of their host plants they may cause damage of economic importance. The parasitoid complex of *P. blancardella* is well known and contains over 30 species. Recent studies show that chemical cues from plants play an important role in the foraging behaviour of parasitoids. To test if the braconid wasp *Apanteles c.f. circumscriptus* (Nees) also responds to plant odors they were exposed to different odours in a Y-tube olfactometer. The wasps were experienced on a leafminer-infested plant for half a day before the bioassay. Each wasp was picked up with a freshly cut infested leaf and introduced into the Y-tube. A trial was counted as decision if the wasp entered 10 cm into an arm through which an odour was introduced. In the first experiment wasps were given a choice between clean air and air that had been passed over two leaves infested by *P. blancardella*. The wasps showed a very clear preference for the leaf odour, with 22 wasps (91.7 %) making a choice for the odour of leafminer-infested leaves and only 2 (8.3 %) choosing the arm with clean air (12 wasps did not make a choice). In a second bioassay the wasps had to choose between the odours of a *P. blancardella*-infested plant and a healthy control plant. A total of 32 animals (88.9 %) walked into the arm of the infested plant and 4 animals (11.1 %) chose the arm of the healthy plant (12 wasps did not make a choice). In a final experiment, volatiles from infested apple seedlings were collected on an adsorbent and later extracted with methylene chloride. The extract was placed on a filter paper disk. The solvent only was placed on a control disk. Again the odour of infested plants was attractive; 23 wasps (76.7 %) chose the odour of infested leaves, whereas 7 wasps (23.3 %) chose the control odour (6 wasps did not make a choice). The experiments indicate that the wasps use volatiles emitted from the plant-leafminer complex in host location. The wasps can also make a clear distinction between infested and healthy plants. It is possible to collect these volatiles thereby making it possible to determine their chemical nature in further studies.

Spatial and temporal changes in cereal aphid and hymenopteran parasitoid populations after an insecticide application

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The spatial distribution of insect hosts in field situations has very rarely been recorded, and the corresponding distribution of their hymenopteran primary- and hyper-parasitoids or even parasitism rates is even less well known.

As it is known that many insect parasitoids search in a methodical, non-random pattern and that the effectiveness of their role of being biocontrol agents includes a sigmoid functional response to their host patches, then studies investigating the relative abundances of hosts, their parasitoids and parasitism rates are long overdue.

Pesticide applications not only directly harm hymenopteran parasitoids (Elzen 1989) and therefore reduce their biocontrol potential, but they can also completely remove or alter the distribution of hosts, leading to changes from aggregated patches to very small randomly spaced populations. What effect this change in host distribution has upon the biocontrol ability of the surviving and/or re-invading parasitoids is becoming an increasingly important question at a time when general farming policies move towards the use of dose reductions in agrochemical inputs.

This paper reports on a field experiment conducted in a winter wheat crop in southern England with spatially explicit sampling points over 4 hectare square plots. Two doses of deltamethrin (field rate and a reduced dose representing 1/20th of the field rate) were applied with a control left unsprayed. Records of cereal aphid, primary parasitoid and hyper-parasitoid numbers were collected via visual counts, suction sampling and sticky trap catches over the following 36 days after treatment.

To date, the findings show that compared to the control, an overall reduction of the aphid and hymenopteran populations occurred after the insecticide applications. Recovery of these populations in the treated areas occurred in random patches and does not seem to have followed the uniform invasion of new recruits from the edge to the centre of the plots as shown by other invertebrate species.

Further analysis of the data will be conducted to investigate the more intricate relationships between the reinvasion and aggregation of the aphid and hymenopteran populations.

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First record of Mymarommatoidea (Hymenoptera) for the mediterranean basin

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The Chalcidoidea (Hymenoptera) of several areas of ecological interest of Eastern Spain was studied through 1992. Several specimens of the superfamily Mymarommatoidea were captured at the Sierra Calderona with Malaise trap at the end of August.

The Malaise trap was placed on a Mediterranean bush where the cork oaks were predominant. A fire on early September destroyed the area and it was impossible to study the phenology of this group.

Mymarommatoidea, until recently a family of Chalcidoidea, has a great significance on phylogenetical studies of Hymenoptera. They are rarely caught and have been found mainly in amber on tropical zones and temperate deciduous forests. This is the first record for the Mediterranean area. An electron-microscopy study of their morphology is presented.

Biological Control of a Canadian Canola Pest, the Bertha Armyworm (*Mamestra configurata*), with the European Parasitoid *Microplitis mediator*

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INTRODUCTION

The bertha armyworm, *Mamestra configurata* Walker, has been an important pest of rape and canola (*Brassica napus* and *B. campestris*) crops on the Canadian prairies. Damage is primarily caused by direct larval feeding on the seed-containing pods but defoliation of plants can also result in losses.

The objective was to augment control of the bertha armyworm effected by the primary native parasitoid species, an ichneumonid wasp (*Banchus flavescens* Cresson) and a tachinid fly (*Athrycia cinerea* (Coquillett)), by introducing a parasitoid which attacks younger (first to third) instars of bertha armyworm, has a short development time permitting at least two generations per year, and attacks a variety of hosts allowing it to maintain high field populations between population increases of bertha armyworm. The braconid wasp, *Microplitis mediator* (Haliday), which parasitizes the cabbage cutworm (*Mamestra brassicae* (Linnaeus)) in Europe, is a parasitoid that is easy to rear in the lab and fulfills the requirements mentioned above (Arthur and Mason 1986).

METHODS

Importations of European *M. mediator*, collected by Dr. Klaus Carl, I.I.B.C. Delémont, were made in 1990–1992. The parasitoids were reared in the laboratory during the winter on *M. configurata.* Parasitoid release localities were selected based on infestations the previous year and the potential that the crop would be sprayed after parasitoid release (no spraying was desired). In 1990, twenty adult (10 o o 's and 10 o o's) *M. mediator* were released in mid-July from a shelter. An additional 10 adults were released in Alberta due to the outbreak conditions in the northeastern canola growing regions of that province and the very low Saskatchewan populations.

In 1991–93 between 1000 and 4000 *M. mediator* were released at each of five locations. In addition to setting out caged adults, parasitized host larvae (150 per cage) feeding on canola plants were placed in isolation cages. Once pupation of the parasitoid occurred (i.e. on senescent leaves and debris) the cages were removed. In 1991 and 1992 additional *M. mediator* were released in Alberta due to the continued bertha armyworm outbreak.

RESULTS AND DISCUSSION

Post release larval surveys in 1990–93 did not yield any hosts parasitized with *M. mediator*. However, a single specimen of *M. mediator* (identification confirmed by Dr. M. Sharkey, Agriculture Canada, Ottawa) was recovered in a Malaise trap set out in Saskatchewan in 1992 at a site where this species had been released the previous year.

In Saskatchewan, in all years except 1990, surveys indicated that host larval populations were low (maximum <5/m^2). The native parasitoids, *B. flavescens* and *A. cinerea*, were found in up to 83% (N>5) of the bertha armyworm larvae collected. In Alberta host populations were high in 1990–92. No *M. mediator* have yet been recovered from Alberta (Dosdall, pers. comm.).

The lack of *M. mediator* emerging from host larvae collected in the surveys after release is not surprising. In 1991–93 larval populations were extremely low and finding bertha armyworm larvae was difficult. Also, after a release, initial parasitism is expected to be very low (except when inundative release is done). If the parasitoid becomes established chances of locating a host containing *M. mediator* will increase as the parasitoid population increases. The broad host range of *M. mediator* increases the probability that the species will become established. The 1992 larval survey showed that numerous potential alternate hosts are available to *M. mediator*. The alfalfa looper (*Autographa californica* (Speyer)), the celery looper (*Anagrapha falcifera* (Walker)), the wheat head armyworm (*Faronta diffusa* (Walker)), the variegated cutworm (*Peridroma saucia* Hubner) and at least two other noctuid species (*Euxoa divergens* (Walker) and *Lithacodia* sp.) were collected (the first two species were more abundant than bertha armyworm) during the surveys.

The establishment of *M. mediator* will depend on the insects' capability to find hosts and successfully survive in the Canadian prairie environment. Follow up surveys are needed to confirm establishment of the parasitoid. Further study of potential alternate hosts for *M. mediator* is needed to determine probability of establishment in the bertha armyworm and the preference/effects on non-target species.

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Host location using vibrations by a leafminer's parasitoid: interpreting the vibrational signals produced by the leafmining host

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Parasitoids of tentiform leafminers have to locate a mobile target, the host, inside the mine. Different sensory modalities of the parasitoid are involved in the detection. Previous work strongly indicates that vibrations emitted by the larva or pupa could be used for locating and hitting the host (Casas 1989).

Vibrations emitted by a larva of the tentiform leafminer *Phyllonorycter malella* (Lep., Gracillariidae) show characteristic patterns contrasting to other vibrations in the environment. A detailed description of these vibrations has been done by Meyhöfer et al. (unpubl.). Three different behavioural categories of the instars are described in that publication: moving larva, showing frequencies in the vibrational signal of up to 5 kHz, wriggling larva, and wriggling pupa both showing frequencies of up to 16 kHz. The vibrational signals of the larva could not be linked to a behavioural detail on the time scale. Therefore we tried to interpret the vibrational signals as a density function of the peak intensity.

The moving behaviour of each of five tissue feeding larvae was recorded during 20 seconds by a laser vibrometer. The signal was analysed in the frequency domain using a time frame of 50 ms. We specially searched for a significant increase in the peak intensity compared to the background noise. A confidence interval of twice the SD of the mean background noise was chosen. The frequencies of the peak intensities were analysed by building a histogram with 20 classes each of a 0.25 kHz. About 79% of the peaks belong to the frequency classes below 1 kHz. Most of them (66%) could be found in the class of 0–0.25 kHz. A second cluster could be found in the frequency class of 2.25–2.5 kHz which contains about 21% of the peaks.

Interpreting these results the information in the frequency domain lies in a narrow range of values mostly below 1 kHz. Vibrational signals containing frequencies of up to 5 kHz do occur, but as seldom events on the time scale at which parasitoid and host interact. Behavioural observations of both partners will show us if the parasitoid *Sympiesis sericeicornis* (Hym., Eulophidae) is able to extract the vibrational information from the abundant but low quality signals below 1 kHz or if it has to rely on the clear but rare high frequency signals.

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Effects of the cyromazine on immature stages of *Opius concolor* Szépl. (Hymenoptera: Braconidae)

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The cyromazine is a IGR that applied on adults of the mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera: Tephritidae), produces a big population reduction. As in another IGRs, its effects on the natural enemies, are unknown.

The indirect effects on the preimaginal development of the parasitoid *Opius concolor* Szépligetti (Hymenoptera: Braconidae), when cyromazine is applied on *C. capitata* are studied. In the experiment, three increasing concentrations of cyromazine, applied on the last larval instar of the fruit fly, were tested.

Insect pest and parasitoid distribution in a field of oilseed rape (*Brassica napus* L.)

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Knowledge of in-field host / parasitoid distribution is important for integrated pest management. Comparative distributions give insights into interspecies interactions and allow sampling or spraying regimes to be refined. Oilseed rape is attacked by cabbage seed weevil *Ceutorhynchus assimilis* (Payk.) (Coleoptera: Curculionidae) and brassica pod midge *Dasineura brassicae* (Winn.) (Diptera: Cecidomyiidae). This study is a comparison of spatial and temporal distributions of these pests and their parasitoids.

Thirty yellow water traps were arranged in and surrounding a field of winter oilseed rape and emptied weekly. Insect counts were entered into mapping software which interpolated the data to produce contour maps of insect distribution. Data were analyzed using indices of aggregation.

Contour maps are shown of: *C. assimilis* and its larval ectoparasitoid *Trichomalus perfectus* (Walk.) (Hymenoptera: Pteromalidae); *D. brassicae* and its egg-larval endoparasitoid *Platygaster* sp. (Hymenoptera: Platygastridae). Implications are discussed.

Cereal aphids and their parasitoids on triticale in Central Poland

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Triticale, which is a hybrid of wheat and rye, is very little studied with respect to its colonization by aphids and their natural enemies. The present paper was prepared in an attempt to bridge this gap. The study was carried out in 1991-1993 in crop fields at Kepa near Warsaw. It involved the species composition of aphids, their number dynamics, parasitization by parasitoids, and the composition of parasitoid community. It has been found that triticale was colonized by three aphid species: *Sitobion avenae* F., *Rhopalosiphum padi* L. and *Metopolophium dirhodum* Walk.

The parasitization of aphids by parasitoids ranged from several to more than ten percent (maximum 23.2%). The community of parasitoids attacking aphids in triticale fields consisted of 6 species of the family Aphidiidae: *Aphidius uzbekistanicus* Luzhetzki, *A.rhopalosiphi* De Stefani-Perez, *A.ervi* Haliday, *A.picipes* Nees, *Ephedrus plagiator* Nees and *Praon volucre* Haliday. In all the study years, *A. uzbekistanicus* was dominant (in successive years: 85.3%,93.9% and 97.1%). Primary parasitoids were attacked by hyperparasitoids at a very high rate.In all the study years, *Dendrocerus carpenteri* Curtis highly predominated. This study has shown that triticale was colonized by the same aphids and their parasitoids as other cereals, especially rye and wheat.

Frequence and geographical distribution of thelytokous parthenogenesis in European species of *Trichogramma* (Hym.: Trichogrammatidae)

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Among 37 European species of *Trichogramma* currently known, 9 exhibit cases of thelytokous parthenogenesis. Three species are entirely thelytokous (*T. cacoeciae* Marchal, *T. cordubensis* Vargas & Cabello, and *T. oleae* Voegelé & Pointel). The other 6 species (*T. agrotidis* Voegelé & Pintureau, *T. daumalae* Dugast & Voegelé, *T. dendrolimi* Matsumura, *T. embryophagum* Quednau, *T. evanescens* Westwood, and *T. semblidis* (Aurivillius)) show three types of populations: thelytokous, bisexual, and with thelytokous and non thelytokous individuals.

In *Trichogramma,* thelytokous parthenogenesis is often due to an intracellular microorganism, *Wolbachia trichogrammae* Louis & Pintureau. High temperatures have a detrimental effect on this symbiot. The presence of the microorganisms was proved in *T. cordubensis, T. oleae* and *T. evanescens,* and is suspected in 5 of the other 6 species. In *T. cacoeciae, Wolbachia* are not present and thelytoky is probably genetic.

The study of the geographical distribution of the strictly thelytokous species shows that species with *Wolbachia* (*T. oleae* and *T. cordubensis*) are localized in the South of Europe, and that the species without *Wolbachia (T. cacoeciae)* is present everywhere. The study of the distribution of the partially thelytokous species (entirely or partially thelytokous populations) shows that thelytokous individuals (probably with *Wolbachia*) are present in France (*T. agrotidis, T. evanescens,* and *T. semblidis*), in the Netherlands (*T. dendrolimi*) and in Bulgaria (*T. daumalae* and *T. embryophagum*). Some of the localities are relatively northern.

From this distribution, it is possible to express some hypotheses on the adaptative signification of thelytoky with *Wolbachia.* In the southern zones, thelytoky can affect all the individuals but the hot season eliminates microorganisms and allows reproductive cycles (in summer, reproduction is bisexual when hosts are rare). In the more northern zones, climate does not allow reproductive cycles and populations have an advantage in including thelytokous individuals (rapid growth of the population when hosts are frequent) and individuals with a bisexual reproduction (genetical recombinations, fecundation of the thelytokous females).

Immunity interactions between *Diadegma armillata* and two host species of the *Yponomeuta* genus

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Diadegma armillata is an ichneumonid parasitoid which develops in *Yponomeuta* (Lepidoptera) hosts. Within the *Yponomeuta* genus, *Y. cagnagellus* belongs to the early diverged species which manifest an immunity reaction against *D. armillata* while *Y. malinellus* is one of the more recently diverged species which are usually unable to encapsulate the parasitoid (Dijkerman 1990). However, in populations from South of France, *D. armillata* parasitizes both Yponomeuta species. The immunity relationships between *D. armillata* and its *Yponomeuta* hosts were investigated in those regional strains. *Y. cagnagellus* and *Y. malinellus* larvae were infested by *D. armillata* females which had been collected on one or the other *Yponomeuta* species.

Dissection revealed significant differences: a) in the aptitude of the *Yponomeuta* species to encapsulate the parasitoid: *Y. malinellus* usually did not develop a defense reaction against *D. armillata* while a large proportion of the *Y. cagnagellus* larvae encapsulated the parasitoid; b) in the aptitude of the two *D. armillata* populations to escape host defenses: *D. armillata* originating from *Y. cagnagellus* was more effective in suppressing the encapsulation reaction of any *Yponomeuta* species than *D. armillata* originating from *Y. malinellus*.

It is worthy to note that the most successful *D. armillata* population was associated in the field with the more resistant host, *Y. cagnagellus*, while the *D. armillata* population showing the less ability to escape host defenses developed in the susceptible host, *Y. malinellus*. It is suggested that the *D. armillata* population which used to encounter resistant hosts of *Y. cagnagellus* species may have been selected to improve its ability to avoid host defenses.

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Data on a Spanish species of Ichneumoninae (Hym., Ichneumonidae)

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Neotypus intermedius Mocsáry, 1883 is a Spanish species belonging to the tribe Listrodomini and is parasitic on *Lampides boeticus* Linnaeus, 1767 (Lepidoptera, Lycaenidae).

After *N. intermedius* was described, it has not been further studied in Europe. In the present study, status of the genus *Neotypus* Foerster (1869) is discussed and compared with related species. A new European species key for the genus *Neotypus* Foerster is proposed.

Biology of a Pimplinae parasite of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae)

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Itoplectis melanocephala Sedivi (Hymenoptera: Pimplinae) is a solitary endoparasite of several lepidoptera pupae. It is recorded for first time in Spanish fauna. *I. melanocephala* has been found parasiting *C. suppressalis* Walker.

C. suppressalis is a major pest of rice in East Asia, India and Indonesia. This pest was detected for first time in Europe in 1933, concretely in Spain. It has been accidentally introduced by man. Many natural enemies have been reported in the literature in Asia but *non* in Europe because the pest has newly been introduced. *I. melanocephala* is the first postembrionary parasite recorded in Europe of *C. suppressalis*.

The life history of *I. melanocephala* is very similar of *other Pimplinae*. Eggs hatch in one day and the larva molts 4 times in 5–7 days. Pupation requires 11–12 days; thus, the total developmental period lasts 17–20 days. Females live approximately 30 days and males 20.

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The manuscript shall be typewritten on one side of the paper only. It shall be double spaced and have margins of at least three centimetres. Each of the following elements of the manuscript shall begin on a new page: (1) the title, (2) abstract, (3) the text, (4) summary, (5) list of references, (6) tables, (7) figure legends.

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ACKNOWLEDGEMENTS

Acknowledgement shall be made only to persons who have contributed substantially to the results of the research. Authors are responsible for ensuring that individuals named are recognized as being identified with the results and conclusions described in the article.

TABLES

Each table shall be typed double spaced on a separate sheet of paper. They shall be numbered consecutively with Arabic numerals and have a concise descriptive heading. Abbreviations in tables shall be explained in footnotes, using the following symbols in this order: [1]), [2]), [3]), [4]), [5]).

Avoid horizontal and vertical rules (lines) in tables. Tables shall be self explanatory as far as possible without the reader having to refer to the text.

FIGURES

All illustrations, line drawings as well as photographs, shall be considered as figures. Figures shall be numbered consecutively with Arabic numerals. Letters, numerals and symbols must stand out clearly and be in relation to each other. Make sure they are large enough to take reduction. Before preparing line drawings the author should decide wether they are to be of 1 column, 1½ columns, or 2 colums width so that lettering, etc., after reduction, will be the same size on all drawings. Photographs should be submitted as near to their printed size as possible. If enlargement or reduction is significant in an photograph, the scale should be given on the back and not in the legend. The legend should make the general meaning comprehensible without reference to the text. Figure legends shall be typewritten together on one or more sheets of paper.

REFERENCES

Reference indicators in the text will follow the Harvard style, with the author's name and the year of publication: Høeg (1971), or (Høeg 1971). The text reference indicator to a work by two authors is given by naming both authors: Oen & Vestrheim (1985) or (Oen & Vestrheim 1985). If three or more authors, the text reference indicator is given by adding et al. to the first named author: Aase et al. (1977) or (Aase et al. 1977).

In the list of references authors' names are listed alphabetically. Two or more works by the same author are listed chronologically and if published in the same year must be distinguished by the appendage a, b, etc. after the year of publication. The corresponding appendage must also appear in the text indicator.

Høeg, O.A. 1971. Vitenskapelig forfatterskap. 2. utg. Universitetsforlaget, Oslo. 131 s.

Oen, H. & S. Vestrheim 1985. Detection of non-volatile acids in sweet cherry fruits. Acta agriculturae scandinavia 35: 145-152.

Strømnes, R. 1983. Maskinell markberedning og manuell planting. Landbrukets årbok 1984: 265-278.

Uhlen G. 1968. Nitrogengjødsling til ettårig raigras. Jord og avling 10(3): 5-8.

Aase, K.F., F. Sundstøl & K. Myhr 1977. Forsøk med strandrøyr og nokre andre grasartar. Forskning og forsøk i landbruket 27: 575-604.

Notice that:

- Only the first named author's initials and surname are inverted.
- The ampersand symbol is used between author's names.
- The date after the author's name is the year of publication.
- The issue number is given in parentheses after the volume number and only in cases where each issue of a particular volume begins on p. 1.
- A colon is used before page numbers in journal articles.
- The year of publication suffices where the volume number is missing.
- In references to a book, the name of the publisher and the place of publication are given after the title of the book. If more than one edition of a book has been published, the edition used must be indicated. The number of pages should be given.
- It is recommended not to abbreviate the titles of periodicals, but in cases where this has nevertheless been done, abbreviations should be in accordance with the World List of Scientific Periodicals and/or BUCOP British Union Catalogue of Periodicals.

ABBREVIATIONS

Use standard abbreviations where available, otherwise abbreviations given in the text should be explained at their first mention. Quantities and units of measurement shall be in accordance with «Systéme International d'Unites« (SI).

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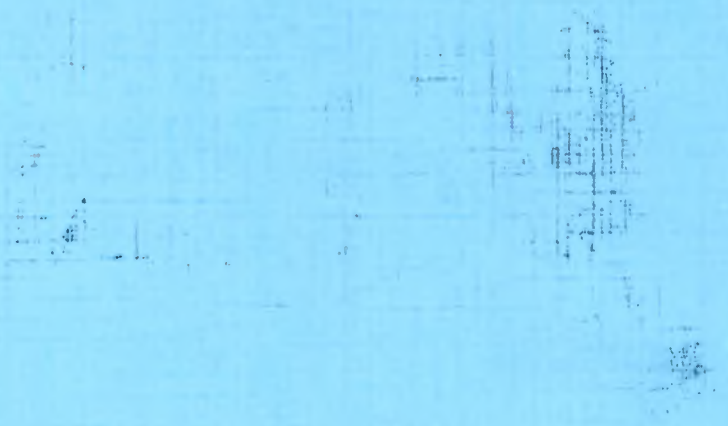

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