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The drawing on the cover is from Kjell Aukrust's *«Guttene på broen»*.

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SELECTION FOR LITTER SIZE IN MINK

IV. Effect on postweaning growth and fur characteristics

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The correlated responses in postweaning growth of males and females and the fur characteristics of male skins are presented from a six generation selection experiment for litter size in dark mink. No general trend in body length, skin size or fur characteristics could be observed resulting from divergent selection for litter size at birth. However, during the last two generations, most of the fur traits favoured the line selected for increased litter size at birth, when the traits were expressed as deviations from the control line. In the last generation, significantly lower average body weight at pelting was observed in both males and females in the high line compared to the low line; 85 grams and 68 grams, respectively. A significantly lower average in hair quality and in general fur quality was observed for male skins in the low line during the last generation. The length of both guard fur and underfur increased in all lines during the experiment, while body length at pelting decreased. Heritability and genetic correlations were estimated for the traits recorded.

Keywords: Correlated response, fur characteristics, litter size, mink, postweaning growth, selection.

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The growth of the mink, finally termed as pelt size, and fur characteristics are traits of great importance in fur production, but in order to include all economically important traits in the complete selection programme, it is necessary to know whether or not these traits are affected by artificial selection for litter size.

Information about these correlated effects of selection for litter size in mink is not particularly widespred. However, some studies have been published on the effects of litter size on body weight, body length and skin length (Venge, 1960; Rimeslåtten, 1961; Jonsson, 1971; Hoogerbrugge & Baud, 1975; Reiten, 1978; Barabasz & Jarosz, 1978; Einarsson, 1980). Most of these studies were based on field data. Genetic parameters of the relationship between litter size and other economically important traits are not known.

Part I of this series of papers (Einarsson, 1987a) points out that one of the principal aims of the present selection experiment for litter size in mink was to seek correlated effects in postweaning growth and fur characteristics when se-

lecting for litter size only. Divergent selection was practised through a high and a low line, and in addition a randomly selected line was kept as a control. The background and the design of the experiment, including analyses of the base population, are given in Part I (Einarsson, 1987a) in this series. Results on direct response and correlated responses on postpartum and preweaning observations are given in two previous papers (Einarsson, 1987b,c).

This present paper gives the correlated responses in postweaning growth and fur characteristics. Genetic and phenotypic parameters for body size at pelting and fur characteristics are also presented.

MATERIAL AND METHODS

Experimental design

In 1978, from a foundation stock of 231 litters, three lines were selected and kept closed until the completion of the experiment in December 1984. One line was selected for increased litter size (H), another line for decreased litter size (L) and the third line was randomly selected to serve as a control (C). The selection criterion was a pedigree index based on litter size at birth, i.e. total number of kits at the first examination. The breeding animals were kept for one year only, except for the last year when half of the females were two year old.

The animals were kept under conventional ranch facilities and management conditions. From weaning to pelting one male and one female were kept in the same cage. The grower diet, was fed from the end of June to mid-December, and consisted of approximately 34 percent protein, 46 percent fat and 20 percent carbohydrate, as percent of metabolisable energy.

In Part I of this series a description is given of the base population, experimental design, animal management and feed composition (Einarsson, 1987a).

Number of animals

The numbers of mink observed for body weight, body length and pelt characteristics are presented in Table 1. Pelt characteristics for females are given for the last generation only, as during the other years the pelted females represented only a selective material of the previous generation. Only pelted males were included in the genetic analysis of body weight and body length at pelting.

Traits recorded

The individual body weights were recorded at six intervals from two to five weeks apart, from weaning to pelting.

Pelting of the animals was conducted in the period from November 28 to December 8, usually within the space of three days in each year. Body length at the time of pelting was recorded on live animals as length in centimetres from the nose tip to the base of the tail. Skin length and skin weight were recorded on dried skins ready for marketing.

Pelt characteristics were recorded for both sexes, but were presented for males only, except for the last year. This was done because after the selection of the breeding females, the number of females left to be pelted was so reduced that they were no longer representative of the line. The observations on male skins were therefore thought to represent the line more effectively, particularly if there was a strong correlation between litter size and pelt characteristics. However, during the last generation when all the progenies were pelted, observations on females were also included.

All the fur characteristics were recorded on the skins after drying. The length of the guard fur and the underfur was measured in millimetres on the back side, about 8 cm from the base of the tail. The fur density was subjectively judged from zero to 10 points (best). Hair quality, indicating the smoothness and fineness of the hairs, was judged using the same numerical scale as for fur density. Fur colour was also judged subjectively, by a scale from 1 point (black) to 7 points (pale). Metallic, which is a disorder of the guard hairs, was judged on a scale from zero to 5 points (severe metallic). Wet belly, caused by a urinary tract disorder, was judged using the same numerical scale as for

metallic. All the fur characteristics mentioned above were judged and recorded at the experimental farm.

The general fur quality of the pelt was judged according to the standard quality grading of the Oslo Fur Auctions using subjective grading from zero to 4 points (best), where 4 = Saga selected, 3 = Saga, 2 = grade I, 1 = grade II and 0 = grade III.

All the skins that were evaluated for fur characteristics were randomly mixed with mink skins from the entire farm and could not be identified by line. They could only be identified later on by their individual number. A broader description of the traits is given in Part I (Einarsson, 1987a).

Mink that died in the period from weaning to pelting were autopsied at the National Veteri-

Table 1. Number of animals with body weights at weaning, body weight and body length at pelting and fur characteristics recorded.

Year	Line	Body weight recorded at weaning, no. of		Body weight and body length at pelting, no. of		Fur characteristics, no. of	
		males	females	males	females	males	females
1979	L	103	114	103	110	81	
	C	99	119	98	117	75	
	H	132	114	126	113	105	
1980	L	102	101	97	100	76	
	C	127	111	122	107	103	
	H	135	107	135	107	113	
1981	L	88	92	85	89	66	
	C	114	95	113	93	98	
	H	132	135	127	130	111	
1982	L	77	77	74	75	60	
	C	91	76	82	74	67	
	H	99	86	93	83	78	
1983	L	56	84	52	82	42	
	C	85	90	84	89	68	
	H	57	50	56	45	38	
1984	L	117	95	107	93	109	94
	C	128	105	117	95	119	102
	H	113	117	107	112	109	112
Sum	L	543	563	518	549	434	
	C	644	596	616	575	530	
	H	668	609	644	590	554	
Total		1 855	1 768	1 778	1 714	1 518	308

nary Institute, Oslo, but the results are not presented as they give no useful information relating to the present experiment.

Statistical methods

The regression coefficient was studied to give an analysis of trends over generations within the lines, by using the following model

$$Y_j = a + b \cdot X_j + e_j$$

where

- Y_j = a given dependent variable for the jth individual
- a = a constant
- b = the sample regression coefficient of Y_j on X_j
- X_j = the independent variable, generation number as a fixed effect
- e_j = the random error associated with the jth individual

The effects of year, line and interaction between them were analysed for the different traits recorded. The model used was

$$Y_{ijk} = a + g_i + l_j + c_{ij} + e_{ijk}$$

where

- Y_{ijk} = an observation on the trait
- a = a constant
- g_i = a fixed effect of ith generation (year)
- l_j = a fixed effect of jth line
- c_{ij} = interaction between ith generation and jth line
- e_{ijk} = the random error associated with the ijk$t$h mink where trait was recorded

Where the interaction was non significant in the model, the analyses were then based on a model excluding the element of interaction. The results of the analyses were based on type III sum of squares (SAS, 1985), where the other elements in the model were included before the given element was analysed.

When analysing differences in LS-means within the last generation the model included the effect of the lines only; similarly, the year (generation) was included when analysing differences between the first and last years within the line.

The genetic parameters were estimated by using a nested analysis including the trait of the progeny within the dam and within the sire. The estimated parameters presented were based on the sire component and included pelted males only.

RESULTS

Growth curves of the kits within sex, line and generation are presented in Figure 1. The intervals between the time of weighing varied throughout the experiment and the curves presented in Figure 1 can therefore not be regarded as general growth curves for mink. However, they allow for the comparison of average body weight between the lines within sex and year. One of the largest differences in average body weight between the lines was observed in the last year at the time of pelting, when males in the L-line were on average 85 grams heavier than males in the H-line ($p<0.05$). The average female body weight at pelting in the H-line was significantly lower compared to both the L-line ($p<0.01$) and the C-line ($p<0.01$).

The means and standard deviations of body weight and body length at the time of pelting are given in Table 2, together with skin length and skin weight. There were only small variations in body length between individuals, as indicated by the low standard deviation.

The average values and standard deviation of pelt characteristics for all lines during the entire experiment are given in Table 3.

Effect of year, line and interaction

The analyses of variance for the traits given in Tables 2 and 3 are presented in Table 4, together with the average value of the traits for the whole experiment.

A significant effect of generation was found for all traits, except for the density of guard fur.

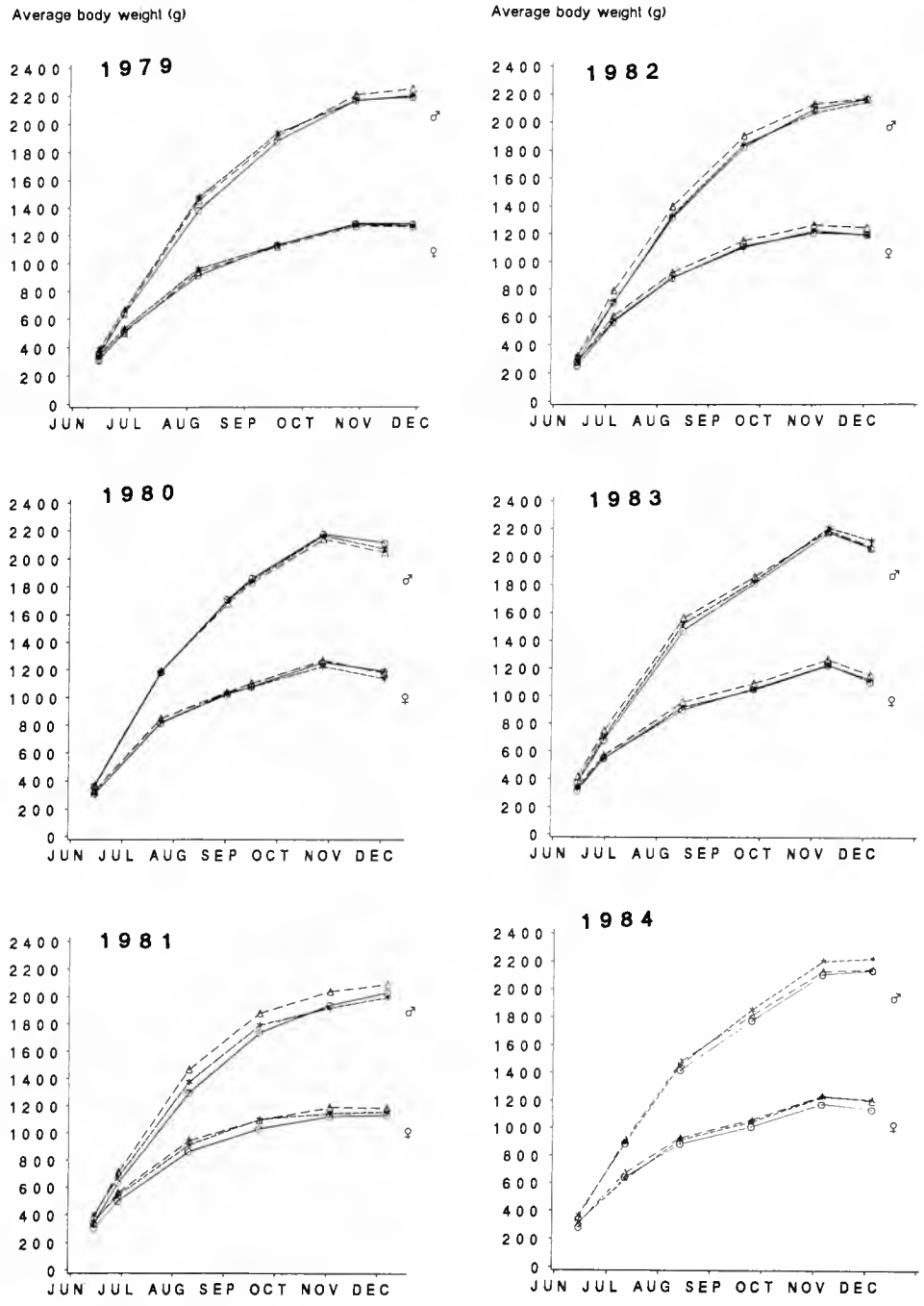

Figure 1. Growth curves of mink from weaning to pelting, including six genrations. The upper group of curves denotes the males, the lower one the females, as marked. Filled line and circel denotes the H-line, broken line and triangle the C-line and dashed line and star the L-line.

The effect of line was significant only for body length at pelting and for skin weight.

The component of interaction between year and line was not significant for body weight at pelting, density of the underfur and hair quality, but significant for the other traits.

Body weight at pelting was significantly higher in the first generation compared with all the following generations, and in the last generation it was significantly higher than in 1980, 1981 and 1983. During the first four generations the body length at pelting was reduced in all three lines by an average of close to 0.5 cm per year, but is then stabilized in the last two years. Significant differences were observed in body length between the both lines in 1981 and 1982, with the longest males in the C-line and in the L-line for the respective two years.

Average skin length of the males was significantly different between the years, except when comparing 1983 to 1981 and 1982.

Table 2. Average body sizes and skin sizes in the three lines during the experiment. Observations are given for males only, except for 1984, where both sexes are included.

Year	Line	Body weight at pelting, grams		Body length at pelting, cm		Skin length, cm		Skin weight, grams	
		mean	SD	mean	SD	mean	SD	mean	SD
1979	L	2 203	*231*	44.5	*1.59*	73.7	*3.46*	153	*21.1*
	C	2 252	*217*	44.5	*1.42*	72.8	*3.12*	151	*17.6*
	H	2 192	*288*	44.2	*1.54*	73.0	*4.26*	151	*22.8*
1980	L	2 074	*232*	44.1	*1.34*	72.8	*3.26*	150	*17.4*
	C	2 050	*241*	43.8	*1.47*	71.6	*3.49*	148	*18.5*
	H	2 118	*218*	44.1	*1.44*	72.7	*3.31*	149	*17.0*
1981	L	1 999	*266*	43.3	*1.40*	69.6	*4.45*	140	*21.1*
	C	2 091	*295*	44.0	*1.51*	71.0	*4.04*	151	*22.9*
	H	2 034	*274*	43.2	*1.46*	69.3	*4.46*	136	*20.6*
1982	L	2 138	*314*	42.7	*1.47*	70.5	*4.52*	150	*24.2*
	C	2 163	*327*	43.4	*1.52*	71.0	*4.93*	155	*30.6*
	H	2 160	*316*	43.3	*1.50*	71.2	*4.31*	155	*25.3*
1983	L	2 118	*285*	43.4	*1.71*	69.1	*5.15*	138	*26.1*
	C	2 075	*289*	43.5	*1.39*	70.1	*3.96*	151	*24.1*
	H	2 063	*314*	43.4	*1.50*	71.5	*3.53*	155	*17.2*
1984	L	2 219	*320*	42.8	*1.41*	72.2	*4.50*	147	*22.6*
	C	2 141	*305*	42.9	*1.54*	70.9	*4.36*	150	*23.3*
	H	2 134	*290*	42.9	*1.38*	71.9	*4.00*	147	*22.1*
Females in the last generation									
1984	L	1 200	*177*	37.4	*1.40*	59.9	*4.20*	80.5	*15.4*
	C	1 191	*166*	37.5	*1.35*	59.6	*3.29*	80.8	*12.3*
	H	1 129	*154*	37.4	*1.23*	59.2	*3.14*	77.3	*9.5*

The average skin length of the males was reduced during the first three generations, then it stabilized and increased slightly in the last generation.

The skin weight was affected significantly by generation, line and interaction. The average skin weight of the males was low in the selection lines in 1981 and in the L-line in 1983. No general trends were observed.

The average length of the guard fur increased in all the lines during the first four generations, stabilized in the fifth and then slightly decreased in the last generation. The average length of the underfur also increased significantly during the first generations, but stabilized during the last two generations.

No significant effect of generation or line was found in the density of the guard fur. There was, however, a significant effect of generation in the density of the underfur due to poorer density in the first and the last generations.

The average classification of hair quality showed no clear trend throughout the experiment. The lowest average score was observed in 1982 and 1984, when it was significantly lower than in the other generations.

The average colour score decreased during the first years, indicating that the skins became darker. However, in the last generation the highest average colour score for the whole experiment was observed. The change in colour was significant in the first three generations. The high score in the last generation was significantly different from all the other generations, except for the first one.

The frequency of metallic showed high variation, as indicated by the standard deviation which was in the same order as the means themselves. However, no clear trends were observed during the experiment either regarding metallic or the disorder wet belly.

The general fur quality, judged at the Fur Auctions, was lowest in the low line during the last three generations of the experiment. The classification Saga Selected and the sum of Saga Selected and Saga were lowest in the L-line in the last generation for both male and female skins as shown in Figure 2.

Within-line differences between the first and the last generation

Analyses of the differences between the first and the last generation for the traits recorded are presented in Table 5. The differences are expressed as LS-means together with the level of significance. In the following paragraphs only significant differences are discussed.

Body length was significantly shorter in all lines in the last generation, which emphasizes the trend for reduced body length during the experiment. Male body weight at pelting was significantly lower in the last generation within the C-line, but was the highest in the lines at the first generation.

The skin length was on average, shorter in the last generation in both the L- and C-lines, but without reflecting any reduction throughout the experiment.

Both guard fur and underfur were on average longest in the last generation in all lines. This again strengthens the observation of increase in fur length, especially during the first generations of the experiment.

The hair quality of the male skins was significantly reduced in the L-line in the last generation, which emphasizes the impression of a slightly reduced hair quality in that selection line during the experiment.

Significant differences were found for metallic between the first and the last generation in the L-line and for wet belly in the other two lines. However, there were great variations from one generation to another in these traits but no pattern of change was observed.

General fur quality of the male skins was significantly higher both in the C- and H-lines in the last generation, reflecting an increase in general fur quality in the last two generations in these two lines.

Between-line differences within the last generation

In the last generation the differences in LS-means between lines were analysed. The traits included are those given in Tables 2 and 3, and the results are presented in Table 6 for both males and females.

Table 3. Average values and standard deviation of fur characteristics in the three lines during the experiment. Figures are presented for males only, except for last generation where both sexes are included. (The upper series of numbers within line denotes the mean while the lower denotes the standard deviation.)

Year	Line	Length of guard fur, mm	Length of underfur, mm	Density of guard fur, points	Density of underfur, points	Hair quality, points	Fur colour, points	Metallic, points	Wet belly, points	General fur quality, points
1979	L	22.8	13.3	5.21	4.63	5.07	5.06	0.96	1.26	2.63
		1.61	*1.15*	*0.89*	*0.87*	*1.07*	*0.84*	*0.94*	*1.31*	*0.85*
	C	22.6	13.5	4.91	4.48	4.81	5.03	0.97	1.77	2.49
		1.90	*1.36*	*0.92*	*0.89*	*0.95*	*0.85*	*0.88*	*1.51*	*0.91*
	H	23.1	13.8	4.98	4.50	4.82	4.90	1.30	1.90	2.39
		1.89	*1.30*	*0.92*	*0.76*	*1.05*	*0.98*	*0.99*	*1.52*	*0.93*
1980	L	23.2	13.7	4.95	4.84	4.72	5.01	0.83	0.66	2.03
		1.50	*1.07*	*0.85*	*0.94*	*0.81*	*0.99*	*1.04*	*0.96*	*0.82*
	C	23.6	13.9	5.17	4.88	4.68	4.63	1.31	0.84	2.14
		1.68	*1.17*	*0.86*	*0.95*	*0.94*	*0.92*	*1.34*	*1.23*	*0.85*
	H	23.6	13.9	5.18	4.84	4.91	4.66	1.28	0.65	2.29
		1.64	*1.01*	*0.72*	*0.83*	*0.89*	*0.98*	*1.35*	*0.90*	*0.76*
1981	L	24.7	14.5	5.08	4.97	4.62	4.24	0.80	0.76	2.88
		1.79	*1.08*	*0.92*	*0.80*	*1.16*	*1.20*	*0.88*	*0.98*	*0.74*
	C	24.0	14.0	5.08	4.86	4.84	4.11	0.91	0.61	2.70
		1.63	*1.21*	*1.00*	*0.86*	*0.99*	*1.02*	*1.01*	*0.92*	*0.79*
	H	24.4	14.1	4.90	4.60	4.76	4.49	0.97	0.56	2.64
		1.66	*1.17*	*0.94*	*1.00*	*1.11*	*1.15*	*1.12*	*0.85*	*0.79*

1982	L	25.1	15.0	5.18	4.90	4.47	4.35	1.02	1.39	2.38
		1.62	*1.12*	*0.85*	*0.99*	*1.08*	*1.36*	*0.95*	*1.31*	*0.74*
	C	24.7	14.7	5.06	4.75	4.55	4.13	1.07	1.00	2.48
		1.81	*1.05*	*0.97*	*1.03*	*1.08*	*1.01*	*1.15*	*1.14*	*0.83*
	H	24.5	14.4	5.03	4.78	4.71	3.94	0.99	0.79	2.38
		1.52	*1.19*	*0.76*	*0.95*	*1.02*	*1.01*	*1.09*	*1.04*	*0.94*
1983	L	24.6	14.2	5.31	4.93	4.95	4.24	0.71	0.86	2.63
		1.61	*1.06*	*0.78*	*0.71*	*1.13*	*1.08*	*1.01*	*1.26*	*0.70*
	C	24.7	14.6	4.82	4.92	4.93	4.19	0.82	0.49	2.85
		1.61	*1.10*	*0.79*	*0.71*	*1.07*	*1.01*	*0.95*	*0.80*	*0.71*
	H	25.3	14.6	4.97	4.79	4.87	4.16	0.84	0.63	2.81
		1.20	*0.98*	*0.72*	*0.78*	*0.96*	*0.97*	*0.72*	*0.94*	*0.52*
1984	L	24.2	14.6	4.99	4.37	4.18	5.08	1.44	1.15	2.50
		1.63	*0.82*	*0.93*	*1.13*	*0.99*	*0.85*	*1.30*	*1.63*	*0.78*
	C	23.9	14.5	4.83	4.64	4.55	5.29	1.10	0.35	2.80
		1.66	*0.98*	*0.99*	*1.11*	*1.10*	*0.89*	*1.17*	*0.78*	*0.83*
	H	23.8	14.3	5.06	4.61	4.59	4.97	1.17	0.61	2.77
		1.31	*0.83*	*1.03*	*1.11*	*1.04*	*0.94*	*1.04*	*1.10*	*0.74*
Females in the last generation										
1984	L	22.6	13.7	5.22	5.16	4.99	4.82	0.89	0.07	2.59
		1.24	*1.42*	*1.04*	*1.05*	*1.11*	*0.97*	*1.23*	*0.39*	*0.87*
	C	22.4	14.0	5.36	5.41	5.33	5.04	0.33	0.05	2.81
		1.31	*0.84*	*0.83*	*0.99*	*1.05*	*0.76*	*0.67*	*0.26*	*0.94*
	H	22.4	13.8	5.42	5.21	5.43	4.81	0.50	0.08	2.72
		1.73	*0.82*	*1.00*	*0.89*	*0.93*	*0.92*	*0.72*	*0.41*	*0.87*

See text for description of points

Table 4. Average value of the traits, degrees of freedom (d.f.) and mean squares in percent for generation, line, interaction between them and error on body size, skin size and fur characteristics of male skins.

Trait	Average value	d.f. tot.	Mean square in percent			
			genera-tion	line	inter-action	error
Body weight at pelting, g	2 125	1 776	83.6***	1.0	9.7	5.7
Body length at pelting, cm	43.6	1 776	85.2***	7.2 *	5.8 **	1.8
Skin length, cm	71.5	1 515	81.3***	3.7	11.3 **	3.8
Skin weight, g	149	1 515	43.5***	30.3	20.2 ***	6.0
Length of guard fur, mm	23.9	1 515	89.8***	4.5	3.9 *	1.8
Length of underfur, mm	14.2	1 515	88.3***	0.3	9.0 ***	2.4
Density of guard fur, p	5.03	1 514	13.9	41.7	29.3	15.2
Density of underfur, p	4.71	1 514	67.8***	10.3	12.7	9.3
Hair quality, p	4.71	1 514	68.4***	9.9	13.6	8.1
Fur colour, p	4.65	1 513	88.6***	4.6	4.8 **	2.0
Metallic, p	1.07	1 513	51.6***	16.0	21.8 *	10.6
Wet belly, p	0.89	1 512	76.6***	7.4	13.4 ***	2.6
General fur quality, p	2.53	1 491	80.1***	7.10	8.90*	3.9

p = points.
* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

Table 5. Significance of difference in LS-means for body size, skin size and fur characteristics of males within lines between the first and the last generation.

Trait	Differences in LS-means, comparing data from 1979 and 1984		
	L	C	H
Body weight at pelting, g	-16	113**	58
Body length at pelting, cm	1.66***	1.54***	1.33***
Skin length, cm	1.53*	1.92*	1.07
Skin weight, g	5.94	0.51	4.46
Length of guard fur, mm	-1.37***	-1.30***	-0.76***
Length of underfur, mm	-1.26***	-0.97***	-0.49***
Density of guard fur, p	0.22	0.08	-0.08
Density of underfur, p	0.26	-0.16	-0.11
Hair quality, p	0.89***	0.26	0.29
Fur colour, p	-0.02	-0.26	-0.07
Metallic, p	-0.48**	-0.13	0.13
Wet belly, p	0.11	1.42***	1.21***
General fur quality, p	0.13	-0.31**	-0.38***

p = points.
* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

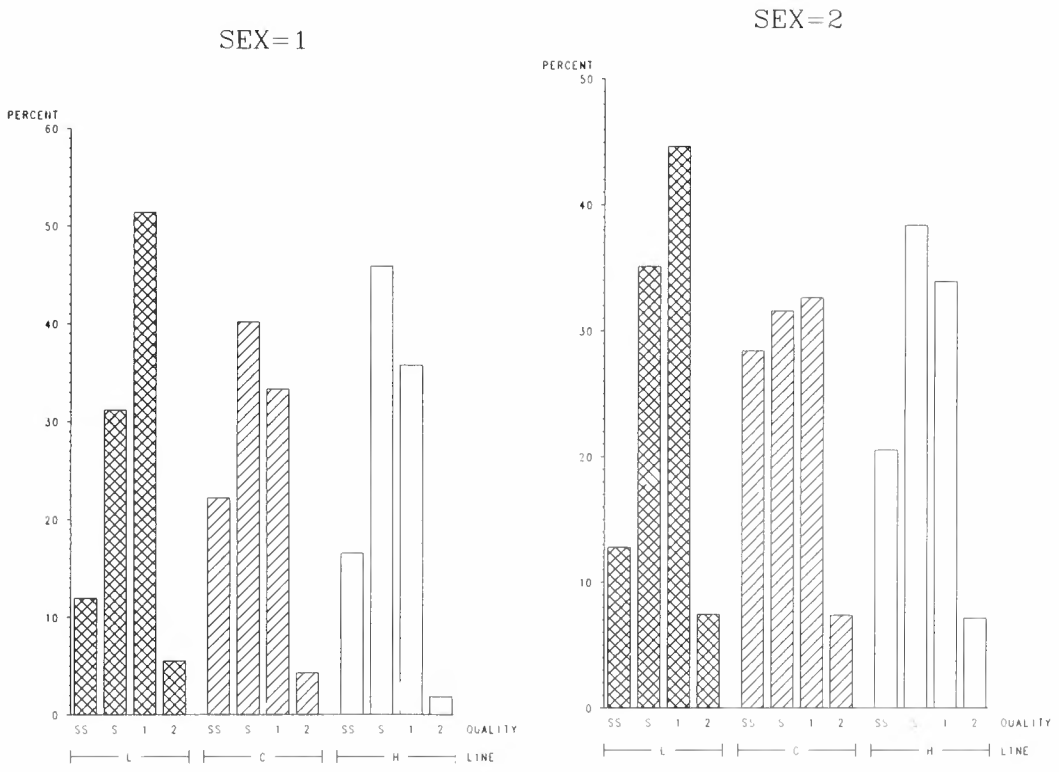

Figure 2. Distribution of general fur quality, judged at the Oslo Fur Auctions, according to the commercial quality classes. The results are from the last generation, presented for each line separately, with males to left and females to right in the figure.

Male body weight at pelting was significantly lower in the H-line compared to the other selection line in the last generation, though this was not reflected either in body length or in skin length. Body weight at pelting for females was significantly lower in the H-line, compared to the other two lines.

As pointed out earlier, there was an increase in the hair length of both guard fur and underfur during the experiment. In the last generation the underfur of male skins was significantly shorter in the H-line than in the other selection line and the underfur in female skins was longer in the C-line than in the L-line.

In the last generation the male skins in the H-line were significantly darker than in the control line.

Some significant differences in both metallic and wet belly were observed, favouring both the H- and C-lines as opposed to the L-line and, as already pointed out, there were great variations in these traits during the experiment.

General fur quality of male skins was found to be significantly better in the high- and the control lines compared to the low line. No significant differences were observed for the female skins, but the figures indicate the same trend as observed in male skins. The distribution of the different quality classes for male and female skins in the last generation, are given separately in Figure 2.

In Figure 3 the direct response of litter size at birth and correlated responses in skin length and some fur characteristics on male skins are pre-

Table 6. Significance of difference in LS-means for body size, pelt size and fur characteristics of both sexes between the three lines in the last generation.

Trait	Line differences					
	Males			Females		
	H – C	C – L	H – L	H – C	C – L	H – L
Body weight at pelting, g	-6	-79	-85*	-62**	-6	-68**
Body length at pelting, cm	-0.07	0.14	0.07	-0.14	0.12	-0.02
Skin length, cm	1.00	-1.31*	-0.31	-0.34	-0.34	-0.68
Skin weight, g	-3.15	3.33	0.18	-3.47*	0.27	-3.20
Length of guard fur, mm	-0.03	-0.34	-0.37	-0.04	-0.17	-0.21
Length of underfur, mm	-0.17	-0.12	-0.29*	-0.23	0.33*	0.10
Density of guard fur, p	0.23	-0.16	0.07	0.06	0.14	0.20
Density of underfur, p	-0.03	0.27	0.24	-0.20	0.25	0.05
Hair quality, p	0.04	0.37*	0.41**	0.10	0.34*	0.44**
Fur colour, p	-0.32**	0.21	-0.11	-0.23	0.22	-0.01
Metallic, p	0.07	-0.34*	-0.27	0.17	-0.56***	-0.39**
Wet belly, p	0.26	-0.80***	-0.54**	0.03	0.03	0.00
General fur quality, p	-0.03	0.30*	0.27*	-0.09	0.22	0.16

p = points.
* = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

sented for the selection lines, expressed as deviations from the control line during the experiment. Figure 3 shows that no drastic changes were observed in fur characteristics as a correlated effect of selection for litter size at birth. There are, however, some negative tendencies in the line selected for decreased litter size with regard to such traits as density of the underfur, hair quality and in particular general fur quality. In the line selected for increased litter size a better density of guard fur and a darker colour were observed compared to the control line. On average the body weight was somewhat reduced when selecting for increased litter size, but this reduction only slightly affected the body length and did not affect the final skin length.

Genetic parameters

The heritability estimates for body size, skin size and fur characteristics of males are presented in Table 7. The analyses were conducted over generations and the results are given separately for each line and for the entire material. It should be stressed that the data included in these analyses are based only on those males that were pelted. The estimates given in Table 7 were based on the sire component and included an average of 5.5 male progenies per sire in the low line, 6.8 in the control line and 7.4 in the high line. The standard errors are given for the entire data base only, but were about twice as high within the lines. The level of significance given in Table 7 was based on the significance

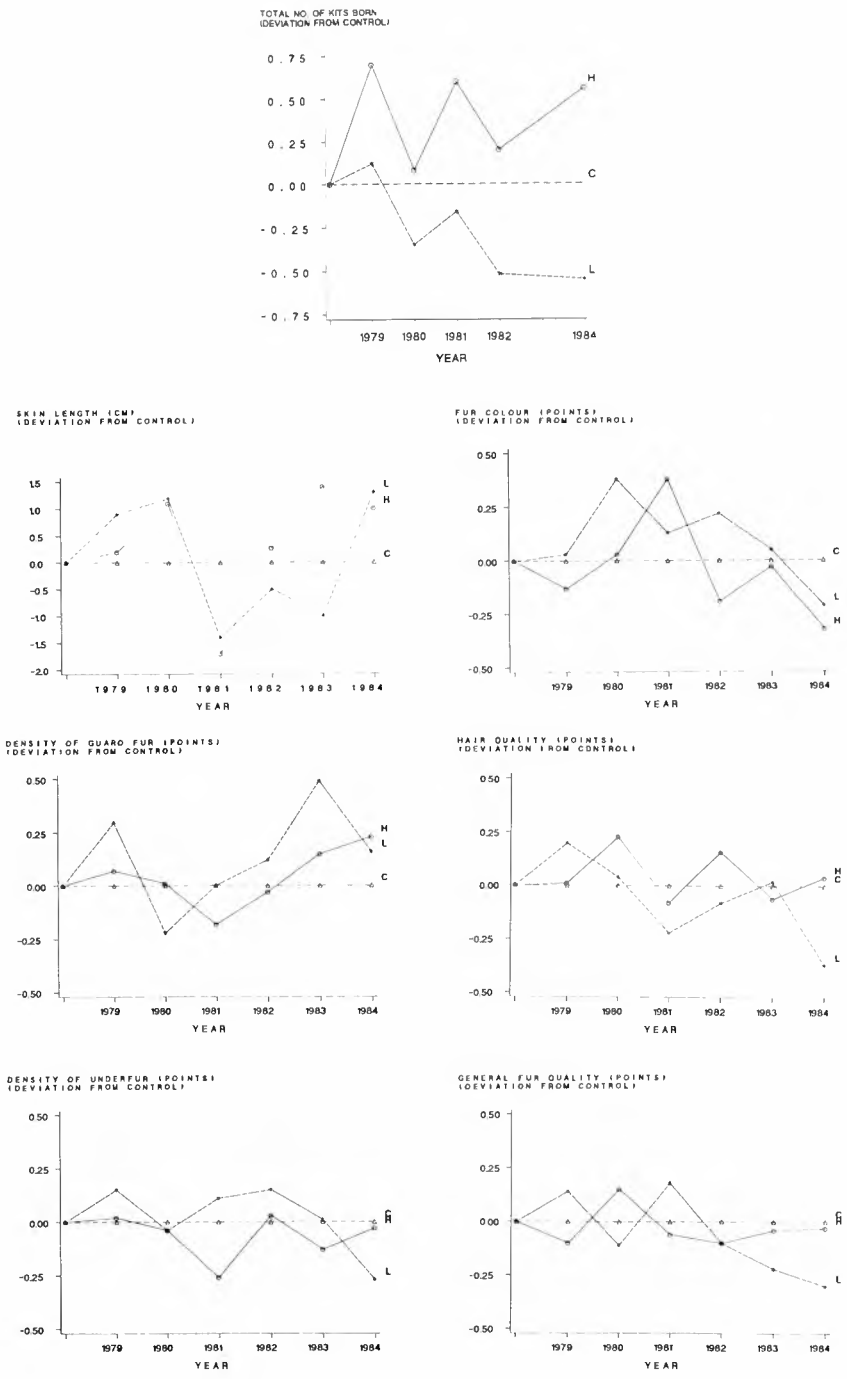

Figure 3. Direct response in litter size at birth (figure at the top) and correlated responses in skin length and some fur characteristics of male skins. The responses in the H-line and the L-line are expressed as deviations from the C-line for the six generations throughout 1979 to 1984.

Table 7. Heritability estimates for body size, skin size and fur characteristics of males. The estimates are based on the sire component and estimated over years. The level of significance given is based on the sire component in the hierarchical model.

Trait	L-line h^2_S	C-line h^2_S	H-line h^2_S	All lines h^2_S	All lines SE
Body weight at pelting	0.18	0.53***	0.43**	0.28**	± 0.12
Body length at pelting	0.51**	0.70***	0.59***	0.57***	± 0.15
Skin length	0.21*	0.49**	0.54***	0.36***	± 0.13
Skin weight	0.24*	0.41**	0.59***	0.36***	± 0.13
Length of guard fur	0.89***	0.93***	0.88***	0.75***	± 0.15
Length of underfur	0.99***	0.56***	0.61***	0.64***	± 0.13
Density of guard fur	0.01	0.00a	0.00a	0.00a	± 0.09
Density of underfur	0.42*	0.33*	0.49***	0.44***	± 0.12
Hair quality	0.30*	0.01	0.00a	0.15*	± 0.10
Fur colour	0.52**	0.75***	0.58***	0.50***	± 0.13
General fur quality	0.15	0.19	0.31*	0.25**	± 0.10

p = points.
* = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.
a denotes that the variance component of the sire was negative.

Table 8. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) for body size and skin size of males. The estimates are based on the sire component.

Trait	Line	Trait 1	Trait 2	Trait 3	Trait 4
1. Body weight at pelting	L		-0.97	-0.18	-0.39
	C		0.39	0.78	0.52
	H		0.30	0.96	0.89
	All		0.05	0.63	0.46
2. Body length at pelting	L	0.50		0.90	0.52
	C	0.51		0.54	0.15
	H	0.55		0.60	0.35
	All	0.52		0.55	0.53
3. Skin length	L	0.76	0.58		0.44
	C	0.77	0.55		0.57
	H	0.80	0.58		0.72
	All	0.78	0.57		0.50
4. Skin weight	L	0.66	0.52	0.80	
	C	0.67	0.47	0.75	
	H	0.71	0.50	0.78	
	All	0.68	0.50	0.77	

Table 9. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) for fur characteristics of male skins. The estimates are based on the sire component.

Trait	Line	Trait						
		1	2	3	4	5	6	7
1. Length of guard fur	L		0.95	0.32	-0.22	-0.33	-0.87	0.23
	C		0.86	a	0.17	0.21	-0.57	0.12
	H		0.89	a	0.70	a	-0.70	0.75
	All		0.96	a	0.30	-0.15	0.37	0.40
2. Length of underfur	L	0.72		-0.83	-0.27	-1.00	-0.51	-0.11
	C	0.66		a	0.19	0.10	-0.41	0.41
	H	0.65		a	0.85	a	-0.35	0.83
	All	0.68		a	0.34	-0.69	-0.45	0.46
3. Density of guard fur	L	-0.06	-0.01		-0.95	1.00	-1.00	-0.26
	C	-0.05	0.01		a	a	a	a
	H	-0.05	0.05		a	-1.00	a	a
	All	-0.05	0.02		a	a	a	a
4. Density of underfur	L	0.15	0.18	0.38		1.00	-0.87	-0.24
	C	0.14	0.21	0.33		0.68	-0.65	0.22
	H	0.12	0.22	0.40		a	-0.16	-0.32
	All	0.13	0.21	0.37		0.21	-0.52	-0.09
5. Hair quality	L	-0.12	-0.09	0.45	0.39		-0.39	-0.48
	C	-0.07	0.03	0.47	0.30		-1.00	-1.00
	H	-0.07	0.02	0.42	0.32		a	a
	All	-0.09	-0.01	0.44	0.33		-0.34	-0.44
6. Fur colour	L	0.01	-0.07	-0.28	-0.21	-0.11		-0.45
	C	-0.02	0.02	-0.28	-0.17	-0.06		-0.61
	H	-0.07	-0.05	-0.29	-0.11	-0.06		0.07
	All	0.15	-0.03	-0.29	-0.16	-0.08		-0.09
7. General fur quality	L	0.09	0.11	0.24	0.29	0.25	0.01	
	C	0.08	0.12	0.18	0.25	0.25	0.01	
	H	0.08	0.14	0.14	0.13	0.26	0.05	
	All	0.08	0.12	0.18	0.21	0.26	0.02	

a denotes that the variance component of sire was negative. Figures >1 and <-1 were set equal to 1 and -1, respectively.

of the sire component in the nested model, the F-value expressed as the mean square of the sire divided by the mean square of the dam. Heritability estimates based on the maternal component varied, but were generally higher than those presented. This could indicate a maternal effect on the traits. However, with limited material, varying from 2.2 to 2.5 male progenies per dam, no definite conclusions could be drawn.

The genetic and phenotypic correlations between traits of body size and skin size of male mink are presented in Table 8.

With the exception of body weight at pelting in the L-line all these correlations were found to be positive, which was to be expected for such closely related traits. No explanation was found for the negative genetic correlations in the L-line. Genetic and phenotypic correlations between fur characteristics are given in Table 9. The reliability of the estimates in both Tables 8 and 9 should be judged on the background of the level of significance of the sire component, given in Table 7. The mean square of the sire was almost equal to the mean square of the dam for density of guard fur in the L-line, thus explaining these estimates.

DISCUSSION

Postweaning growth

The body weight at pelting of both males and females was significantly lower in the H-line in the last generation than in the L-line. However, as shown in Figure 1, no line differences were observed in previous generations as a result of the selection for litter size. Table 5 shows that no significant differences were observed in male average body weight at pelting between the first and the last generation within the lines. It should also be noted that even with a low preweaning or weaning body weight, as presented in Part II in this series (Einarsson, 1987c), compensatory growth results in an almost normal body weight by the time of pelting.

No significant differences in body length at pelting were observed between the lines in the last generation. There was, however, a general trend to reduced body length in all the lines during the experiment. The total reduction was about 1.5 cm for the males in all lines, which was in the same order as one standard deviation. No explanation was found for this reduction, but some variation in the stretching of the animals from one generation to the other could be responsible for some of the differences. However, this would not affect the comparison between lines within year. The reduction in body length at pelting could not be explained as an effect of variation in body weight at pelting.

Earlier results showed that an increase of one kit per litter reduced the average body weight at pelting by 20 – 25 grams for males and by 10 – 15 grams for females (Venge, 1960; Hoogerbrugge & Baud, 1975; Reiten, 1978; Einarsson, 1980). The corresponding reduction in body length at pelting was 0.10 – 0.15 cm for the males and 0.05 – 0.10 cm for the females, while the skin length was reduced by 0.32 cm for the males and 0.17 cm for the females (Rimeslåtten, 1961; Reiten, 1978; Einarsson, 1980). Einarsson (1980) pointed out that the reductions in body size by increased litter size are small in litters of 3 to 7 kits only, which shows that the effect of litter size on body size is not linear.

In selection experiments for litter size in mice, no general significant correlated responses in progeny body weight have been found (Bakker et al., 1978). However, when standardizing litter size at birth, positive correlations between litter size and growth have been found, and positive genetic correlations with postweaning gain have been estimated (Joakimsen & Baker, 1977; Eisen, 1978). Increased litter size as a result of selection for increased body weight has also been reported. However, as discussed by Joakimsen & Baker (1977), this is probably due to positive correlation between body weight and ovulation rate and not between body weight and embryo loss. In the mink there should be a distinction between what could be called the «true body size» and the varying degrees of fatness. This is especially important in mink since the animal is fattened towards pelting, which is about the time when the growth of the gonads are initiated. Tauson & Alden (1985) observed

that very fat mink females often had reduced reproduction results, which concurs with other reports quoted by them.

Skin length and skin weight

The skin length of the males was reduced during the experiment, although it showed a small increase in the last generation. Significant differences in the length of the male skins were observed within the L- and C-lines between the first and the last generations. However, Figure 3 shows that there were no differences in skin length between the lines as a result of the selection for litter size.

From the earlier results it could be concluded that skin weight was not affected by selection for litter size. As long as neither body length nor skin length are affected by the selection, the skin weight is not expected to be affected either. Einarsson (1980) found a significant reduction of skin weight by 1.5 – 2 grams for the females when litter size increased by one kit. In the same analysis it was found that the thickness of the leather was not affected by variation in litter size.

Fur characteristics

As discussed earlier, evaluation of the fur characteristics was based on the male skins, as these represented the lines best. Fur characteristics in November were determined on the live animals in the first years, but these observations gave less precise information than the observations made on the skins. Consequently they are not published in this paper.

The lengths of both the guard fur and the underfur increased significantly in the males during the experiment, but no trend was observed as a result of the correlated response for change in litter size. These traits, and especially the length of the underfur, are relatively objectively observed traits. The measuring method was therefore not regarded as an explanation for any large part of the variation. In an experiment by Skrede (1983) it was found that an increased ratio of fat:carbohydrate, which increased the energy and resulted in fatter animals, had a tendency to cause reduced length of the guard fur. However, in the current experiment there was no variation in the ratio of fat:carbohydrate, and no difference in body weight was observed. Selection pressure has always been placed on fur characteristics and on fur length. In this experiment, without selection pressure on fur length, the guard fur might have changed to become the fur type of the original wild mink. It is, however, difficult to accept this as the only explanation, except in the case where there is a high «fitness-value».

The density of both the guard fur and the underfur showed no correlated response, although a minor increase was observed in the H-line during the last generation. The density of the guard fur is difficult to judge and it depends on both the number and the length of the guard hairs. It could therefore be speculated that the increase in density was caused by the increased length of the guard hairs. However, when describing the impression of the density by the hair length rather than by the number of guard hairs, it depends more on the texture (the proportion between the length of the guard fur and the underfur) than on the length of the guard hairs themselves. So although the length of the hairs increased during the experiment, the texture remained unchanged.

The male skins in the H-line became lighter up until 1981, but then darkened and were significantly darkest in the last generation. The changes in colour score in the L-line were almost the same as those in the H-line.

It was also observed that the hair quality in both the selection lines changed irregularly throughout the experiment when compared to the control line, and showed a significantly poorer result in the L-line in the last generation. It is known that melatonin has a positive effect on the development of the winter fur and on the colour, but acts as an inhibiting factor on the gonadotrophic hormones. However, no effect on these fur characteristics was found as a result of increasing or decreasing the litter size. The work of Ellis et al. (1982), however, indicates that male infertility and neonatal kit losses were genetically closely linked to finely-bred dark

mink. This fur type was defined as dark, short and with a silky texture.

The general fur quality of the male skins, judged at the Oslo Fur Auctions, varied irregulary during the first four years and was then reduced in the L-line to become significantly lowest compared to both the other lines. The same impression is shown in Figure 2, where the frequency of skins classified as Saga Selected and Saga is higher for both sexes in the H- and C-lines compared to the L-line.

Concerning the disorders metallic and wet belly, no effect was observed as a correlated response of the selection for litter size. However, in the last generation a significantly higher frequency of these disorders was observed in the H-line.

The evaluation of the fur characteristics at the experimental farm was conducted at the same time on all skins of the farm, which were randomly mixed. Assuming a general improvement in fur characteristics on the rest of the fur farm, the quality of the individual fur characteristics of the skins from the selection experiment could be undervalued throughout the experiment. These effects would only be indicated if comparing results over the generations and not between lines within a generation, or as deviations from the control line over the generations.

Genetic parameters

The genetic parameters were estimated over the generations and possible changes during the experiment will therefore not be discussed. The heritability estimates presented for the males concur well with those found by Reiten (1977b) for the traits of body weight, body length, skin length, skin weight, length of guard fur and underfur, density of underfur and fur colour. Low heritability was found in this present analysis for general fur quality and much lower for hair quality and density of the guard fur. The difference of opinion between the two investigations over these latter traits is thought to have been brought about by a better judgement of the single traits in Reiten's (1977b) experiment.

Reiten (1977b) estimated heritability for body size and fur characteristics, and, quoted several reports – Johansson (1955), Jonsson (1971), Sandh (1975) and Olausson (1976) – with estimates in the same order as he had found. Since that time only a few studies have been published on genetic parameters. Lohi & Christensen (1985) estimated heritabilities at 0.18 for skin length, 0.32 for general fur quality according to a scale from 1 to 15, 0.09 when using quality classes at the fur auction and 0.26 for fur colour. When correcting for sex differences the analysis was based on the sire component of four half siblings of each sex of standard dark. In a Swedish investigation genetic parameters were based on the fur quality of the living animal only. Lagerkvist & Lundeheim (1985) estimated the heritabilities at 0.93 for colour, 0.15 for density of the underfur, 0.23 for general fur quality and 0.23 for body weight in September. These estimates of the standard dark were based on the sire component, and sex was included in the hierarchical model. Reiten (1977b) found only slight differences in the estimates caused by the sex.

Maternal effects on fur characteristics do not appear to be of any significant importance, although this has been reported when heritability estimates based on the maternal component were larger than those based on the sire component (Reiten, 1977b; Lohi & Christensen, 1985; Lagerkvist & Lundeheim, 1985). However, as pointed out earlier, the data are often too limited to draw such conclusions.

Generally high correlations were found between body weight and body length at pelting, skin size and skin weight. Regarding the fur characteristics, high correlations were found between the length of the guard fur and the underfur, which concurs with the phenotypic correlations found by Reiten (1977a).

Positive correlations were found between fur length and density of the underfur. Reiten (1977a) points out that this could be explained to some extent because longer underfur could give the impression of better density, although the number of hairs was the same. Of course this argument would be based on the assumption that there was a minimum number of hairs.

As pointed out earlier the genetic parameters including density of guard fur were unreliable. In this present analysis the density of the underfur is positively phenotypically correlated to both hair quality and general fur quality, in accordance with Reiten's (1977a) results. However, the genetic correlations were found to be negative. This is difficult to explain, especially for general fur quality. The same situation obtains between hair quality and general fur quality, where the phenotypic correlation was positive, while the genetic correlation was negative. It seems very unlikely that there could be negative genetic correlations between these traits, as the density of the underfur and hair quality are two of the most important factors for the collective term «general fur quality».

The results presented indicate that body weight at pelting decreases slightly when selecting for increased litter size at birth in mink, as opposed to decreasing the litter size. It might be expected that further accumulated selection responses in litter size would affect both body size and skin size, but only slightly affect the fur characteristics. However, endocrinological relations on reproductive and fur traits should be investigated in more detail.

The genetic parameters indicate that all size-related traits and fur characteristics could be further improved by selection. These traits could therefore be successfully combined with reproductive traits in a complete selection programme. However, with strong emphasis on litter size, the growth and final size of the animals should be given special attention.

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SELECTION FOR LITTER SIZE IN MINK

V. Development of an applied selection index

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A selection index for litter size in mink has been developed for applications in a selection programme. The selection index includes information for litter size at about three weeks postpartum based on defined relatives and is calculated separately for dam and sire. The pedigree index is half the sum of the parental indices and is standardized to a mean value of 100 with a standard deviation of 10. Adjustments to allow for systematic environmental factors are discussed. The reliability of the index is calculated and the genetic gain by using the index is estimated at about 0.1 kits per year in commercial farming. By using the selection index for litter size in mink, farmers will be able to practise an effective between-litter selection for litter size, as one of the stages in the complete selection programme.

Key words: Breeding programme, litter size, mink, selection, index.

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During recent years, more attention has been given to the development of selection methods in mink (Einarsson, 1978; Rønningen et al., 1980; Einarsson & Elofson, 1981; Elofson & Einarsson, 1984; Christensen et al., 1984). Litter size should be given high priority when establishing breeding strategies. As shown in the selection experiment for litter size in mink, the selection index is an effective tool (Einarsson, 1987a, b) and should be applied in commercial selection programmes. In the earlier papers in this series it is shown that litter size can be changed during selection without negative effects on other economically important traits in the form of correlated responses (Einarsson, 1987b, c, 1988).

In order to estimate the most accurate breeding value of the kits for litter size, an index had to be developed to combine information from relevant relatives. Falconer (1967) states that such a combined selection, in principle, will always be the best method and relatively more efficient with a trait of low heritability, such as litter size. This combined selection will also allow for the inclusion of information from both the dam and the sire side of the kits.

Fertility can be divided into two parts – male and female fertility. The selection index presented in this paper concentrates on female fertility expressed in terms of litter size. However, male fertility should also be included in the selection programme.

MATERIAL AND METHODS

The parameters used in the development of the index for litter size came from personal calculations based on information from commercial mink farms and from the experimental farms of the Agricultural Universities of Norway and Sweden. In addition, parameters from the selection experiment for litter size at birth in mink (Einarsson, 1987a, b, c) and from the relevant literature were also used.

Heritability and standard deviation of litter size at three weeks postpartum were calculated from Swedish sources and consisted of 1 600 standard females at five commercial farms and 111 standard females at one experimental farm. The methods of estimation used were daughter nested within sire in data from the commercial farms and regression of daughter on dam in data from the experimental farm. With the latter method, all age classes of dam were included, but only litters from one-year-old daughters. The data from the experimental farm were also used in the calculation of age correction factors, genetic gain and correlations between the index of the female and the size of its last litter and its dam's index. Some preliminary results from the latter calculations have been presented earlier (Elofson, 1981b).

The material from two commercial Danish mink farms was also used in the calculations of the age correction factors and earlier in estimating genetic parameters for litter size in mink. A more detailed description of this data was given by Einarsson (1981) together with data from the Norwegian experimental farm.

In Einarsson's (1981) report it was claimed that there was no negative correlation between direct and maternal effects on litter size in mink. Further analysis of these data, from the experimental farm in Norway, has been conducted and estimates of some components of covariance between relatives are presented in the present paper.

When calculating the age multiplicative correction factors, the relative value of mean litter size for three-year-old females was fixed at 1.00. The correction factors of the one- and two-year-old females were calculated by dividing their mean litter size into the three-year-old females' mean litter size.

RESULTS

The selection trait

The number of kits alive at a given time postpartum is dependent on different factors (illustrated in Fig. 1). The litter size itself could therefore be regarded as an index that includes several of these factors. If the first examination of litter size was made at a random time during the first 2 – 3 days after birth, it would be unreliable, as the highest kit mortality occurs during the first two days after birth (Einarsson, 1987c). It was therefore decided to define the trait used in the selection index as *number of kits alive at three weeks of age*. This trait takes into account the ability of the female to give birth to the litter, as well as its maternal ability to nourish the kits. It also allows for practical applications, both by covering the variation in time of examination and the possibility of returning the farm report, including the calculated breeding values, before weaning.

It was assumed that litter size was normally distributed, that random mating took place and that no relationship between the sire and the dam occurred.

Parameters and information sources

Estimates of heritability for litter size in mink are given in Table 1, together with means and standard deviations. The average genetic standard deviation from the Swedish data was 1.08 and 0.88 for the experimental farm and the commercial farms, respectively.

An analysis of components of covariance between relatives, in data previously used to estimate heritability for litter size (Einarsson, 1981), showed that the additive maternal variance component was about zero, while the environmental maternal variance component was slightly positive. Both the genetic and the environmental covariances between the direct and the maternal effect were zero or slightly below

zero. The covariance components were found to be in the order from 0.00 to -0.03, even with a slight increase in the maternal variance component. With a fixed value for the heritability and the maternal variance components, the two covariance components varied inversely.

The genetic and phenotypic parameters chosen in the development of the index were:

h^2 = 0.20 – the heritability of the trait litter size

σ_P = 2.05 – the phenotypic standard deviation of the trait

σ_G = 0.92 – the genetic standard deviation of the trait

σ^2_{Am} = 0.0 – the additive maternal variance component of the trait

σ_{om} = 0.0 – the covariance component between direct and maternal effect of the trait

r = 0.25 – the repeatability of the trait

c^2 = 0.05 – common environmental effect for full sibs

It is well known that reproductive success in mink varies between parities, i.e. among the

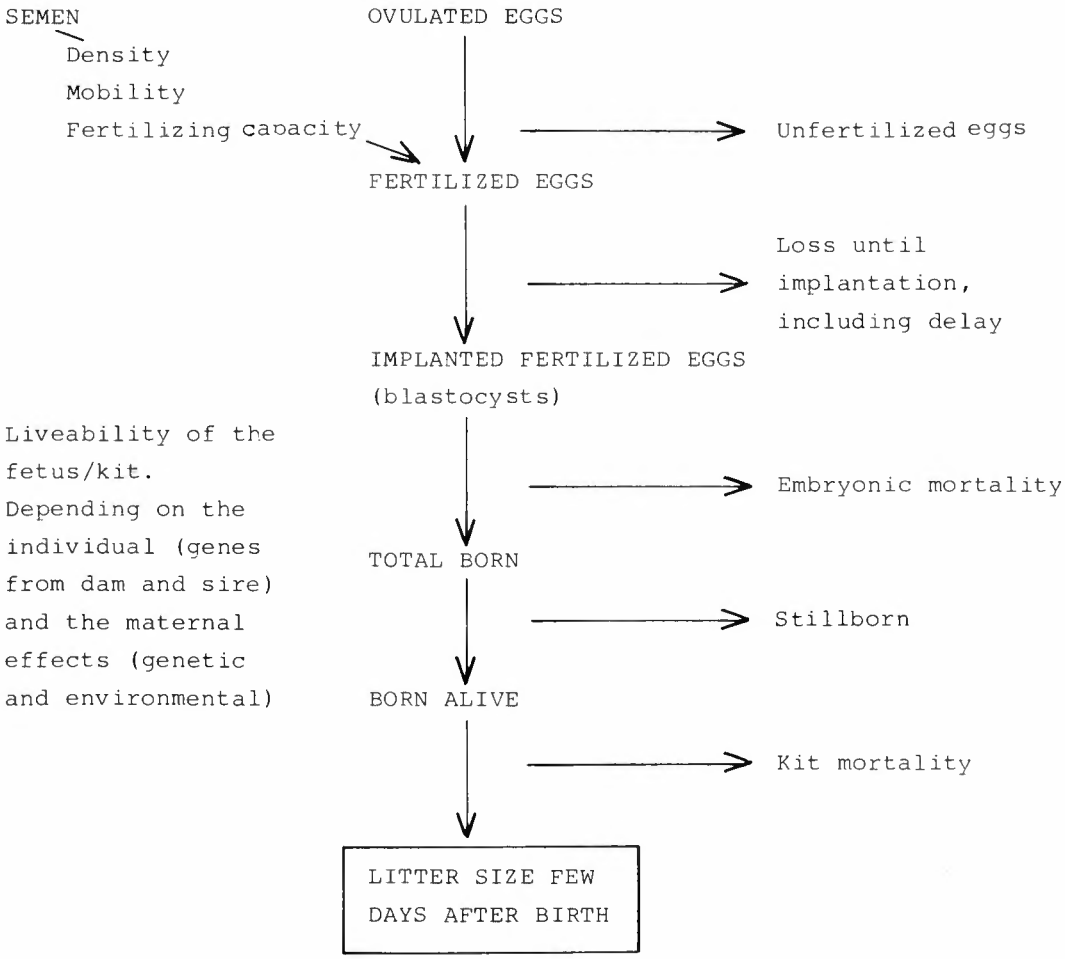


Fig. 1. Factors determing the litter size in mink.

age classes (as shown in Table 2). The multiplicative correction factors for the age of the female on litter size at weaning, from two commercial mink farms are given in Table 2. Based on the average of both farms and including all colour types, the correction factors were 1.09 for one-year-old females and 0.93 for two-year-old females when fixed to 1.00 for three-year-old females. From the Swedish experimental farm the correction factors for litter size at three weeks postpartum were estimated at 1.11 and 0.89 for one- and two-year-old females, respectively. In order to allow for the correction of the effect of the females' age on litter size in the index calculations, the following multiplicative correction factors were chosen:

one-year-old females	1.1
two-year-old females	0.9
older females	1.0

The number of different groups of relatives involved in the model has to be judged according to their contribution to the increase of the correlation between the true breeding value (T) and the calculated index (I), r_{TI}. The evaluation of this problem was based on the works of Lush (1947), Searle (1963) and Henderson (1963), and by using Cunningham's (1968) computer programme. Possible practical complications through using information from several relatives were also taken into consideration.

Information about litter size at three weeks postpartum from the dam herself, the full sisters and the granddam of the litter should obviously be included.

As shown in Fig. 2 these individuals or groups of individuals provide a significant contribution to the r_{TI}-value of the dam index. However, as seen from the same figure there

Table 1. Heritability for litter size, and mean and standard deviation of litter size in mink.

Colour type	Litter size at[a]	Number of females	h^2 [b]	Mean	S.D.	References
Standard	3 w.p.p.	111	0.24	4.6	2.20	exp. farm
Standard	3 w.p.p.	1 600	0.19	4.4	2.03	com. farms
Selection experiment						
Standard	birth	792	0.11	6.72	2.46	Einarsson, 1987b
Literature						
Standard	birth	374	0.24			Venge, 1961
All	birth	9 666	0.07	5.5	1.7	Johansson, 1965[c]
Standard	birth	4 163	0.12	5.0		Narucka & Gedymin, 1978[d]
Standard	3 w.p.p.		0.19			Rosberg, 1978[e]
Standard	weaning	230	0.16		2.2	Pastirnac, 1980
Standard	birth	953	0.22	5.70	2.16	Einarsson, 1981
All	weaning	5 623	0.20	5.55	2.06	Einarsson, 1981
All	3 w.p.p.	17 400	0.26		1.97	Clausen, 1985

[a] 3 w.p.p. = 3 weeks postpartum. Litter size at birth is total number of kits at first examination.
[b] Different estimation methods are used.
[c] Including females with living kits only.
[d] The heritability for number of kits at weaning was estimated at 0.11.
[e] Cited by Rønningen et al., 1980.

are low r_{TI}-values with limited family size or limited information within the family. In these cases it would be of value to include other relatives that could give an additive contribution to increase the correlation between the true breeding value and the calculated index. Information about litter size from the half sisters of the dam was therefore included, although their contribution within a normal family size would be low.

When using information from the sisters of the dam and the sire, the excluding model was used (Liljedahl et al., 1979). In this model the individual's information was excluded from the full sister's mean and information from the full sister's mean was excluded from the half sister's mean.

Selection index

The development of the selection index for litter size in mink was based on the work of Hazel (1943), Osborne (1957a, b) and Henderson (1963). The aggregate genetic value of an individual (the true breeding value, T) can be expressed as:

$$T = \sum_{i=1}^{n} (a_i \cdot G_i)$$

Table 2. Mean and standard deviation of litter size at weaning of different age classes and colour types at two mink farms. Multiplicative correction factors for the age of the female on litter size were calculated as if for three-year-old females fixed at 1.00.

Colour type	Age of female	Farm 1				Farm 2			
		No. of females	Litter size at weaning Mean	SD	Correct factor	No. of females	Litter size at weaning Mean	SD	Correct factor
All	1	1 310	5.21	2.07	1.08	1 366	5.13	2.03	1.10
	2	969	5.98	2.01	0.94	761	6.14	1.98	0.92
	3	633	5.65	2.13	1.00	648	5.66	1.86	1.00
	4	297	5.19	2.01	1.09				
Standard	1	357	4.49	2.08	1.16	512	4.88	2.07	1.10
	2	234	5.22	1.96	1.00	179	6.01	2.14	0.90
	3	169	5.20	2.17	1.00	187	5.38	1.89	1.00
	4	62	5.23	2.07	1.00				
Pastel	1	385	5.41	1.86	1.05	186	5.20	2.06	1.08
	2	179	6.09	1.96	0.93	121	6.20	1.92	0.91
	3	153	5.69	2.16	1.00	197	5.62	1.94	1.00
	4	36	5.39	2.23	1.06				
Demi buff	1	253	5.17	2.06	1.13	354	5.14	2.02	1.12
	2	250	5.89	1.96	0.99	212	6.22	1.76	0.93
	3	183	5.82	1.95	1.00	139	5.78	1.69	1.00
	4	97	5.01	1.93	1.16				
Pearl	1	312	5.80	2.06	1.02	314	5.50	1.89	1.06
	2	295	6.60	1.94	0.90	249	6.12	2.08	0.95
	3	125	5.91	2.19	1.00	215	5.84	1.89	1.00
	4	95	5.37	1.95	1.10				

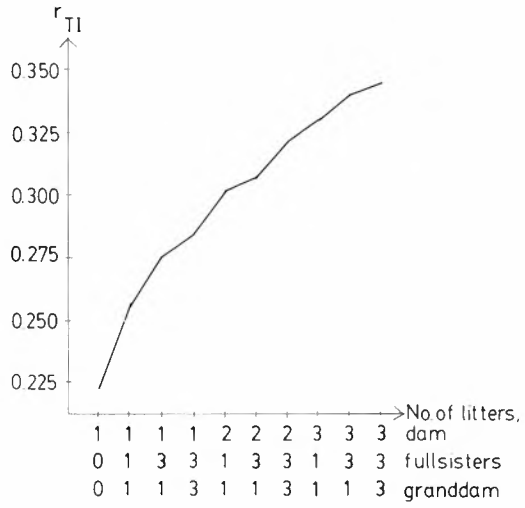


Fig. 2. The effect of increased information sources on the accuracy of the dam index for litter size in mink.

where

a_i = a vector of the relative economic value of one unit of trait i (here the litter size)

G_i = the additive genotype for the trait i (litter size) of the individual

The true breeding value cannot be directly selected since the heritability is less than one, and the selection criterion has therefore to be an estimate of T. The selection index (I) serves this function and is expressed as:

$$I = \sum_{i=1}^{n} (b_i \cdot P_i)$$

where the symbols, when including only litter size and several information sources, are

b_i = partial regression coefficients, expressing the weight of the phenotype for the individual i (or mean of individuals) in the index

P_i = phenotypic record expressed as deviation from the population mean for the trait measured on individual i

Cunningham (1969) points out that the index is the best estimate of T, while it

- maximes the correlation between T and I (r_{TI})
- maximizes the probability of the correct ranking of the individuals on their T-value
- maximizes the genetic changes through selection
- minimizes the mean squared difference between T and I

In the derivation of the selection index (see Cunningham, 1969) we aim at calculating the weighting vectors (b_i) in the index by maximizing r_{TI}, where

$$r_{TI} = \frac{\sigma_{TI}}{\sqrt{\sigma_T^2 \cdot \sigma_I^2}}$$

With a selection index including only one trait and four information sources, as for the dam index we have

$$\begin{aligned}
b_1 \cdot \sigma^2_{P_1} &+ b_2 \cdot \sigma_{P_1P_2} + b_3 \cdot \sigma_{P_1P_3} + b_4 \cdot \sigma_{P_1P_4} = \sigma_{TP_1} \\
b_1 \cdot \sigma_{P_1P_2} &+ b_2 \cdot \sigma^2_{P_2} + b_3 \cdot \sigma_{P_2P_3} + b_4 \cdot \sigma_{P_2P_4} = \sigma_{TP_2} \\
b_1 \cdot \sigma_{P_1P_3} &+ b_2 \cdot \sigma_{P_2P_3} + b_3 \cdot \sigma^2_{P_3} + b_4 \cdot \sigma_{P_3P_4} = \sigma_{TP_3} \\
b_1 \cdot \sigma_{P_1P_4} &+ b_2 \cdot \sigma_{P_2P_4} + b_3 \cdot \sigma_{P_3P_4} + b_4 \cdot \sigma^2_{P_4} = \sigma_{TP_4}
\end{aligned}$$

where

b_i = weighting factors as defined earlier
$\sigma^2_{P_i}$ = phenotypic variance
$\sigma_{P_iP_j}$ = phenotypic covariance (between information sources)
σ_{TP_i} = covariance between the true breeding value and the phenotype

We assume that the variance of litter size is the same for all information sources (P_i) and that the covariance between relatives is of additive genetic origin only, which includes that

$$\sigma_{P_iP_j} = r_{G_{ij}} \cdot \sigma^2_G$$

where $r_{G_{ij}}$ is the additive genetic relationship between the individuals i and j and σ^2_G is the additive genetic variance. Similarly,

$$\sigma_{T_\alpha P_i} = r_{G_{i\alpha}} \cdot \sigma^2_G$$

The covariances given in the four equations can now be replaced and the equations divided by $\sigma^2_{P_i}$. This gives the following equation (only showing the first)

$$b_1 \cdot \frac{\sigma^2_P}{\sigma^2_P} + b_2 \cdot r_{G_{12}} \cdot \frac{\sigma^2_G}{\sigma^2_P} + b_3 \cdot r_{G_{13}} \cdot \frac{\sigma^2_G}{\sigma^2_P} + b_4 \cdot r_{G_{14}} \cdot \frac{\sigma^2_G}{\sigma^2_P} = r_{G_{1\alpha}} \cdot \frac{\sigma^2_G}{\sigma^2_P}$$

as $h^2 = \frac{\sigma^2_G}{\sigma^2_P}$ we have the full set of equations

$$\begin{array}{llll}
b_1 + b_2 \cdot r_{G_{12}} \cdot h^2 + b_3 \cdot r_{G_{13}} \cdot h^2 + b_4 \cdot r_{G_{14}} \cdot h^2 = r_{G_{1\alpha}} \cdot h^2 \\
b_1 \cdot r_{G_{12}} \cdot h^2 + b_2 + b_3 \cdot r_{G_{23}} \cdot h^2 + b_4 \cdot r_{G_{24}} \cdot h^2 = r_{G_{2\alpha}} \cdot h^2 \\
b_1 \cdot r_{G_{13}} \cdot h^2 + b_2 \cdot r_{G_{23}} \cdot h^2 + b_3 + b_4 \cdot r_{G_{34}} \cdot h^2 = r_{G_{3\alpha}} \cdot h^2 \\
b_1 \cdot r_{G_{14}} \cdot h^2 + b_2 \cdot r_{G_{24}} \cdot h^2 + b_3 \cdot r_{G_{34}} \cdot h^2 + b_4 = r_{G_{4\alpha}} \cdot h^2
\end{array}$$

These equations can be solved and b_i found.

In the derivation of the selection index previously shown, only one observation was given for each information source (P_1, P_2, P_3, P_4). However, in the index for litter size we may have several observations on full and half sisters and often repeated observations on dam and granddam. This influence on the variance given in the diagonal in the four equations presented earlier ($b_i \cdot \sigma^2_P$). There is no longer unity and the following element has to be introduced

$$\sigma^2_{P_i} = \frac{1 + (n-1) \cdot r}{n} \sigma^2_P$$

- for repeated observations r is the repeatability of single records (dam and granddam)
- for full sisters $r = 0.5\ h^2 + c^2$
- for half sisters $r = 0.25\ h^2$

The genetic correlations (relationship) within the sister groups are 0.5 and 0.25, respectively, when using the excluding model.

With all the combinations of b-values, using matrix algebra to solve the set of b_i is much simpler and allows for the use of a computer programme (Cunningham, 1968). The general index equation was

$Pb = Ga$, which is solved as
$b = P^{-1} \cdot Ga$

The P-matrix is a symmetric n×n matrix, where n is the number of information sources. It describes the phenotypic variance and covariances between these sources, with the variance as the leading diagonal. The b-values are the unknown factors in the model which are to be calculated. The G-matrix is a simple diagonal n×m matrix,

describing genotypic variances and with one trait, only including the element $r_G \cdot \sigma^2_G(m = 1)$. The single trait selection also simplifies the a-vector and the C-matrix, as 1.0 and σ^2_G, respectively. The matrixes are shown in Figure 3.

The importance of maximizing the correlation between the true breeding value and the index is clearly seen from the formula of genetic change (ΔG).

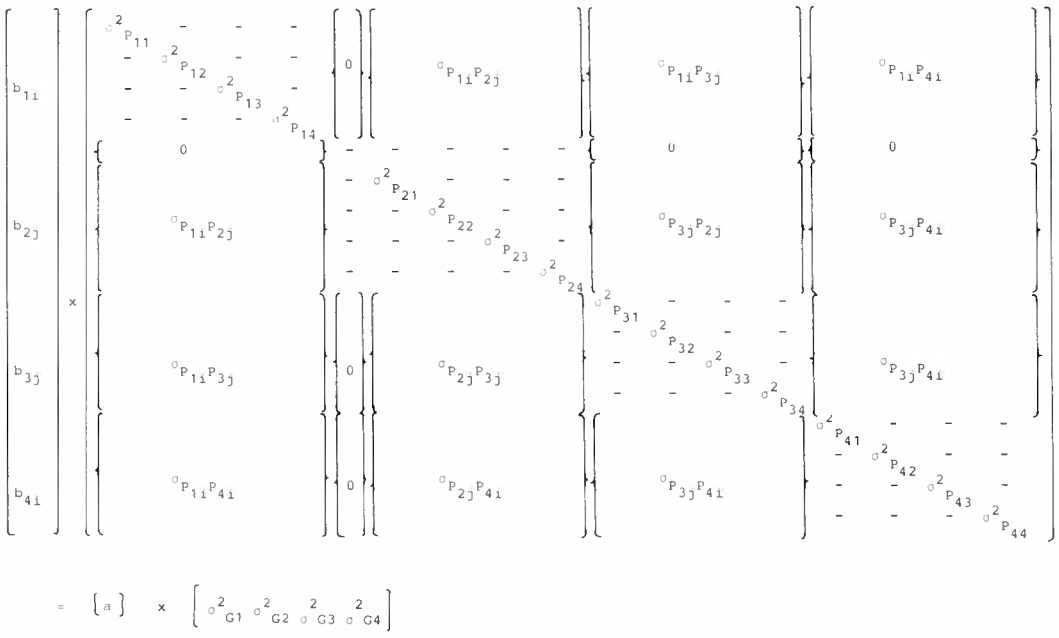
$$\Delta G = \frac{r_{TI} \cdot i \cdot \sigma_G}{L}$$

where

i = selection intensity
L = generation interval

The other symbols were given earlier. This equation, which is one of the most important in the breeding theory, gives all the elements influencing the genetic change in a given trait under selection.

The r_{TI} of the calculated pedigree index is half the square root of the heritability of the in-



$$= \{a\} \times \left[\sigma^2_{G1} \; \sigma^2_{G2} \; \sigma^2_{G3} \; \sigma^2_{G4} \right]$$

P_1 = litter size of the dam
P_2 = litter size of fullsisters of the dam
P_3 = litter size of halfsisters of the dam
P_4 = litter size of granddam
i = number of recorded litters per female
j = number of individuals with recorded litter size (excluding model)
σ^2_P = phenotypic variation
σ_{PiPj} = phenotypic covariation
σ^2_G = genetic variation
b = the weighting coefficients in the index
a = the economical vector

Fig. 3. The matrixes used in the estimation of the b-values included in the selection index for litter size in mink.

dex, since the trait, and thereby the breeding value, is based on the parental observations

$$r_{TI_P} = \sqrt{0.25 \cdot r^2_{TI_D} + 0.25 \cdot r^2_{TI_S}}$$

In the following section the construction of the indexes for the dam and the sire of the litter will be shown separately.

The dam index was calculated as:

$$I_D = b_1 (\bar{P}_1 - \bar{P}) + b_2 (\bar{P}_2 - \bar{P}) + b_3 (\bar{P}_3 - \bar{P}) + b_4 (\bar{P}_4 - \bar{P})$$

$\bar{P}_1$ = The mean litter size of the dam of the litter

$\bar{P}_2$ = The mean litter size for the full sisters of the dam, excluding the dam herself

$\bar{P}_3$ = The mean litter size for the half sisters of the dam, excluding the full sisters

$\bar{P}_4$ = The mean litter size of the maternal granddam of the litter

$\bar{P}$ = The litter size as population mean within farm per year

b_i = Partial regression coefficients, i = 1, 2, 3, 4

The information on the litter size of both the dam and the granddam will often be the mean of deviations from repeated observations over years, i.e. $(\bar{P}_1 - \bar{P}) = \Sigma (\bar{P}_{1i} - \bar{P})$. The deviations are given for each year, as deviations from the rolling means.

In addition to the dam index, a sire index was also calculated as the sire contributes the same number of genes as the dam to each progeny. The information sources were the same as in the dam index, but of course without the information for the sire per se. The matrixes used were in principle similar, but simpler than those presented in Figure 3, because of the exclusion of the sire information. The sire index was

$$I_S = b_5 (\bar{P}_5 - \bar{P}) + b_6 (\bar{P}_6 - \bar{P}) + b_7 (\bar{P}_7 - \bar{P})$$

$\bar{P}_5$ = The mean litter size for the full sisters of the sire

$\bar{P}_6$ = The mean litter size for the half sisters of the sire, excluding the full sisters

$\bar{P}_7$ = The mean litter size of the paternal granddam of the litter (see also explanations given on dam index)

The information sources used in the calculation of the pedigree index are shown in Figure 4.

Assuming there was no relationship between the sire and the dam of the litter, then the breeding value of the kits for litter size, *the pedigree index*, was estimated according to the formula:

$$I_P = \tfrac{1}{2} (I_D + I_S)$$

The pedigree index was standardized with a mean of 100 and a standard deviation of 10, according to the formula:

$$I = \frac{I_P - \mu_{I_P}}{\sigma_{I_P}} \cdot 10 + 100$$

where

I = the new standardized pedigree index for the litter

I_P = the first calculated and unstandardized pedigree index for the litter

μ_{I_P} = the mean of the unstandardized index in the population (for each year and defined colour types)

σ_{I_P} = the standard deviation of the unstandardized index in the population

The dam and the sire indexes both have an expectancy of zero since they are calculated as deviations from the population mean (P). If the standardization were to be done on the dam and the sire index separately and the pedigree index calculated as half of this sum, then they would have to be weighted according to their average r_{TI}-value.

The correlation between the dam's index and

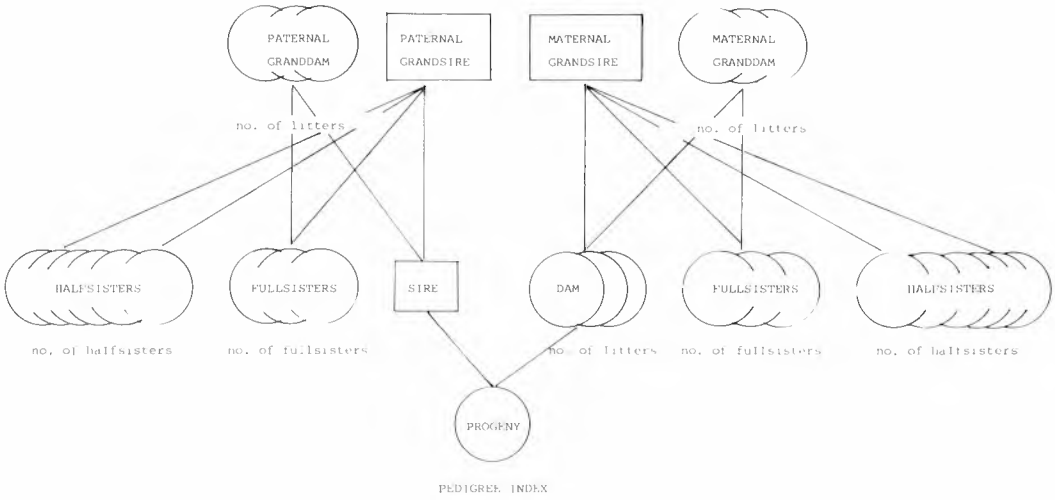


Fig. 4. The relatives involved in the calculation of the pedigree index for litter size in mink.

her daughter's index was about 0.25 as seen in Table 3. The correlation between the index of the female and her last litter was also calculated and decreased for older females because the first litter is the most valuable additive information source for the females.

The regression of the female's first litter on her dam's index was calculated at 0.15. This concurred well with the expected value of 0.16. The regression coefficient was not significant, but the material consisted of only 135 young females whose mothers had produced two litters only.

The expected genetic gain by using the selection index for litter size was analysed and is presented in Table 4. The generation intervals were set at 1.6 and 2.05 years for the sire-offspring and the dam-offspring, respectively, based on field analyses involving 99 farms (Ericson, 1983). The average accuracy of the selection index was fixed at 0.345. In order to allow selection for other economically important traits also, the selection intensity was calculated on the assumption that there were 3 or 4 times as many females and 6 – 8 times as many males as those needed for replacement, according to the pedigree index of litter size. The replacement rate was fixed, while culling intensity after first litter, selection intensity and selection criteria were varied among five alternatives. The first alternative in Table 4 is probably the most realistic from a practical point of view. It more than doubles the genetic gain in litter size compared to the second alternative, where the kits were selected only according to the size of the litter in which they were born, which today is the most common selection procedure. The other alternatives giving a gain of 0.15 kits per year are also realistic and could be practised in farms with low reproductive status.

Table 3. The correlations between the index of the female and the index of her dam.

Age of the female, years	No. of females	Correlation female index – dam index
1	292	0.23***
2	164	0.37***
3	68	0.25*
Total	524	0.24***

* = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

Table 4. Estimated genetic gain in litter size per year with a given replacement rate and variations in culling intensity after first litter, selection intensity and selection criterion.

Selection criteria	Alternatives of selection criteria				
	Index	Litter size at birth	Index	Index	Index
Replacement rate of females, % ...	40	40	40	40	40
Culling intensity after first litter, %	25	25	20	50	25
Selection for other important traits in females.....	1:4	1:4	1:3	1:3	1:3
Estimated genetic gain, kits per year	0.11	0.05	0.15	0.14	0.16

DISCUSSION

Development of the selection index

The heritability estimates obtained in the present analysis for litter size at 3 weeks postpartum are in good accordance with the results of Einarsson (1981), who used the same methods of estimation. The averages for these two investigations were 0.20 for heritability, 2.11 for the phenotypic standard deviation and 0.94 for the genetic standard deviation. These heritability estimates for litter size in mink concurred relatively well with the results of Moore (1954), Venge (1961), Rosberg (1978, cited by Rønningen *et al.* 1980), Pastirnac (1980) and Clausen (1985). In the investigations of Johansson (1965) and Narucka & Gedymin (1978) the heritability of litter size was found to be about 0.1. Different methods of estimation were used in the investigations cited, and the assumptions given for the different methods were not always confirmed, as discussed by Einarsson (1981). In the present selection experiment for litter size in mink, the realized heritability for the index including litter size at birth was about 0.14 which indicated a realized heritability for the trait litter size at birth of 0.11 (Einarsson, 1987b). Placed against this background the heritability for litter size in mink is recommended to be set at 0.15 – 0.20 in a breeding programme. Using a selection index including one trait only, as described in this paper, variation in the heritability estimate will not affect the ranking of the litters. However, if two or more traits are included, then the ranking of individuals would be biased.

In pig breeding, negative correlations between direct and maternal effects for litter size have caused problems in the effort to improve the trait (Revelle & Robinson, 1973; Vangen, 1980). In selection experiments with mice it was shown that maternal factors affect the genetic change in litter size and therefore should be experimentally controlled in some way (Vangen, 1986). It is therefore essential to find out if such effects also exist in mink. Einarsson (1981) discussed these problems both from a physiological point of view and by using different methods of estimating the heritability. There was no indication of negative correlation between direct and maternal effects for litter size, either genetic or environmental. This was also the result after further analysis of the data, presented in this paper, regarding maternal variance and covariance components. However, a hypothesis about disturbance of the oogenesis is discussed in Part II in this series (Einarsson,

1987b), which can result in maternal effects on reproduction under special conditions.

In addition to the additive variation, the correlation between relatives can also include some non-additive effects and systematic environmental effects. The two most common methods of estimating the heritability, regression of daughter on dam and daughter nested within sire, do not include either the genetic- or the dominance maternal variance component. In addition, the within dam systematic environment, as a direct or maternal component, should be included for full sibs. Johansson (1965), discussed the method of calculating the repeatibility for litter size in mink and stated that the regression of the second litter on the first litter and so on, was a better method than using intra-female correlation, as he found that the former method gave some higher estimate. The repeatability was estimated at 0.16 – 0.22 (Moore, 1954; Venge, 1961; Johansson, 1965).

Repeated observations on the same individual would increase the value of r_{TI}. With information from only one litter per dam, as practised when selecting kits according to the litter size where they were born, the r_{TI} is equal to $\frac{1}{2} \cdot \sqrt{h^2}$. This corresponds to $r_{TI} = 0.22$ when using a heritability of 0.20. By using the mean of two litters the accuracy increases to 0.28, and further to 0.32 with the mean of three litters. Only a small proportion of the females would under normal, commercial conditions, be allowed to produce four litters or more. Due to this fact and the diminishing additional value of further information, the maximum number of litters per female used in the index calculations was limited to three. With more than three litters an average would be estimated for all the litters available, but the weight would be given as of three litters only.

Both the litter size for one full sister of the dam, and one litter information from the granddam, would contribute with the same increase in the value of r_{TI}, since they each have a relationship of 0.5 to the dam. When one of these observations is added in the index, assuming the dam has only one litter, the r_{TI}-value increases from 0.22 to 0.24. By including both relatives each with one litter information the r_{TI}-value increases to 0.26.

On a commercial mink farm, an average of two or three full sisters would be selected according to the selection index for litter size. Furthermore, each dam and granddam would on average have information about two litters. This would increase the r_{TI}-value by about 40 percent when including the dam side only. In such a family structure, information about half sisters' litter size would contribute only minimally to the accuracy of the index. Information about litter size from five half sisters provides about the same information as one litter from the granddam. In addition the granddam could have repeated observations. However, when the information is limited to only one litter per dam and granddam, and no full sister giving birth, it would be worthwhile having data from the half sisters in the index of both the dam and the sire. These data were therefore included in the index calculations. Any further information from ancestors other than those included is of no practical interest for increasing the accuracy of the selection index for litter size in mink.

In the calculations of the b-values it was practical, from a quantitative point of view, to define the information flow. The number of repeated observations on litter size for dam and granddam was set at a maximum of three. With the excluding model 0, 1, 2 or 3 full sisters' litter information was used and 0, 1 – 3, 4 – 6 and more than 6 litter information was used from the half sisters. This included a total of 144 different combinations of relatives to be used in the dam index.

The use of the excluding model, as presented in this paper, involves an extra stage of calculation, with the dam having to be excluded from the full sister's mean and the full sister's from the half sister's mean. However, using the including model will give even more complicated matrixes than presented in this present paper (Liljedahl et al., 1979).

It is widely known that the reproductive results in mink vary between parities, or age classes. As seen from Table 2 the largest litters were found in two-year-old females and the smal-

lest litters in one-year-old females. The multiplicative age correction factors of 1.1, 0.9 and 1.0 for one-year, two-year and three-year or older females, respectively, were used. These figures corresponded very well with those calculated from Johansson's (1965) data. The multiplicative age correction factors for 10 000 females of different colour types were calculated at 1.10 and 0.93 for one- and two-year-old females, with females of three years and older fixed at 1.00. It should be emphasized that the multiplicative age correction factors presented are based on selected material and will therefore not express the biological difference in litter size between unselected mink of different age classes.

In order to increase the accuracy in the breeding evaluation it is important to define and allow for significant systematically environmental effects on the phenotypic records. As presented earlier this was accomplished for the age effect of the females. There are several reports on the effect of the mating system and date of mating on the litter size (Elofson, 1984). However, in the breeding evaluation it is assumed that all females are given the same opportunity within the mating system used on the farm. The differences in the individual acts of mating are indications of the female's willingness to accept mating. If there is a genetic background of the effects mentioned, the females will be selected in a positive direction as a correlated effect of litter size. At the present time, no corrections to the fertility records have been carried out on the effects of different mating systems or dates of mating.

Clausen (1985) reported on significant effects of farm, colour type, female's age, the last date of mating and the mating system on the litter size at weaning. Because of index calculations within farm and colour type, and correction for age, the first three effects are considered, while the latter two factors, which were discussed previously were not taken into account in the present calculations.

In the construction of the index it was assumed that the variance of litter size was similar for the different information sources, although earlier it was shown that the variance decreased slightly for the dams compared to the daughters (Einarsson, 1981).

The proposed construction of the female index contains only accumulated, adjusted deviations in litter size, based on defined colour types and on a within-year basis. Besides the accumulated devation, the dam of the female has the selection index available. The accuracy of the dam's selection index is of course higher than it would have been had only her own fertility records been used in predicting her true genetic value. It was decided, however, that the dam's accumulated adjusted records, not her index, should be used in the calculation of the female index of her offspring. The reason for this is that the expectation of the dam's accumulated deviation depends on the selection intensity each year, and therefore will the dam's deviations not be the same for each year. When the dam's index is being used the accumulated information on the dam's side is utilized as far back as the selection indices have been calculated. An accumulative effect is then obtained of all the selection events from all the earlier generations. The expectation of the contribution to the female index from the dam will therefore be dependent on how many generations back the selection indices have been calculated. The expected contribution will also tend to increase somewhat each year as the selection indices are calculated. Also, individuals for whom there is no information about their granddam will be increasingly at a disadvantage. It should be pointed out that in mink production the reproduction results come in one stage per year and not stage by stage throughout the year. It seems therefore to be of no advantage to use the «direct updating method» (Christensen et al., 1984).

Using the r_{TI}-value in the weighing of the information is the correct procedure as far as the accuracy and the variation of information is concerned. The problem that arises is mainly due to the accumulating differences in levels. A further step would be to use the pedigree index of the female kit, and later to combine this with her own and her siblings' reproduction results.

The BLUP-procedure (Henderson, 1975) has been considered, but has not been chosen in place of the proposed conventional selection index. Breeding work in mink is based on a within-farm selection, which excludes the farm effect. In mink breeding the replacement rate is relatively high, about 40 percent for the females and 60 percent for the males and normally only three generations are present at the same time.

The breeding season is relatively concentrated, with one information flow per year only and the mink males on average are only used on five females per year. There is not supposed to be any bias on reproduction parameters from maternal components, or on the variance or covariance components.

The conditions mentioned indicate that reliable herd averages are obtained where systematic environmental effects such as farm effects, seasonal effects, maternal effects, age effects, etc., are low or have been elimated, The high replacement rate of the males as well as the uniform breeding season diminished the need for taking genetic trend into account with regard to overlapping generations.

Kit mortality is usually high during the first three days after birth. In a breeding programme, it is necessary to have a fixed time for counting the litter size, as random registration of litter size during the first two days will be unreliable. It is also important that the litter size is registered some days after birth as this indicates the female's ability not only to give birth but to give birth to a litter of live kits and to prove her maternal ability. On the other hand, the registration of the litter size should not be too late as the calculated index should be sent back to the farm before weaning at about six weeks of age. Litter size at about three weeks postpartum therefore seems to be appropriate to serve as the selection trait although the age of most of the kits will vary between two and four weeks. An index including both litter size at birth and at a later time was not thought to be reliable, because of the problems of exact registration at birth.

The correlation between the female's own calculated index and her litter size was about 0.9 for one-year-old females and correlation between the index of the female and her dam about 0.24. Clausen (1985) found in his analysis that using the estimated breeding value for litter size in mink was four times as efficient a selection criterion compared to litter size where kits were born, when predicting the female's litter size in the first year.

The calculation of the index is carried out in several stages which may differ somewhat from one system to another. A detailed description of the specifications in the National Field Control for Fur Bearing animals in Norway is given by Einarsson et al. (1986). The National Field Control has its basis in the National Breeding Programme (Einarsson et al. 1983).

A few points are given here on the organization of the litter information when calculating a selection index for litter size. A register for all active breeding males and females in the farm must be established. It should be updated once or twice per year depending on mating and litter information being given simultaneously or at separate times. Information about reproduction should be transferred from the farm to the computer and this can be done by alternative means, either by making use of a central computer, a personal computer or some combination. The average litter size (population mean) of similar colour types within the farm should be calculated for each year, in order to find the female's deviation from the mean in litter size. The litter size as a deviation from the mean should be corrected for age effect. For one-year-old females the deviation in litter size for full sisters and half sisters and the numbers of full sisters and half sisters should be calculated. The reproduction results for older females should then be updated with respect to the deviation in litter size, the number of litters and new data from sisters. The granddams should be identified and information from them included. It is then possible to calculate the dam index by using the b-values which are chosen from the matrix according to the code for number of the dams' litters, number of full sisters, number of half sisters and number of the granddam's litters. The corrected deviations from the information sources

are then weighted according to their value of information, expressed as the b-values.

The sire index is calculated by including the average deviation of litter size and number of full sisters and half sisters, respectively, and the granddam data. By using both the dam and the sire index the pedigree index is calculated as described earlier.

An important assumption for breeding strategy and selection methods is that the ancestors of the individual are identified. By using different males in the remating this assumption might not be fulfilled. As a general rule, the same male should always be used when remating. Problems in male fertility should be solved by methods other than that of using two different males. However, it is also possible to calculate the pedigree index when the sire is unknown. In such a case the sire index should be fixed to the mean value of the sire index in the population.

In the estimation of the heritability for litter size in mink, the effect of the males is assumed to be random. As indicated in Figure 1, male fertility will mainly depend on the fertilizing capacity of the sperm cells, both quantitatively and qualitatively. Along with other factors, this depends on the density, the mobility and the deformities of the sperm cells. This should be taken into account when breeding males are selected and independent culling should be carried out after testes palpation and after semen testing of the males (Kangas, 1976; Fougner, 1980; Elofson, 1981a). A preliminary analysis conducted by the authors of the present paper indicates that male fertility follows a similar distribution to female fertility, and that the heritability seems to be in the same order as found for female fertility, at about 0.2. Male fertility should therefore be selected, for example, by studying their performance given in the farm report. Empty females and females that lost the entire litter prior to weaning should be culled.

Efforts towards developing selection programmes in mink breeding have been limited. There are, however, some reports on multi-trait selection indices (Sandh, 1975; Narucka & Gedymin, 1978; Rønningen et al. 1980). The main problems have been in connection with the inclusion of different fur quality traits in addition to other economically important traits in one index. There are also some problems in getting the farmers' acceptance of only one index as the selection criterion for several traits.

It is common in mink production all over the world for the breeding work to take place within all the farms and without special nucleus herds (a non-pyramid structure). The index presented in this paper is developed for practical use in a multistage selection programme within the farms, and presents only a stage concerned with reproduction. All kits in the same litter will have the same estimated breeding value of litter size. By using the index, the selection for reproduction will therefore be a between-litter selection. The other economically important traits in mink production will have to be included in the selection programme, but will not be discussed in the present paper. However, they will be included by combining selection indices and independent culling of given traits in the complete programme (Einarsson et al. 1983).

According to the calculations given in Table 4 it should be possible to obtain a genetic gain in litter size above 0.1 mink kits per year assuming a generation interval of about two years. In the selection experiment of litter size in mink a genetic gain of about 0.1 mink kits was observed per generation (Einarsson, 1987b). The correlated responses found in decreased pre- and postnatal mortality in the line selected for increased litter size at birth should also contribute to an increased response in litter size at three weeks postpartum. When discussing such effects as natural selection and increased inbreeding during the experiment, Einarsson (1987b) stated that these factors indicated that the response in litter size was underestimated. When also taking into account that the selection differences obtained in the selection experiment are possible to obtain under practical conditions it could be concluded that an index for litter size in mink at three weeks postpartum may result in a genetic gain of about 0.1 kits per year. It was also favourable that no negative trends were ob-

served in other economically important traits when selecting for increased litter size in mink (Einarsson, 1987c, 1988).

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CHANGES IN BLOOD PARAMETERS IN PREGNANT EWES AFTER SHEARING

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Pronounced changes appeared in the blood picture during the first week after shearing of pregnant ewes. Hemoglobin, hematocrit and lactic acid dehydrogenase value differences persisted throughout the pregnancy.

Key words: Shorn ewes, blood parameters.

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The effects of shearing on production and animal behavior, recorded at the University for more than 30 years indicate that lambs tend to be heavier and to increase in weight more rapidly when the mothers have been shorn before lambing (Nedkvitne 1972). Low temperatures of the skin and rectum and higher pulse rates have been seen after shearing (Nedkvitne 1970). In the early studies, some blood analysis values were also seen to be affected (Nedkvitne 1963 & Astrup 1963). Hemoglobin and hematocrit values after shearing. The 1968-69 experiments showed that plasma free fatty acids and plasma glycerol increased immediately after shearing (Astrup et al. 1970, Aulie et al. 1971). An extensive investigation of blood parameters carried out in the following year is presented in this report. Related topics have been studied during housing of the ewes in insulated houses (Nedkvitne 1963, Nedkvitne & Nygaard 1977, Austbø 1985). Other studies of the effects of cold on sheep and other ruminants have been thouroughly discussed and reviewed (Bass & Henschel 1956, Alexander 1975, Young 1981).

MATERIAL AND METHODS

Sheep from the Dala sheep stock of the University Farm were used. The animals were fed and housed in an unisulated shed under equal conditions. Fifteen out of 30 ewes were shorn on the 1st of March 1970, when the average March temperature at Ås was -1,7°C versus normal -1,2°C. The mean on the day of shearing was -0,7°C.

Animals were fed 0.5 kg hay, 3.5 kg grass silage and 0.2 kg concentrate mixture with 15 % crude protein. Magnesium rich mineral mixture was given after shearing, 15 g supplied 150 mg Mg a day. The sheep were fed individually.

Blood analysis

Blood samples taken from the vena jugularis at 0900 hours were left for 2 h in the cold room to coagulate, and centrifuged for 20 min at 1500 rpm in 50 ml swing-out caps of the laboratory Christ centrifuge. Blood plasma samples for the hormone determinations were added heparin as an anticoagulant. Hemoglobin was determined in the whole blood by the cyano methemoglobin procedure,

and hematocrit by centrifugation in graded tubes (Hawk et al. 1947). Free fatty acids (FFA) extracted by means of hydrochloric acid, isopropanol and heptane, according to Dole, were titrated with micrometer syringe and N/100 alcoholic NaOH under nitrogen with Nile blue as indicator (Patterson 1963). Glucose was assayed by the ortho toluidine colorimetric procedure on serum previously deproteinized by trichloro acetic acid. Urea-N was determined in Conway micro diffusion dishes after det urea was split with jack bean urease. Albumin was assayed by precipitation with 2.4 hydroxyazobenzene benzoic acid (HABA) in phosphate buffer, with turbidity at 510 nm in a Beckman spectrophotometer. Bovine albumin in dilutions was uses as standard. Gamma globulin was determined after the procedure of Aschaffenburg (1949), by precipitation with zink sulphate solution and with turbidity recorded as O.D. at 650 nm in 1 cm cuvettes. Lactic acid dehydrogenase (LDH) and transaminase (SGOT) enzymes were assayed by methods from Boehringer's Test Fibel (1966). Calcium, sodium and potassium were measured by emission spectrophotometry in a Zeiss instrument. Magnesium was determined by atomic absorption spectrophotometry (Astrup 1982). Cortisol determinations proceeded according to Basset, Hinks 1969, and Basset 1972. Samples of blood were taken on 11 Jan., 8 Feb., 3, 8, 15, 22, 29 March, 14 April.

Calculations

True diviations from the control group and from the preceding control period were obtained by subtracting differences of groups in the pre-shearing period from those obtained after shearing.

RESULTS AND DISCUSSION

Mean and standard errors of 13 blood parameters one week before shearing are reported in Table 1. Deviations from control group and control period, calculated as described above, are given 6 times from samples in the post-shearing period. In intervals after shearing the changes in the blood picture appear likely to reflect the various mechanisms

Table 1. Blood parameter levels before, and changes following, shearing

	Mean SE		Deviation from control group and period means					
Date of sampling	22/2		3/3	8/3	15/3	22/3	29/3	14/4
Days from shearing	-8		3	8	15	22	29	46
FFA pr µeq/l	945	41	384*	217*	-26	99	35	68
Glucose, mg %	60	1.2	5.4*	-1.0	-1.3	1.1	-1.6	-7.1*
Urea-N, mg %	9.9	0.1	-0.1	-1.1*	0.5	0.6	0.5	0.6
Albumin, %	2.60	0.05	0.20*	0.04	0.11	0.21	0.00	-0.11
γ-globulin,O.D.	0.95	0.03	-0.14*	-0.09	0.07*	0.01	-0.02	0.06
Hemoglobin, %	12.9	0.2	1.3*	1.0	1.4*	1.0*	1.2	0.1
Hematocrit, %	38,0	1.0	5.0*	4.9*	3.7*	2.1	1.5	-0.6
LDH, units	483	16	119*	126*	155*	148*	112*	89*
SGOT, units	84	3	21*	34*	21*	8	5	-3
Ca, mg %	10.2	0.1	-	0.3	-	-	-	-
Mg, mg %	2.03	0.05	-0.08	-0.12*	-0.07	-0.04	-0.06	-0.15*
Na, mg %	337	3	3*	9*	0	-2	-6	-1
K, mg %	17.6	0.1	0.1	0.7	0.2	0.0	0.1	0.0

* Significance at 5 % level or better by test of variance

successive homeostatic counteracting the cold stress in the animals.

The rapid increase in FFA, glucose, and the drop in γ-globulin are reactions now known to appear in sheep subjected to cold stress (Halliday et al. 1969). Blood sugar raises results from mobilizing muscle and liver stores. FFA is mobilized from fat tissues. The increase in sodium and potassium levels may reflect blood volume reduction. Sheep seem specially adaptive (Bailey 1964). A rapid decline of blood serum γ-globulin is known to accompany stress. The immediate changes in blood parameters are the results of hormonal regulations.

These changes are the result of a concerted action and appear to include many hormonal systems. In addition to the adrenal hormones, thyroxine, prolactin, somatotropin, glutathione increase their activity, while insulin is depressed (Basset et al. 1970, Graham et al. 1970, Bass et al. 1970, Blom et al. 1970, Yamamoto 1972, Olsen, Trenkle 1973, Trenkle 1978 and Sasaki, Takahashi 1983). The increase in serum transaminase in the ewes may be a result of raised cortisol activity. Corticosteroids were analysed before and after shearing, and had an expected rise. However, individual variations in the stress hormone level were large (Table 2). From the second week the effects of the hormones fade and other regulations take over (Table 1). Changes i hemoglobin and hematocrit are superimposed on the change through pregnancy. Values are not reported in the table. Correlation coefficients with days during in-door feeding are negative and highly significant, -0.87 and -0.93. The regressions are $y = 12.6 - 0.03\ x$ and $y = 36{,}5 - 0.11\ x$ for hemoglobin and hematocrit, respektively. The values remained higher in the sheared animals group. Thus, erythrocyte stores apparently mobilize to follow the increase of metabolism and oxygen consumption. Lactic acid dehydrogenase activity is raised throughout pregnancy in the shorn animals. This is likely the result of an increase in muscular activity and heat regulation caused by shivering. In experiments with ruminants Kennedy et al. (1976, 1982) found cold to affect rumen activity too. Cold produces an increase in rumen bypass and results in an inferior digestibility of roughages, but better utilization of proteins. The proteins are digested in the gut, rather than in the rumen. The drop in urea-N and the increase in albumin may reflect this situation in our experiment.

Table 2. Plasma corticosteroid levels (μg/100 ml) in 4 ewesbefore and after shearing

Sheep no.	8 days before	3 days after
413	1.8	3.0
414	1.2	5.4
423	0,6	1.8
486	1.6	9.0

Blood glucose values between the groups are not consistent. While temporarily raised after shearing, they are depressed in the shorn ewes shortly before lambing. The drop in glucose values during pregnancy is not shown, but calculated here to give coefficient of correlation -0.83 with days in this period (x) and regression following a straight line: $Y = 54 - 0.26x$. The FFA values in the same period rose to a platau half way in pregnancy, as they did the previous year (Astrup et. al 1970). Magnesium level behaved differently from sodium and potassium. Magnesium level is slightly, but significantly lower one week after shearing and also in the final days of pregnancy. Magnesium is likely in demand, and withdrawn from circulation, possibly to serve increased enzyme activity in energy metabolism. However, since magnesium digestion occurs largely in the rumen (Astrup, Hvidsten 1987), an effect of cold or rumen bypass may also reduce magnesium digestibility. This is in accordance with the low serum magnesium levels seen in cows treated with thyroxine (Astrup 1966). In Table 3

Table 3. Intercorrelation of blood parameters in the ewes from sampling 8 d after shearing

	FFA 1	Gluc 2	Urea 3	Alb 4	γ-glob 5	Hem. 6	Hemc. 7	LDH 8	SGOT 9	Ca 10	Mg 11	Na 12	K 13
FFA	1												
Gluc	-0.45**	1											
Urea	0.06	-0.40*	1										
Alb	0.11	0.24	-0.19	1									
γ-glob	-0.47**	0.29	0.10	0.12	1								
Hem.	0.14	0.21	-0.28	0.30	0.02	1							
Hemc.	0.08	0.35*	-0.22	0.18	0.12	0.71***	1						
LDH	-0.14	0.48**	-0.32	0.31	0.24	0.31	0.35*	1					
SGOT	0.05	0,12	-0.31	-0.36*	0.04	0.38*	0.31	0.25	1				
Ca	-0.32	0.36*	0.06	0.03	-0.01	-0.02	0.09	-0.04	-0.38*	1			
Mg	-0.22	0.12	-0.20	-0.23	0.28	-0.41*	-0.18	-0.18	-0.09	-0.42*	1		
Na	0.03	-0.18	-0.27	-0.29	0.05	0.09	0.17	0.04	0.23	-0.13	-0.16	1	
K	-0.23	0.30	0.09	0.11	0.50**	-0.04	0.02	-0.23	-0.05	0.47*	0.06	0.07	1

correlation coefficients are compared between blood parameters on day 8 after shearing. The strongest connections seem to be between sugar level, free fatty acids, γ-globulin, urea-N, hematocrit value and lactic acid dehydrogenase. Glucose level proves to be a good negative indicator of the increase in energy demand.

In previous experiments growth promotion as a result of shearing was probably a result of increased appetite and feed uptake. The very short lasting excitation of hormones seen after shearing raises the question whether these really are responsible for the long term effects of growth improvement.

SUMMARY

1. Blood parameters were analysed in pregnant ewes before and after shearing.

2. Pronounced changes appeared the first week after shearing.

3. Free fatty acid and glucose value differences between shorn and unshorn ewes disappeared the second week after shearing.

4. Hemoglobin, hematocrit and lactic dehydrogenase value differences between shorn and unshorn ewes lasted longer and persisted throughout the pregnancy.

5. The duration of hormonal imbalance may be too short to effect fetal growth. Increased rate of metabolism, however, may persist through muscular activity. Such activity is evidenced by raised blood pigments and high lactic acid dehydrogenase levels.

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EXAMINATION OF NITRATE REDUCTASE ACTIVITY AT THE SEEDLING STAGE AS A PREDICTOR OF THE GRAIN PROTEIN OF WHEAT AND BARLEY

Nitrate reductase as a predictor of grain protein

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The first leaf of 8-day-old wheat and barley seedlings was tested for nitrate reductase activity. Grain protein, grain protein per cent and yield were previously tested in the various lines used. There was a positive correlation ($r = 0.95$) between nitrate reductase activity and grain protein per cent for 10 barley cultivars and breeding lines of diverse origin and protein content. However, in two more extensive experiments using 40 breeding lines of barley and 39 of wheat, no significant correlation was found between nitrate reductase activity and any of the parameters mentioned above. We therefore conclude that nitrate reductase cannot be used as a reliable selection criterion in breeding programs.

Key words: Grain protein, grain yield, *Hordeum vulgare*, nitrate reductase, selection criteria, *Triticum aestivum*.

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Nitrate reductase (NR) is assumed to be the rate limiting factor in the assimilation of nitrogen (Hageman 1979), and the use of this enzyme as a criterion for the selection of cultivars with high grain protein has been suggested (Dalling and Loyn 1977, Hageman 1979). Such biochemical selection criteria at the seedling stage could be useful in a breeding program.

Dalling and Loyn (1977) examined 24 wheat cultivars and found a correlation between high NR activity in the first leaf of 21-day-old plants and grain nitrogen yield. Six out of the seven cultivars with the highest NR activity were also among the top seven when the plants were ranked for grain nitrogen yield. Duffield *et al.* (1972) found a positive correlation between NR activity

and grain protein per cent in a hard winter wheat cross. However, Rao *et al.* (1977), working with two wheat genotypes which differed in NR activity, and Deckard and Busch (1978), working with eight wheat parents and 25 different crosses, concluded that NR activity could not be used as a single criterion in selecting genotypes with high grain protein.

Although a positive correlation is usually found between NR activity and grain protein (Hageman 1979), NR activity has not been used as a selection criterion in breeding programs. The aim of the present work was to test the usefulness of NR activity as a selection criterion for wheat and barley lines grown in Norway.

MATERIALS AND METHODS

Plant materials and analysis of grain protein and yield.

Ten barley cultivars and breeding lines of greatly diverse protein content were used in an initial experiment. These had been analysed in detail in the genetic studies of Olsen (1979). In a more extensive experiment 40 current breeding lines of barley and 39 of wheat were used. The grain protein was analysed by the Kjeldahl method and yield was determined in field plots or in single plants grown in rows in the field (Olsen 1979).

Assay of nitrate reductase

Seeds were sown in vermiculite and watered with Hoagland solution. The seedlings were kept at 21°C and given 12 h light (Philips 33, 65 Wm^{-2}, 400-700 nm) and 12 h darkness. The leaves were harvested during the photoperiod, 2-3 h after the light was switched on, and during the dark period, 10-11 h after the light was switched off. Twenty different lines were grown in the same batch and one sample was harvested from each line and tested for NR activity. The data presented are the mean values from three batches. NR activity was tested using the in vitro assay. The reduced form of nicotinamide adenine dinucleotide was used as the electron donor, and the extract was incubated for 10 min. For details see Lillo (1983) and Lillo and Henriksen (1984).

RESULTS

The NR activity together with grain weight per plant and grain protein per cent of the 10 barley cultivars and lines are listed in Table 1. The correlation coefficients between these parameters are given in Table 2. There was a significant positive correlation between NR activity tested during the day or night and grain protein per cent. Furthermore, there was a significant negative correlation between NR activity and grain yield or grain protein per plant.

Similarly, data for the 40 barley and the 39 wheat breeding lines were also obtained (data not shown). In the 40 barley lines grain protein per cent varied from 8.9 to 13.0 while grain yield varied from 2280 to 4810 kg ha^{-1}. The NR activity (mean value of day and night) varied from 15.9 to 25.1 mol NO_2^- (g fresh wt)$^{-1}h^{-1}$. In the 40 wheat lines protein per cent varied from 11.5 to 15.4 while grain yield varied from 5320 to 7450 kg ha^{-1}. The NR activity (mean value of day and night) varied from 23.8 to 36.2 mol NO_2^- (g fresh wt)$^{-1}h^{-1}$. No significant correlations were found between NR activity and grain protein, grain protein yield or grain yield for these lines (Table 2).

DISCUSSION

We found a significant positive correlation between the NR activity of the first leaf and grain protein per cent of the ten barley lines which included the high protein lines 'Hiproly' and 'Notch

Table 1. NR activity, mol NO_2^- (g fresh wt)$^{-1}$h^{-1}) grain protein %, and grain weight per plant of 10 selected barley cultivars and breeding lines. Data for grain protein % and yield are from Olsen (1979).

Cultivar	During day	During night	Mean value	Grain protein %	Grain weight g plant^{-1}
Møyjar	16.9	10.1	13.5	12.7	10.0
Hiproly	21.5	13.3	17.4	19.0	2.4
Notch II	18.4	13.4	15.9	18.6	1.5
Min. 990-5	18.0	11.6	14.8	15.4	6.8
Carlsberg II	17.0	10.4	13.7	12.3	9.5
Bomi	16.8	10.1	13.5	12.3	11.5
Line 29	17.9	10.4	14.2	13.4	8.9
Line 86	18.3	9.4	13.9	13.4	8.2
Risø 56	17.3	11.9	14.6	13.8	6.2
Risø 1508	17.0	10.1	13.6	13.4	7.9

II'. However, there was a negative correlation between NR and grain protein or yield in these lines because high protein per cent and low grain weight are highly correlated. Grain protein, not grain protein per cent, would therefore be the more important selection criterion. This implies that low NR activity, would be the proper selection criterion in this case. This is in contrast to Hageman's (1979) statement that correlation of seedling NR and yield have always been positive and significant.

In a more extensive experiment with the 40 barley lines there was no significant correlation between NR and yield or protein. Neither was there any correlation between NR and yield or protein for 39 wheat lines tested. In these breeding lines the span in protein per cent was considerably less than in the ten specially selected barley cultivars/breeding lines. This can at least partly explain the different results. Our findings contrast with those of Dalling and Loyn (1977), who investigated 24 wheat cultivars and found a positive correlation between high NR activity and grain nitrogen yield. On the other hand, Eilrich (Hageman 1979) found a positive correlation between NR activity and grain nitrogen for 10 selected wheat cultivars only, but not in the case of 5 cultivars of diverse origin.

Table 2. Correlation coefficients between NR activity grain protein %, yield and grain protein.

	10 barley cultivars			40 barley lines			39 wheat lines		
	day	night	mean	day	night	mean	day	night	mean
Grain protein%	0.822*	0.909*	0.952*	0.138	0.284	0.219	-0.171	-0.071	-0.133
Yield	-0.729*	-0.908*	-0.900*	-0.221	-0.318	-0.295	0.180	-0.166	0.116
Grain protein	-0.689	-0.904*	-0.881*	-0.084	-0.240	-0.182	0.011	-0.253	-0.074

* Significant on the 1 % level

Deckard and Busch (1978) and Rao and co-workers (1977) did not find that NR activity was a useful selection criterion for wheat. Although positive results have been presented by some scientists, and although such positive results can be obtained in special materials, we conclude from our studies that NR activity cannot be used as a reliable selection criterion in our breeding programs for wheat and barley.

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TRICHOTHECENES AND ZEARALENONE IN NORWEGIAN BARLEY AND WHEAT

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Randomly selected barley and wheat samples of the 1984 crop from the major cereal growing areas of Norway were analyzed for mycotoxins with GC-mass spectrometry, HPLC and ELISA. All of the 102 samples contained nivalenol with six samples above 100 ng/g. Fourteen of the samples contained more than 100 ng/g deoxynivalenol, while two samples had more than 1000 ng/g of this mycotoxin. Low levels of zearalenone were detected; only two samples contained the T-2 toxin, and none contained fusarenon X

Trichothecenes, zearalenone, barley, wheat, mycotoxins

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Fungi are known to produce several toxins harmful to man and animals. There are many well-documented cases of toxicosis in domestic animals due to consumption of fungus-infected cereals (Ueno 1983). Toxic metabolites are produced by a number of fungi, but in a temperate climate most toxicoses are caused by *Fusarium* spp. (Ueno et al. 1971, Smalley & Strong 1974, Mirocha et al. 1976).

Zearalenone (ZEN) is an oestrogenic mycotoxin produced by *Fusarium graminearum* Schwabe. The structure of ZEN was characterized by Urry et al. (1966). Hyperoestrogenism induced by zearalenone may cause abortion and reduced fertility in domestic animals (Mirocha et al. 1967).

ZEN has oestrogenic effects on humans also. Schoental (1983) reported precocious sexual development in Puerto Rico associated with ZEN contaminated food. Zearalenol, a metabolite of ZEN, has a four times greater oestrogenic effect than ZEN (Pathre & Mirocha 1976).

Trichothecenes are closely related sesquiterpenoids produced by fungi from the genera *Fusarium, Trichothecium, Myrothecium and Stachybotrys*. More than 40 naturally occurring trichotecenes are known. The T-2 toxin (T-2),

nivalenol (NIV), deoxynivalenol (DON), and diacetoxyscirpenol (DAS) are all produced by *Fusarium* spp. and are considered the most dangerous of the trichotecenes (Ueno 1977a, 1977b). They are cytotoxic to eucaryotic cells and their biological activity is closely related to their high lethality to animals by causing cellular damage to actively dividing cells, potent immunosuppression and inhibition of protein and DNA synthesis (Ueno 1980, 1983, 1987).

«Scabby grain intoxication» in man and farm animals is mainly caused by consumption of cereals contaminated with *F. graminearum* which produces ZEN and also NIV and DON and other trichothecenes. Other *Fusarium* spp. are also important sources of mycotoxins in cereals (Mirocha & Christensen 1974, Ueno 1980).

In the United States DON is the major toxicant in scabby grain (Mirocha et al. 1976, Vesonder et al. 1978, 1979). DON is also the most important mycotoxin in grains in Canada (Trenholm et al. 1981, Scott et al. 1981, Hart & Braselton 1983, Scott 1984, Côté et al. 1984), United Kingdom (Gilbert et al. 1984), Austria (Vesonder & Ciegler 1979), Italy (Bottalico et al. 1984), and South Africa (Marasas et al. 1977, 1979). Both NIV and DON have been detected in cereal grain from Japan (Kuroda et al. 1979, Kamimura et al. 1981, Yoshizawa 1983, Yoshizawa & Hosokawa 1983, Dohi et al. 1984) and France (Jemmali et al. 1978).

ZEN is widely distributed throughout the world in cereals, mixed animal feeds and other products (Mirocha et al. 1974, Stoloff 1976, Bennett & Shotwell 1979, Cohen & Lapointe 1980, Scott et al. 1978, Lovelace & Nyathi 1977, Yoshizawa et al. 1979, Jemmali 1978 et al.). There are reports of co-contamination of NIV, DON and ZEN in grain from Korea (Lee et al. 1985) and Japan (Tanaka et al. 1985c). Also cereal flours, popcorn and health food have been contaminated with NIV, DON, and ZEN in Japan (Tanaka et al. 1985d).

Studies in Finland have revealed that 3000-9500 ng/g ZEN caused infertility in a herd of dairy cows; ZEN concentrations as high as 40 000 ng/g were detected in an oat sample associated with mycotoxicosis in Finland (Ylimäki et al. 1979).

In the 1984 crop, DON was found in 57% of wheat and in 14% of barley samples in Sweden (Pettersson et al. 1986). The DON concentrations were from 50 to 1180 ng/g in grain harvested in the wet autumn of 1984, while in the crop from the drier 1982 harvest the DON concentration ranged between 40 and 260 ng/g (Pettersson et al. 1986). Isolates of *F. culmorum* (W.G. Smith) Sacc. from Swedish cereals produced both ZEN and DON (Holmberg and Pettersson 1986).

In random samples of Norwegian grain and feed samples, 18% contained ZEN (Yndestad & Olberg 1981). Trichothecenes have also been identified in Norwegian cereals, DON and T-2 being the most common in previous studies (Yndestad 1984)..

Olberg (1985) reported that specific mycotoxicosis symptoms were observed in a Norwegian pig herd fed a premixed feed containing 3750 ng/g ZEN. The symptoms included reduced weight gain, prolapse of the vagina and swollen vulva. In dairy cow herds with abortion and diarrhoea symptoms, the trichothecenes DOS and DON were detected in addition to ZEN (Olberg 1985).

MATERIAL AND METHODS

Samples of barley and wheat

Altogether 102 samples from barley and wheat seed cultivated in 1984 under the Norwegian State Seed Certification Programme were analyzed for mycotoxin contamination. The samples were randomly selected to obtain a geographical distribution representing the important cereal growing areas of Norway. Myco-

toxins in the barley and wheat samples were analyzed as described below.

Isolation of Fusarium spp.
Plant materials were surface disinfected and plated on potato dextrose agar supplemented with pentachloronitrobenzene and aureomycin as described by Nash & Snyder (1962). *Fusarium* isolates were stored at 4°C on autoclaved, moist soil in bottles. The isolates were identified according to the manual of Nelson et al. (1983). Different fungal isolates were cultured on rice grains for four weeks before the infected grains were subjected to mycotoxin analysis.

Standards of trichothecenes and zearanlenone
4-acetyl-NIV and 3-acetyl-DON were isolated from culture fil trates of *Fusarium* spp. (strain Fn-2B) (Ueno et al. 1971) and *F. graminearum* (Ishii et al. 1985), respectively. NIV and DON were prepared by hydrolysis in methanol-NH4OH. T-2 toxin was isolated from culture filtrates of *F. sporotrichoides* Sherb. (strain M-1-1). ZEN was obtained from Makor Chemicals Ltd., Jerusalem, Israel.

Analysis of nivalenol, deoxynivalenol, zearalenone and T-2 toxin
NIV, DON and ZEN were analyzed according to methods reported elsewhere (Tanaka et al. 1985a,b,d), and which can be briefly summarized as follows. The samples (20 g) were extracted with acetonitrile-water (3:1), defatted with n-hexane, and after ethanol was added to the aqueous acetonitrile layer, the solvents were evaporated to dryness in a rotary evaporator. The residue was purified using a chromatographic procedure with a Florisil column. The amounts of NIV and DON in the column eluates were derivatized with a trimethyl-trimethylchlorosilane/ethyl ecetate (1:0.2:9), and then estimated by GC (Shimadzu Model GC-8AE, Shimadzu Ltd., Kyoto) with 63Ni electron capture detection and confirmed by GC-mass spectrometry (GC-MS) (Hitachi Model M/80A, Hitachi Ltd., Tokyo). A portion of the Florisil column eluate was subjected to analysis for ZEN by HPLC (Shimadzu Model LC4A) with fluorescence detection (Shimadzu Model RF-530). The chromatographic separation was carried out using a silica gel column (Nucleosil 50-10), and the elution solvent was 90% watersatura ted chloroform/cyclohexane/acetonitrile/ethanol (50:15:2:1). The column temperture and solvent flow rate were set at 30°C and 1 ml/min, respectively. ZEN in positive samples was confirmed by GC-MS. The detection limits of the methods employed were 2 ng/g for NIV and DON, and 1 ng/g for ZEN.

The ELISA of T-2 toxin was carried out as follows. 100 µl of T-2 HG-BSA (Ohtani et al. 1987)(1 µg/ml) dissolved in a coating buffer (0.4 g Na_2CO_3, 0.73 g $NaHCO_3$ and 0.04 g NaN_3 in 250 ml of deionized water) was added to each well of a 96-well microtiter plate and incubated at 37°C for 2 h. After the wells were washed three times with PBS-Tween (0.05% Tween 20 in 50 mM phosphate buffered saline, pH 7.4), 100 µl of 0.5% BSA in PBS (50 mM phosphate buffered saline, pH 7.4) was added to each well and incubated at room temperature for 1 h. Before use, the plate was washed four times with PBS-Tween. Fifty µl of the cleanup sample solution or T-2 standard in 10% ethanol solution and 50 µl of the T-2.1 MAb (Chiba et al., 1988)(1 µg/ml) dissolved in PBS-Tween were added to each well of the plates, and then incubated at 4°C for 16 h. After washing the plate four times with PBS-Tween, 100 µl of alkaline phosphatase-labelled sheep anti-mouse IgG (diluted to 1:100 (v/v) in PBS-Tween) was added to each well of the plate. The plates were incubated for 1 h at room temperature and washed four times with PBS-Tween. Then, 100 µl of the enzyme substrate (1 mg/ml p-nitrophenylphosphate in 1 M diethanolamineHCl buffer containing 0.5 mM NaN_3 and 0.3 mM $MgCl_2$, pH

Table 1. Concentrations of trichothecenes and zearalenone in barley samples from South-Eastern Norway (ng/g)

Sample no.	Origin of sample		Trichothecenes			Zearalenone
	County	Commune	DON	NIV	T-2	
1	Oppland	Østre Toten	30	30	ND	ND
2	Hedmark	Åsnes	ND	57	ND	ND
4	Akershus	Vestby	ND	51	ND	ND
7	Buskerud	Ringerike	ND	42	ND	5
13	Buskerud	Ringerike	31	68	ND	ND
16	Akershus	Nes	ND	41	ND	ND
18	Hedmark	Ringsaker	ND	69	ND	ND
21	Akershus	Frogn	58	38	ND	ND
22	Akershus	Frogn	15	35	ND	ND
23	Akershus	Frogn	32	55	ND	ND
24	Akershus	Frogn	ND	36	ND	ND
25	Akershus	Enebakk	ND	15	ND	ND
27	Akershus	Ås	30	13	ND	ND
28	Akershus	Vestby	27	36	ND	ND
36	Østfold	Våler	ND	60	ND	2
38	Akershus	Aurskog-Høland	65	52	46	1
40	Buskerud	Ringerike	ND	21	ND	ND
45	Oppland	Østre Toten	ND	36	ND	ND
46	Telemark	Bø	9	31	ND	ND
52	Østfold	Våler	ND	68	ND	ND
56	Østfold	Rakkestad	8	21	ND	ND
84	Østfold	Rakkestad	35	34	22	ND
85	Østfold	Rakkestad	223	46	ND	ND
103	Oppland	Østre Toten	28	156	ND	ND

DON = deoxynivalenol, NIV = nivalenol, T-2 = T-2 toxin, ND = not detected

9.8) was added and incubated for 1 h at 37°C. The optical density at 405 nm in the wells was recorded with a microplate photometer. The detection limit of the present ELISA is 10 ng/g of T-2 toxin.

RESULTS AND DISCUSSION

Nivalenol contamination

All the Norwegian barley and wheat seed lots that we analyzed were contaminated with NIV (Tables 1-4). The average concentration of the mycotoxin showed little variation between geographical regions and the NIV levels were similar in barley and wheat (Table 6). Six of the 102 samples contained more than 100 ng/g nivalenol. The highest level recorded was 887 ng/g in a wheat sample from Telemark County.

The low detection limits with the methods we employed may explain the apparent discrepancy with the results published by Yndestad (1984). He detected NIV in 12.5% of his samples of Norwegian cereals and animal feed using a method with a detection limit of 200 ng/g.

Deoxynivalenol contamination

The level of DON varied in the barley and wheat samples (Tables 1-4). Very high levels (above 1 000 ng/g) were recorded in two wheat samples from Vestfold County, two wheat samples from Sør-Trøndelag County, and one barley sample from Nord-Trøndelag County. Nineteen of the 102 samples contained more than 100 ng/g of DON. About half the samples from South-Eastern Norway contained DON, while 84% of the barley

Table 2. Concentrations of trichothecenes and zearalenone in barley samples from Central Norway (ng/g)

Sample no.	Origin of sample County	Commune	Trichothecenes DON	NIV	T-2	Zearalenone
32	Nord-Trøndelag	Stjørdal	28	22	ND	3
33	Nord-Trøndelag	Stjørdal	ND	46	ND	3
57	Nord-Trøndelag	Stjørdal	ND	24	ND	ND
58	Nord-Trøndelag	Steinkjær	ND	36	ND	3
60	Sør-Trøndelag	Trondheim	ND	37	ND	ND
61	Sør-Trøndelag	Melhus	675	258	ND	2
62	Nord-Trøndelag	Steinkjær	2139	74	ND	4
63	Nord-Trøndelag	Stjørdal	240	63	ND	1
64	Nord-Trøndelag	Levanger	27	27	ND	ND
65	Sør-Trøndelag	Trondheim	59	69	ND	2
66	Sør-Trøndelag	Trondheim	18	51	ND	2
67	Nord-Trøndelag	Steinkjer	26	52	ND	ND
68	Nord-Trøndelag	Steinkjer	6	59	ND	2
69	Nord-Trøndelag	Steinkjer	71	48	ND	ND
70	Nord-Trøndelag	Steinkjer	50	27	ND	ND
71	Sør-Trøndelag	Trondheim	165	43	ND	ND
72	Nord-Trøndelag	Stjørdal	31	27	ND	ND
73	Sør-Trøndelag	Skaun	55	50	ND	ND
87	Sør-Trøndelag	Trondheim	624	33	ND	ND
88	Sør-Trøndelag	Melhus	624	53	ND	4
89	Sør-Trøndelag	Skaun	43	47	ND	ND
90	Nord-Trøndelag	Frosta	30	31	ND	2
91	Nord-Trøndelag	Stjørdal	63	43	ND	2
92	Nordland	Alstadhaug	44	42	ND	3
93	Nord-Trøndelag	Stjørdal	94	40	ND	3

DON = deoxynivalenol, NIV = nivalenol, T-2 = T-2 toxin ND = not detected

lots and 92.3% of the wheat lots from Central Norway had detectable levels of DON (Table 5).

Yndestad (1984) found DON to be the most common mycotoxin in the materials he studied. He detected DON in 29% of the animal feed and cereal lots sampled.

Zearalenone

Only low levels of ZEN were found in the barley and wheat samples (Tables 1-4). Altogether 20 of the 102 seed lots were contaminated with ZEN, and with one exception the levels varied from 1 to 5 ng/g. One wheat sample from Hedmark County contained 23 ng/g ZEN. Fourteen out of 25 barley samples from Central Norway had detectable levels of ZEN.

Olberg (1985) detected ZEN in very low concentrations in 1% of 205 randomly selected Norwegian barley and oat samples. In 116 samples of mouldy animal feed she found 20%.with moderate to high ZEN concentration. Twenty mouldy barley samples contained an average of 2 351 ng/g ZEN with one sample containing 15 000 ng/g ZEN. The detection limit for the method employed by Olberg was 20-25 ng/g.

T-2 toxin and Fusarenon X

Only 2 of the 102 barley and wheat samples contained the T-2 toxin (Tables 1-4). None of the samples were contaminated with fusarenon X (data not shown).

The T-2 toxin was detected in 25% and fusarenon X was found in 12.5% of samples analyzed by Yndestad (1984). In

Table 3.Concentration of trichothecenes and zearalenone in wheat samples from South-Eastern Norway (ng/g

Sample no.	Origin of sample County	Commune	Trichothecenes DON	NIV	T-2	Zearalenone
3	Akershus	Vestby	ND	78	ND	ND
5	Akershus	Vestby	ND	53	ND	ND
6	Akershus	Vestby	237	59	ND	ND
8	Buskerud	Ringerike	ND	26	ND	ND
9	Vestfold	Sandefjord	57	35	ND	ND
10	Østfold	Rolvsøy	21	16	ND	ND
11	Østfold	Onsøy	ND	23	ND	ND
12	Østfold	Rolvsøy	ND	55	ND	ND
14	Hedmark	Stange	ND	42	ND	ND
15	Hedmark	Sør-Odal	389	112	ND	23
17	Oppland	Gjøvik	ND	40	ND	ND
19	Hedmark	Ringsaker	ND	36	ND	ND
20	Akershus	Frogn	ND	36	ND	ND
26	Akershus	Ås	27	19	ND	ND
34	Østfold	Skjeberg	ND	25	ND	ND
35	Østfold	Skjeberg	ND	15	ND	ND
37	Østfold	Råde	ND	71	ND	ND
39	Hedmark	Vang	ND	43	ND	ND
41	Østfold	Rygge	175	22	ND	ND
42	Østfold	Sarpsborg	ND	36	ND	ND
43	Østfold	Sarpsborg	ND	28	ND	ND
44	Østfold	Rygge	ND	17	ND	ND
47	Vestfold	Lardal	166	887	ND	ND
48	Hedmark	Grue	459	32	ND	ND
49	Østfold	Rygge	ND	39	ND	ND
50	Oppland	Østre Toten	ND	46	ND	ND
51	Østfold	Rygge	ND	34	ND	ND
54	Hedmark	Vang	ND	104	ND	ND
55	Hedmark	Vang	8	73	ND	ND
74	Østfold	Rakkestad	29	18	ND	ND
75	Akershus	Ås	50	27	ND	ND
76	Vestfold	Sem	1571	25	ND	ND
77	Hedmark	Stange	19	29	ND	ND
78	Akershus	Nes	150	33	ND	ND
79	Østfold	Rygge	20	26	ND	ND
80	Østfold	Rakkestad	30	26	ND	ND
81	Vestfold	Borre	25	19	ND	ND
82	Vestfold	Tønsberg	1161	22	ND	ND
83	Buskerud	Hole	30	21	ND	ND
86	Østfold	Rakkestad	152	39	ND	ND

DON = deoxynivalenol, NIV = nivalenol, T-2 = T-2 toxin, ND = not detected

hay made up 23 of the 24 samples contaminated with trichothecenes.

Trichothecenes and zearalenone production in the laboratory

Fusarium-species cultivated on rice grains in the laboratory produced all the five different mycotoxins we analyzed for (Table 7). The toxin levels were generally lower than in naturally produced barley and wheat.

Abbas et al. (1987) obtained 125 *Fusarium* isolates from arctic regions and North, Central and Southern Norway. They increased the isolates on a solid rice medium and fed the infected

Table 4. Concentration of trichothecenes and zearalenone in wheat samples from Central Norway (ng/g)

Sample no.	Origin of sample County	Commune	Trichothecenes DON	NIV	T-2	Zearalenone
29	Nord-Trøndelag	Snåsa	54	44	ND	ND
30	Sør-Trøndelag	Melhus	1776	150	ND	ND
31	Nord-Trøndelag	Stjørdal	44	53	ND	ND
59	Nord-Trøndelag	Steinkjær	10	33	ND	ND
94	Sør-Trøndelag	Skaun	210	43	ND	4
95	Nord-Trøndelag	Verdal	26	44	ND	2
96	Sør-Trøndelag	Melhus	3193	71	ND	ND
97	Nord-Trøndelag	Frosta	ND	38	ND	ND
98	Nord-Trøndelag	Frosta	11	30	ND	ND
99	Nord-Trøndelag	Frosta	29	44	ND	ND
100	Nord-Trøndelag	Frosta	20	56	ND	ND
101	Nord-Trøndelag	Frosta	8	70	ND	ND
102	Nord-Trøndelag	Frosta	24	71	ND	ND

DON = deoxynivalenol, NIV = nivalenol, T-2 = T-2 toxin, ND = not detected

Table 5. Summary of deoxynivalenol content in Norwegian barley and wheat samples

Geographical region	Crop	Total no of samples	Percentage with DON	DON concentration ng/g Range	Average	SD
South-Eastern	Barley	24	54.2	8-223	45.5	55.8
"	Wheat	40	50.0	8-1571	307.2	500.0
Central	Barley	25	84.0	6-2139	243.4	484.0
"	Wheat	13	92.3	8-3193	419.9	962.3

DON = deoxynivalenol

substrates to rats. Sixty-four percent of the isolates caused a decrease in weight and some caused congestions, and haemorrhage of various tissues including stomach, intestine, thymus, and haematuria in the bladder.

The number of known toxic metabolites produced by *Fusarium* spp. is increasing with improved bioassays and biochemical separation methods. Abbas et al. (1984) compared metabolites in tests on human and mouse skin fibroblasts, rat skin toxicity and rat feeding tests. Cytotoxicity values of fungal extracts obtained on cultured cells correlated well with weight loss in rat feeding experiments.

The formation of mycotoxins is not an essential factor in the pathogenesis of *Fusarium* spp., but Manka et al. (1985) found that highly pathogenic isolates of *F. culmorum* and *F. graminearum* produced DON, Ac-DON and ZEN in considerable quantities.

Millar et al. (1985) compared resistance and mycotoxin production among wheat, rye and triticale cultivars. Resistant cultivars contained low concentrations of DON after artificial infection with *F. graminearum*. This could be due to reduced synthesis or higher degradation of the mycotoxin.

The fungicide tridemorf influences production of trichothecenes in cultures of *Fusarium* spp. (Moss & Frank 1985).

Table 6. Summary of nivalenol content in Norwegian barley and wheat samples

Geographical region	Crop	Total no. of samples	Percentage with NIV	NIV concentration ng/g		
				Range	Average	SD
South Eastern	Barley	24	100.0	13-156	46.3	28.4
«	Wheat	40	100.0	15-887	59.7	136.0
Central	Barley	25	100.0	22-258	53.8	45.0
«	Wheat	13	100.0	30-150	57.5	31.1

NIV = nivalenol

Table 7. Production of trichothecenes and zearalenone on rice grains by *Fusarium* species isolated from Norwegian crop plants

Fungus species	Isolate no.	Host plant	Concentration ng/g				
			DON	Ac-DON	NIV	FX	ZEN
F. acuminatum	1	Oats	-	-	-	-	0.50
	2	Oats	8.24	3.01	-	-	-
	3	Oats	-	4.67	-	-	-
F. avenaceum	1	Barley	-	-	3.07	0.68	-
	2	Barley	-	2.47	-	-	-
	3	Grass	-	1.56	-	-	-
F. oxysporum	1	Grass	-	-	-	-	1.05
	2	Grass	-	1.22	-	-	-
	3	Barley	-	0.52	-	-	-
	4	Barley	-	-	0.80	-	-
F. sambucinum	1	Barley	0.52	-	-	-	-
	2	Barley	-	-	0.80	-	-
	3	Grass	-	1.41	-	-	3.10
	4	Grass	0.15	0.53	-	-	1.50
	5	Grass	0.59	0.66	-	-	-
	6	Grass	0.62	-	-	-	-
	7	Grass	4.62	-	-	-	-

DON = deoxynivalenol, Ac-DON = 3-acetyl-deoxynivalenol, FX = Fusarenon X, NIV = nivalenol, ZEN = zearalenone, - = not detected

At a 6-8 ppm concentration the fungicide enhanced the growth of *F. sporotrichoides* and inhibited production of T-2 and DAS. However, high concentrations, which inhibit growth by about 50%, stimulated the formation of T-2 toxin in the shake flask culture.

The present investigation has demonstrated that contamination with NIV and DON is a common phenomenon in Norwegian-grown barley and wheat. In some samples the mycotoxin content is above the acceptable level for human consumption or animal feed. This public health and veterinary problem deserves further study.

In 1984 the Norwegian cereal yield average.surpassed 4 t/ha for the first

time (Wolden 1984). The precipitation was below normal in July and August in the major cereal growing regions of Norway.

Thus, the samples analyzed by us are probably representative of quality barley and wheat in a good growing season.

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PARTIAL RESISTANCE TO POWDERY MILDEW IN WHEAT SEEDLINGS

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The seedlings of 20 spring and winter wheat cultivars were inoculated in greenhouse with 8 different isolates of powdery mildew. The latent period and the percentage of leaf area infected were observed. A partial resistance in exess of the level found in the spring wheat cultivar Runar was detected in 5 spring wheats and 3 winter wheats. No interactions found between isolates and cultivars indicated the absence of race specific resistance genes.

Key words: *Erysiphe graminis*, durable resistance, horizontal resistance, latent period, *Triticum aestivum*, virulence.

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Breeding for resistance to powdery mildew (*Erysiphe graminis* D.C. f. sp. *tritici* E. Marchal) in wheat has mostly been based on single genes (also called major genes). In most cases, the durability of this type of resistance when introduced into new cultivars has been short-lived. New virulent races have appeared soon after these cultivars have been grown extensively. In recent years there has been an increased interest in breeding for lasting horizontal resistance in accordance with the concept of Van der Plank (1968); this is called durable resistance (Johnson 1979) or partial resistance (Jørgensen 1987). Numerous investigations have been devoted to studies of partial resistance in barley (Asher 1982, Jones et al. 1982, Knudsen 1984, Heun 1986) and in wheat (Shaner 1973, Rouse et al. 1980, 1984, Royer et al. 1984, Hautea et al. 1987). Bennett (1981) reported background seedling resistance in English cultivars of wheat having major genes that had been overcome by new virulent strains of the pathogen. Jørgensen (1987) assumed that partial resistance is governed by many additively acting genes. According to Johnson (1981), durable resistance includes no statement or implication about the genetic control of the resistance, its mechanism, its degree of expression, or its race specificity. Partial resistance may not always be durable, and may depend on major genes (Martin & Ellingboe 1976, Royer et al. 1984). According to Johnson and Law (1975), durability of partial resistance will only be proved when a cultivar is grown extensively for several years.

The purpose of this investigation has been to find the level of resistance in some Scandinavian wheat cultivars and the degree of interaction between cultivars and isolates of powdery mildew. According to the definition by Van der Plank (1968), partial or horizontal resi-

stance can be inferred from statistical analyses when there is no interaction between host and parasite.

MATERIALS AND METHODS

Twenty cultivars or lines of spring and winter wheat were investigated (Table 1). Cv nos. 1, 2, 5, 11, 13, 14, 18, and 19 are from the Department of Crop Science, while nos. 6, 7, 9, 10, 15, 16, and 17 are from the Department of Genetics and Plant Breeding, Agricultural University of Norway. No. 12 is from the State Research Station Møystad, Norway; no. 3 is from the Agricultural Research Center Jokioinen, Finland, no. 20 from Hankkija Plant Breeding Station, Finland, and no. 4 from Weibullsholm Plant Breeding Station, Sweden. No. 8, a susceptible check cultivar, was apparently introduced into the United States from Mexico (Briggle, pers. comm.).

The isolates were collected from commercial fields in the wheat growing area of South Eastern Norway, consisting of the cultivars Runar or Reno with the Pm4b resistance gene, and from wheat nurseries grown at the State Research Stations at Ås, Ottestad and Vang. All the isolates were virulent on the resistance genes Pm1, Pm2, Pm3a, Pm4b, and pm5 and avirulent on Pm4a. For resistance genes Pm3b, Pm3c, Pm6, Pm8, and Mld some isolates possessed virulence and others avirulence.

The cultivar Møystad has the resistance gene Pm1, Rida has pm5 (Skinnes & Elen unpubl.), while Runar, A 35-195, and A 35-213 all have Pm4b (Aastveit 1968, Strand 1975). The other cultivars have no known resistance genes. Accordingly, it was possible to study the effects of resistance genes other than the known race specific genes in the experiment.

The plants were grown in greenhouse in 5 cm pots filled with a clay/peat mixture; 16 pots of 3-5 plants of each cultivar in each of two experiments. In experiment I, the plants were sown in the autumn, and grown for about two weeks at 15-20°C in low light intensity. Experiment II plants were sown in winter and grown for three weeks at 12-13°C in normal light intensity. At stage 12 (2 leaves, Zadoks et al. 1974), groups consisting of 2 replicates of each cultivar were put into eight 40x50 cm plastic boxes with lids; randomization of pots was done within the boxes. Each of the eight boxes were inoculated with a different mildew isolate. The plants were incubated for 48 hours at 15°C and then kept at about 20°C until assessment for disease. The density of the inoculum was not measured exactly, but it was much higher in the first experiment than in the second.

Disease was assessed by observing the latent period and percentage of leaf area infected at the beginning of sporulation.

RESULTS AND DISCUSSION

The results of experiment I, performed in the autumn, and experiment II, performed in the winter differed. There was a more intensive disease development in the first experiment than in the second (Table 1). The mean latent periods were 5.4 days in experiment I and 9.1 days in experiment II. The corresponding results of leaf area infected were 29.2% and 15.3%, respectively. The differences between experiments were mainly caused by the differences in the conditions in which the plants were grown before inoculation. The density of the inoculum was lower in the second experiment than in the first; according to Aust & Kranz (1974) this should not cause a delay of more than 8 hours in the latent period, but in our case the latent period was 3.8 days longer. Plants in the second experiment were grown under cooler conditions and consequently the growth period was prolonged. According to Tapke (1951), plants of barley and wheat grown under cold conditions before inoculation

Table 1. Latent period and percentage of leaf area infected in 20 wheat cultivars and lines inoculated with powdery mildew.

Cultivar/line		Growth habit	Latent period, days		% leaf area infected	
			1. exp.	2. exp.	1. exp.	2. exp.
1	Rida	W[1)]	5.3	10.1	31	7
2	Mø 76-29	W	5.3	9.3	22	11
3	Aura	W	5.3	8.2	33	20
4	Folke	W	5.3	8.4	32	9
5	Mø 70-69-43	W	5.1	10.3	32	6
6	A 35-213	S[2)]	5.3	8.5	30	15
7	A 35-195	S	5.3	7.7	32	25
8	Little Club	S	5.4	8.1	30	16
9	T 50-11	S	5.3	8.7	29	17
10	T 50-39	S	5.3	8.2	28	22
11	Runar	S	5.2	8.7	35	15
12	Møystad	S	5.8	10.7	23	7
13	Mø 75-711	S	5.3	8.3	30	26
14	T 9054	S	5.3	8.7	33	16
15	MS 57-8	S	5.3	8.3	31	30
16	MS 57-144	S	5.8	10.5	27	13
17	MS 273-150	S	5.8	10.4	22	8
18	T 2038	S	5.4	9.8	26	13
19	T 8020	S	5.8	10.0	25	12
20	Ruso	S	5.3	9.4	33	18
	Mean		5.4	9.1	29.2	15.3

[1)]Winter type
[2)]Spring type

are more resistant to mildew than plants grown at temperatures between 16 and 21°C. The results in Table 1 show that the differences between resistant and susceptible cultivars increased from the first to the second experiment.

Table 2 shows results from analyses of variance performed for the latent period and the leaf area infected, with experiments as blocks. No significant differences between isolates nor significant interactions between isolates and

Table 2. Analyses of variance for the effects of inoculation of 20 wheat entries with 8 isolates of powdery mildew.

Source	DF	Latent period		Leaf area infected	
		MS	F	MS	F
Total	319	4.55		143.1	
Experiment	1	1,115.00	1,432.00 ***	15,468.0	253.00 ***
Isolate	7	7.08	2.94 ns	836.5	1.78 ns
Error I (ExI)	7	2.41		468.7	
Cultivar	19	4.66	5.98 ***	321.7	5.26 ***
CV x isolate	133	0.48	0.61 ns	42.5	0.70 ns
Error II	152	0.78		61.1	

*** Statistical significance $p \leq 0.001$

cultivars were found for the two characters observed. There were, however, very significant differences ($p<0.001$) between cultivars for the latent period as well as for the percentage of leaf area infected.

According to Van der Plank (1968), the non-significant interaction between isolates and cultivars means that the cultivars do not differ in effective race specific major genes, so the resistance detected may be caused by race non-specific minor genes. However, Parlevliet & Zadoks (1977) pointed out that in the case of minor gene interaction, variance is predominantly composed of main effects, and with a normal experimental error this type of interaction is not likely to be discerned as statistically significant. So, it cannot be ruled out that in this experiment the partial resistance could have been caused by race specific minor genes.

It is worth noting that significant differences were found between cultivars in the level of partial resistance despite changes in growing conditions and amount of inoculum between the two experiments. This means that manifestation of genotypic differences in resistance is relatively stable across different testing environments, and accordingly should be predictive for field conditions.

The analyses of variance performed for the isolates taken separately showed no statistically significant differences between cultivars, which indicates the necessity to minimize the experimental error to an acceptable level when investigating for partial resistance. This is in accordance with Knudsen (1984), who used many replicates in his investigation of partial resistance to mildew in barley. Knudsen (l.c.) also reduced the experimental error by using a technique whereby horizontal leaves were sprayed with a uniform suspension of spores.

Table 3 shows that the average latent period varied from 6.5 days in A 35-195 to 8.2 days in Møystad. The latter was also the least infected cultivar, while MS 57-8 suffered the heaviest attacks. The Norwegian wheat cultivar Runar which is the most extensively grown, is used here as a standard. Six cultivars had a significantly longer latent period than the standard, five of these also being less infected. In addition, two other cultivars suffered a lesser attack than Runar, so altogether eight cultivars possessed higher levels of partial resistance than the standard. Five of these were Møystad or its derivates, the other three being winter wheats. The Pm1 gene found in Møystad is absent in the offspring (Skinnes & Elen unpubl.). However, it is not effective against the isolates used and accordingly cannot be responsible for the partial resistance in these lines.

Table 3. Latent period and percentage of leaf area infected in 20 wheat entries inoculated with powdery mildew; the means of latent periods are arranged in descending order.

	Cultivar/line	Latent period, days	% leaf area infected
12	Møystad	8.2[1)]	14.9[1)]
17	MS 273-150	8.1[1)]	15.0[1)]
16	MS 57-144	8.1[1)]	19.8
19	T 8020	7.9[1)]	18.2[1)]
5	Mø 70-69-43	7.7[1)]	18.9[1)]
1	Rida	7.7[1)]	19.2[1)]
18	T 2038	7.6	19.4[1)]
20	Ruso	7.4	25.5
2	Mø 76-29	7.3	16.7[1)]
9	T 50-11	7.0	22.8
14	T 9054	7.0	24.8
11	Runar	7.0	25.0
6	A 35-213	6.9	22.4
4	Folke	6.8	20.6
10	T 50-39	6.8	25.2
13	Mø 75-711	6.8	28.0
15	MS 57-8	6.8	30.3
8	Little Club	6.7	23.2
3	Aura	6.7	26.1
7	A 35-195	6.5	28.8
	Mean	7.3	22.2
	LSD5%	0.6	5.4

[1)]Statistically significant different from Runar

The latent period and percentage of leaf area infected were closely correlated with a highly significant phenotypic regression coefficient, $r = -0.8084^{***}$. This result indicates that these two characters are governed by similar genetic control or environmental factors. As suggested by Asher & Thomas (1984), if genetic, they may represent different expressions of resistance that affect fungal development.

On the basis of the results presented here the following conclusions may be drawn:

1. No race specific effects of resistance against the isolates tested have been detected.
2. Significant differences were found between cultivars in the latent period and the percentage of leaf area infected.
3. In some varieties the level of partial resistance exceeded the level in the commercially grown cultivar Runar.
4. The differences in partial resistance were consistent with differences in the growing conditions for the seedlings prior to infection.

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COMPOSITION OF THE DIESTERS OF ALKANE-2,3-DIOLS OF THE UROPYGIAL GLAND OF WILLOW PTARMIGAN *(LAGOPUS LAGOPUS)*, BLACK GROUSE *(LYRURUS TETRIX)* AND CAPERCAILLIE *(TETRAO UROGALLUS)*

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Solberg, Y., and Steen J. B. Composition of the diesters of alkane-2,3-diols of the uropygial gland of Willow Ptarmigan *(Lagopus lagopus)*, Black Grouse *(Lyrurus tetrix)* and Capercaillie *(Tetrao urogallus)*. Norwegian Journal of Agricultural Sciences 1: 67-71. ISSN 0801-5341

The structure of the alcoholic and acidic moieties of the lipids excreted by the uropygial glands of *Lagopus lagopus*, *Lyrurus tetrix* and *Tetrao urogallus* was determined by gas chromatography and combined gas chromatography-mass spectormetry. In *lagopus lagopus* C12 diols were the major neutral component, whereas C14 and C14, and C18 and C20 diols predominated in *Lyrurus tetrix* and *Tetrao urogallus*, respectively. Smaller amounts of other diols and normal alcohols in the range C8 to C22 were also observed in all three bird species. With regard to the structure of the diols, only components with the two hydroxyl groups in the 2,3-position have been identified in our material. *Cis-trans* alkonol isomers have been found in the two species *Lagopus lagopus* and *Tetrao urogallus*, whereas *Lyrurus tetrix* only showed the presence of *trans*-isomeric 2,3-diols. The acidic moiety of the uropygial gland lipids consisted primarly of the C8, C10 and C12 fatty acids, with smaller amounts of the C9, C11 and C13 to C18 acids. All the fatty acids except of C18 were unbranched and saturated. In addition, Squalene, Cholesterol and Cholestanol have been detected in the neutral moieties of the lipid fractions. Free alcohols or fatty acids have not been detected in the unhydrolysated lipid secretions.

Key words: *Lagopus lagopus*, Lipid composition. *Lyrurus tetrix*, *Tetrao urogallus*, Uropygial gland.

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The surfaces of higher animals, plants and insects are covered by a thin layer of lipids. In birds, the surface fat originates in the uropygial or preen gland and differs significantly from that produced elsewhere in the body. Chemical investigations of the composition of this secretion have firmly established that it consists of mono or diester waxes. A complex misture of fatty acids and diols

in the hydrolysates of the esters has been reported. The relative proportion of these constituents appears to vary considerably according to species (Kolattukudy 1972, Saito & Gamo 1972, Jacob 1976).

In contrast to waterfowl, in which wax esters proved to be the major preen lipid, species of birds belonging to the Galliformes (hens, turkeys, pheasants) have preen lipids largely composed of diesters of alkane-2,3-diols (Jacob 1976). The acidic fraction of hydrolysates of the preen lipids from chicken (Tang & Hansen 1976) consisted of saturated C10 to C20 fatty acids. The major components of the neutral fraction were C22, C23 and C24 alkane-2,3-diols (*cis-trans* isomers). It was also found that the alkane-2,3-diol fraction consisted of *trans* and *cis* isomers. The preen gland of turkey (Sawaya & Kolattukudy 1972, Hansen et al. 1969) consisted of the four major diols C20-C23, all of *cis* configuration. The fatty acids were saturated and contained the homologous series C10 to C20 with C18, C19 and C14 constituting the major components in the order listed (Jacob 1976). In the Stubble Quail *(Conturnix pectoralis)* (Sawaya & Kolattukudy 1972, Sawaya 1973, Abalain et al. 1983), the alkane-2,3-diols identifies were mainly C14 to C24 and they were *cis* isomers with C18, C22, C23 and C24 as the major components. The preen lipids of the Ring-necked Pheasant *(Phasianus colchicus)* (Sawaya & Kolattukudy 1972, Sawaya 1973, Sawaya & Kolattukudy 1973) contained only one major diol, C18-2,3-diol (85 %) with C17, C19 and C20 as minor components. In the Mikado Pheasant *(Phasianus mikado)* (Sawaya 1973), the alkane-2,3-diol fraction consisted of several components, with C18 and C20 as the major components and C16, C17, C19 and C21 as the minor ones. These diols were found to be of the *trans* form. In contrast to the Mikado Pheasant, the Great Curssow *(Crax rubra)* (Sawaya 1973) has diol lipids containing a mixture of C22 to C25 diols of both *cis* and *trans* forms. In the Great White Erget *(Erget alba)*, the diol waxes compared very well with those of the Green Pheasant, having the C18 diol as the major diol component (Saito & Gamo 1970).

No studies appear to have been made of the composition of the content of the uropygial gland secretion from wild birds in Norway. The present paper discribed the separation and identification of the constituent alcohols and fatty acids from the preen gland secretions of the Willow Ptarmigan *(Lagopus lagopus)*, Black Grouse *(Lyrurus tetrix)*, and Capercaillie *(Tetrao urogallus)*, using gas chromatography (GC) and gas chromatography/mass spectormetry (GC/MS).

MATERIALS AND METHODS

Gland material. After the birds were killed and frozen, the whole uropygial glands were dissected out, freed of external adipose tissue and internal wax and crushed finely with quartz sand. Subsequently the crushed glands were subjected to extraction of the lipid mixture with methanol/chloroform (2 + 1, v/v) for 24 h. The organic solution was removed from the residual tissue, which was rinsed with the methanol/chloroform solution. The combined extracts were evorated to dryness under diminished pressure. The free fatty acids and the diols were recovered together and hydrolysed.

Hydrolysis of lipids and preparing of derivatives. An aliquot of the gland extract was dissolved in methanol and refluxed for 3 h with 2M aqueous potassium hydroxide. The cooled solution was then diluted with water, saturated with NaCl and the alcoholic fraction extracted into hexane. After acidification, the fatty acids (FA) were similarly extracted. Aliquots of this solution were derivatized as described

below. The compounds obtained from the hydrolysed extracts were separated and quantified as free and acetylated alcohols and methyl esters of the fatty acids. Fatty acids and alcohol derivatives were obtained as described by Solberg (1986). Gas chromatographic separations were carried out as given in the same publication. The column oven temperature in the gas chromatograph was programmed from 95 oC to 300 oC at 3°C/min;

Reference compounds. In order to confirm the stereochemistry of the diols, 2,3-alkenes of chain length C10, C12, C14 and C16, containing approximately 13.5 % *cis* isomers, were synthesized and their structures ascertained by proton nuclear magnetic resonance and mass spectrometric analyses (MS). The mixture of olefins was oxidized to 2,3-alkane-diols using osmium tetraoxide (Christie 1982) in the presence of toluene/dioxane (8+2).

RESULTS AND DISCUSSION

The preen lipids of the birds *Lagopus lagopus*, *Lyrurus tetrix* and *Tetrao urogallus* were examined using a combination of GC and MS of the alcohols and fatty acids released by hyrolysis. The classes of lipids contained in the three species of birds studied were very similar but the chain lengths were different.

Mono-alcohols - The chain lengths of the mono-alcohols were found to be mainly C16 and C18 in *Lyrurus tetrix* and *Tetrao urogallus*, With minor amounts of C14 and C17 alcohols. *Lagopus lagopus* contained the mono-alcohols C14 and C16 as the major components, with trace amounts of C17 and C18 alcohols (Table 1). All the isolated mono-alcohols were of a unbranched and saturated nature and represented a greater part of the unsaponifiable fraction. Furthermore, a high proportion of the sterol components Cholesterol (Cholest-5-en-3β-ol) and

Table 1. Constituent alcohols of the uropygial gland lipids of *Lagopus lagopus*, *Lyrurus tetrix* and *Tetra urogallus*. The values below represent the total of the two isomers and are expressed as relative proportional values within each species

Compounds	Birds		
n-ALCOHOL	*L. lagopus*	*L. tetrix*	*T. urogallus*
C 14	4	1	1
C 16	4	14	19
C 17	1	3	3
C 18	1	15	4
ALKANE-2,3-DIOL			
C 12	38	11	7
C 13	1	17	11
C 14	10	33	25
C 15	-	9	8
C 16	1	11	10
C 17	-	3	3
C 18	2	7	32
C 19	trace	1	6
C 20	2	2	25
C 21	1	3	5
C 22	trace	3	1

In addition, trace quantities of the C10, C11, C23 and C24-2,3-diols have been detected in the gland secrete of the birds *Lyrurus tetrix* and *Tetrao urogallus*.

Cholestanol (Cholestane-3β-ol) was detected in the lipid secretions from all three species. The characteristic and most abundant ions present in the obtained mass spectra of these compounds were m/z 386 (M+), 368 (M-18), 353 (M-33), 301, 275, 255, 231, 213, 207, 199, 187, 173, 159, 145, 133, 119, and m/z 388 (M+), 373 (M-15), 370 (M-18), 355 (M-33), 316, 257, *233*, *215* respectively. The MS results of Cholestane-3β.ol are in accordance with those of authentic components (Budzikiewicz et al. 1964).

Alkane-2,3-diols. GC and MS analyses of the diol-diacetates of *Lagopus lagopus* showed that the secretion from this bird contained two major components, *Dedecane-2,3-diol* (C12) and *Tetradecane-2,3-diol* (C14).

The other minor alkane-diols present were components with chain lengths of C13, C16, C18, C20 and C21. Trace quantities of C19 and C22 were also observed. Periodate/permanganate oxidation (Christie 1982b) of the diol-mixture from *Lagopus lagopus* gave C10 and C12 fatty acids as the major constituents. This is in accordanse eith the prevalent alkane-2,3-diols present in this species.

Lyrurus tetrix was found to contain four major diols with chain lengths of C14, C13, C12 and C16 and a wide range of minor diol compounds, including C22. All the diols of the secretion of this species were found to be of the *trans* from only. The different diols are listed in order of decreasing quantities.

The major diols in the preen glands of *Tetrao urogallus* were found to be C18, C14, C20 and C16, of both *trans* and *cis* forms. In effect the diol composition of this species was different from that obtained from *Lagopus lagopus* and from *Lyrurus tetrix* investigated in our work. It is of interest that the chain lengths of the diols detected in the secretions of *Lagopus lagopus* and *Lyrurus tetrix* did not resemble those of birds previously referred to in this paper, whose preen gland lipids contained major diols with chain lengths of C18 and upwards. Table 1 shows the mono-alcohol and alkane-2,3-diol composition of the lipid esters from the three bird spesices.

The simple MS fragmentation pattern observed in the free alkane-2,3-diols in the present work is readily interpreted. The molecular ion was never detected, and the heaviest ions observed were (M-45) and (M-64). which indicated loss of ($HOCHCH_3$) and (C2 H8 02) respectively from the molecular ion. Prominent informative fragmentation ions were also formed by removing the (AcOCHCH3) fragment from the molecular ions of the acetyl derivatives. All the results indicated a 2,3-position of the two hydroxyl groups.

Fatty acids. With the exception of C18:1 all the fatty acids of the preen lipid hydrolysates of the three birds investigated were of an unbranched and sturated nature, C8:0, C 10:0 and C12:0 predominating. The components of the *Lagopus lagopus* secretion showed that it contained C12:0, C18:1 and C14:0 as the major compounds and a series of minor ones. The predominant FA components in the *Lyrurus tetrix* and *Tetrao urogallus* secretions were found to be C10:0, C8:0 and C12:0, and C12:0, C10:0 and C8:0 respectively, written in order of decreasing amounts. All the FA in the range from C8:0 to C16:0 were of a straight chain and saturated nature. Our results resemble those obtained previously in which normal fatty acids frequently were seen as part of the preen gland lipids of *Galliformes* (Jacob 1976). The survey of the Fa isolated from the three bird species in the present work is given in Table 2.

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Table 2. Proportional amounts of the fatty acids in the acidic fraction of the uropygial gland lipids of *Lagopus lagopus*, *Lyrurus tetrix* and *Tetrao urogallus*

FA	*L. lagopus*	*L. tetrix*	*T. urogallus*
C 8:0	2	28	13
C 9:0	1	14	1
C 10:0	4	32	22
C 11:0	3	8	2
C 12:0	11	13	24
C 13:0	3	1	-
C 14:0	5	1	1
C 16:0	3	-	1
C 18:0	4	-	2
C 18:1	6	-	2
C 18:2	4	-	2
C 18:3	1	-	2

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