One moment in time. Gene expression analysis of honey bees; nurse bees v.s. foragers

Tove Rimestad

Department of animal and aquacultural sciences
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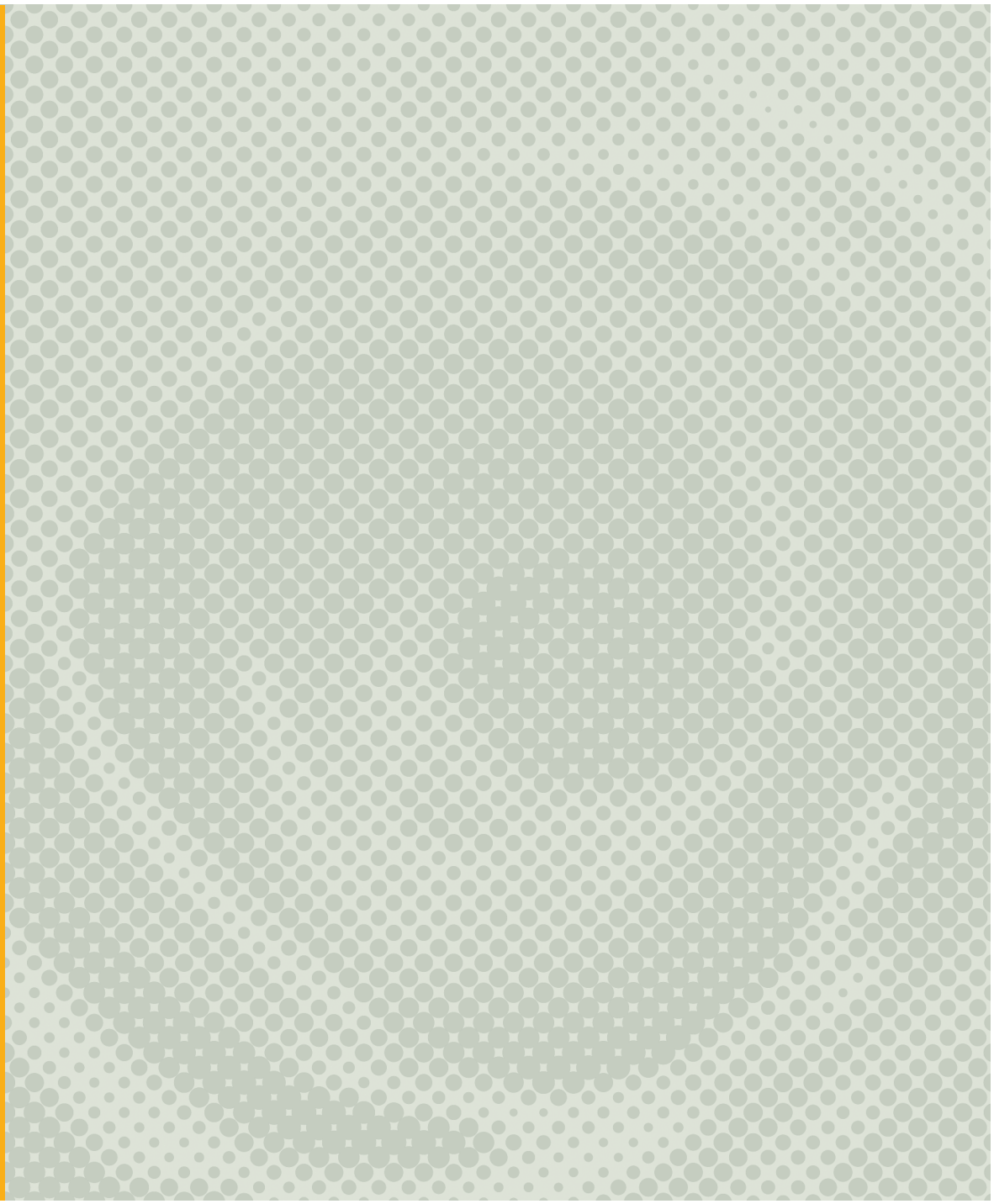
Norwegian University of Life Sciences



One moment in time

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Contents

Preface

This thesis is a part of a master study at the Department of Animal and Aquacultural Sciences at Norwegian University of Life Sciences. The study was funded by NFR #186362/V40. The field work was preformed the summer of 2009, the lab work was preformed autumn 2009 and spring 2010. The SOLiD sequencing was performed by Uppsala Genome Centre, Rudbeck Laboratory, summer, autumn 2010. The data analysis was preformed autumn 2010, winter 2011.

Supervisor was Siri-Christine Seehuus and co-supervisor was Dag Inge Våge.

Thanks to Dag Inge for helpful advice and response in the process.

A special thanks to Siri-Christine for guidance and for being available at all hours of the day when the deadline became dangerously close.

A big, big, big thank you to Eli Svendsen and Kristoffer Rimestad-Sævik for being patient angels.

In the lake that's like an ocean
I count about a billion head
All the time
there's a motion
-*Pixies "Palace of the Brine"*

Abstract

Honey bees live in complex societies based on a division of labour. The honey bee workers specialise in different tasks throughout their lives, starting off as nurse bees and ending as foragers. The nurse bees and foragers display interesting phenotypic differences that do not have its origins in differences at genotype level, but in differences in gene expression. This thesis presents the results from an expression analysis done on honey bee workers comparing the expression profiles of nurse bees and foraging bees. We confirm patterns previously described in differences in expression of genes involved in energy storage, nutrient and energy metabolism, immune defence and communication. There are also a few surprises such as the expression of two hexamerins previously not reported in adult bees.

Introduction

The honey bee, apart from being of high agricultural value as a crop pollinator and a honey producer, is of high value as a model organism (*1, 2*). The bee provides an excellent model for sociogenomics and in studying interactions between nature and nurture (*3*). The honey bee is a eusoscial insect that lives in colonies of typically about twenty to forty thousand individuals (*4*). All bees in the colony are offspring of the queen bee whose main task is to lay eggs and keep the colony together (*5*). The maintenance of the hive is the sole domain of the worker bees which are functionally sterile females with morphological specializations adapted for nursing, building and foraging for nectar, pollen and water. In spring and summer the colony also consists of about a thousand reproductive, haploid males (*4*), drones, which leave the hive to mate with virgin queens on their nuptial flights (*6*)

The honey bee worker exhibits an interesting case of plasticity of aging (*7*). In favourable seasons they shift from nest tasks to foraging duties after ~2-3 weeks of adult life (*8*). Foraging is associated with an increase in mortality risk and most workers die after 1-2 weeks of foraging activity (*9*). However, the worker bee is able to respond flexibly to changes in intracolonial and extracolonial environment (*10*), enabling workers to nurse brood continuously until >130 days of age (*11*) or initiating foraging flights as early as 4-7 days

after emergence. Plasticity in division of labour is essential for colony function and survival, and the role each worker is assigned is at any time ruled by a wide range of social and chemical cues (*7, 12, 13*). Nurses are continuously pushed from their role by newly emerged workers and pulled over in other tasks by interacting with older individuals of the hive (*7*) . For instance one of the ways foragers recruit new foragers is by regulating the amount of ethyl oleate released via trophallaxis (*14*).

Nurse bees have high levels of nutrient reserves, specifically high levels of stored lipids and proteins (*15*), whereas the foragers are unable to obtain amino acids from the colony's pollen store (*16*) and are fully dependent on nurse bees to feed them according to foraging activity level. Foragers are reported to be associated with increased mechanical senescence (*17*), immunosenescence (*18*), susceptibility to oxidative stress (*19*) and accumulation of oxidative damage in brain (*20*).

In the search for understanding underlying mechanisms of the plasticity of aging in the worker bee, there is a need for examining the differences in gene expression profiles between the two bee polyphenisms or discrete phenotypes. The main metabolic tissue of the honey bee is the fatbody, an oedipoues tissue found in the abdominal cavity and head, forming a lace-like, white tissue of cells lying in the hemocoel beneath the cuticula (*5, 21, 22*). The cells (trophocytes and oenocytes) of the fatbody are versatile and can change activity depending on the hormonal or nutritional state of the bee (*23*). The metabolism of lipids, nitrogenous compounds and carbohydrates takes place in the fat body and these may also be stored as energy supply in the form of fat, protein and glycogen (*5*). Hemoglobin, vitellogenin and blood sugar levels are produced and regulated in the fatbody making it functionally similar to mammalian liver (*24*). The largest proportion of fatbody tissue is contained in the abdomen. Abdomens of nurses and foragers where therefore chosen for whole transcriptome analysis to compare the main differences in expression profiles between the two phenotypes. Within the timeframe of a master thesis a thorough secondary analysis of the all the significant differences was not possible, I therefore chose to give an overview of the differences highlighting differences storage proteins, nutrient/energy metabolism, immune system activity and communication.

Material and methods

Honey bees

Newly emerged honeybee workers were marked with a spot of paint and introduced in the production colonies of *Apis mellifera carnica* in the apiary of the University of Life Sciences (Aas, Norway). After eight days the marked workers engaging in nurse tasks were collected. To be defined as a nurse the bee had to be observed with both head and thorax in a larvae containing cell. The colonies were checked for returning marked foragers every day and these were marked with a second spot of paint. When the larger majority of marked bees had made the transition to foraging the double- marked bees were gathered at the entrance of the hive.

In both groups intestines were removed immediately after collection. The bees were then flash frozen on dry ice and transferred to -80˚C for storage until further processing.

RNA extraction, Lab protocol

RNA was extracted from worker abdomens using the RNeasy Mini Kit (#74106 Qiagen) with small modifications to the original protocol. In brief; Tissue was disrupted and homogenized with Precellys 24 steel bullets. The lysate was centrifuged for 3 minutes at full speed. The supernatant was mixed with 70% ethanol, transferred to a RNeasy spin column before centrifuging and washing according to the RNeasy protocol. DNase stock solution was prepared according to protocol and 20 µl DNase in 60 µl RDD buffer was added to the columns and left to incubate for 15 minutes at room temperature. After incubations the RNA was washed and gathered according to the RNeasy protocol. Samples were then frozen to -80˚C in 20 µl alquilots. Each alquilot was tested for concentration and quality both by use of the Agilent RNA 6000 Nano Assay (Bioanalyzer) and Nanodrop. For sequencing only samples meeting the requirements of no RNA degradation and a total RNA concentration above 7,5 µg were used (see table 1).

Table 1. *An overview of the samples chosen for SOLiD whole transcriptome sequencing*

Dato	Prøve ID	Prøve	ul ut	Notater	Bioan	Nanodrop	ug/ul	tot kons
13.10.2009	cs01	Trekk	23	Solid	ok	386,2	0,3862	8,8826
13.10.2009	cs02	Trekk	23	Solid	ok	451,4	0,4514	10,3822
13.10.2009	cs03	Amme	23	Solid	ok	450,6	0,4506	10,3638
14.10.2009	cs05	Amme	31	Solid	ok	523,2	0,5232	16,2192
14.10.2009	cs06	Amme	26	Solid	ok	525,1	0,5251	13,6526
14.10.2009	cs07	Trekk	26	Solid	ok	731,1	0,7311	19,0086
14.10.2009	cs08	Trekk	26	Solid	ok	553,6	0,5536	14,3936
19.10.2009	cs13	Amme	20	Solid	ok	851,1	0,8511	17,022
19.10.2009	cs14	Amme	26	Solid	ok	493,4	0,4934	12,8284
19.10.2009	cs16	Trekk	20	Solid	ok	518,3	0,5183	10,366

SOLiD sequencing

The SOLiD sequencing was performed at the Uppsala Genome Centre (UGC), Rudbeck laboratory, using Sequencing by Oligo Ligation Detection 4 (SOLiD 4). The data was subjected to the SOLiD pipeline using Applied Biosystems AB Whole Transcriptome Analysis Pipeline (ABWT) version 1.2. The output presented 50 bp length raw reads that were aligned to an mRNA reference (Gnomon_mRNA.fsa) based on predicted genes from Apimel 4 (courtesy of Simon Taylor, Cigene, Norwegian University of Life Sciences). The contigs from the mRNA reference sequence (the predicted genes) were run in BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and hits showing over 98% similarity were considered as likely to present fitting information on the gene. The individual blast results were sorted

according to contig number and joined with the results from the alignment to form one list containing gene info and number of hits per contig for all individuals (see attachment 1).

Statistics and functional annotation

The number of hits per contig were normalised according to total amount of reads and log2 transformed. To determine which genes were significantly differently expressed between the two groups a Wilcoxon signed rank-test was performed. A pairwise two tailed t-test was also preformed to see if the result was coherent with the rank-test. Genes with a q-value above 0.05 from the rank-test were compiled into two lists; i) Upregulated in nurses compared to foragers, and ii) upregulated in foragers compared to nurses. These lists were submitted in the format of entrez gene ids to DAVID functional annotation tool (http://david.abcc.ncifcrf.gov/summary.jsp). The output from "functional annotation table" was sorted by general function and compiled into one list of genes expressed differentially between the groups. Only the genes DAVID provided information on were included in this analysis.

Results

Out of the contigs making up the mRNA reference 8640 were successfully identified in the BLAST search. Among these 380 genes were considered to be differentially expressed based on the results from the rank-test ($q < 0.05$). Genes with low p-values from the t-test was to a large extent the same that got low q-values in the rank-test. Out of the 380 genes considered differentially expressed 175 were upregulated in foragers compared to nurses and 205 genes in nurses compared to foragers. The functional annotation table provided information on the function of 97 out of the 380 differentially expressed genes (see table 2 and figure 1).

Table 2. ***Differentially expressed genes grouped into functional categories.***

(Marked in red = up in foragers, marked in blue = up in nurse. In the protein names "similar to" is shortened to "st")

entrez id	Protein name	Gene id
Immuneresponse:		
Lysosome:		
494509	malvolio	GB15139
408992	st Niemann-Pick Type C-1 CG5722-PA	GB14749
409709	st Y4C6B.6	GB13722
411429	st CG17119-PA, isoform A	GB15127
413489	st Deoxyribonuclease II CG7780-PA	GB14548
411392	st N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase precursor	GB20000,
antibacterial:		
406144	abaecin	GB18323
406143	defensin	GB19392
406142	hymenoptaecin	GB17538
defence mechanisms:		
410944	st ATP-binding cassette transporter sub-family C member 8	GB13238
Metabolism:		
Energy production and convertion/secondary metabolites:		
550686	st ATP citrate lyase CG8322-PA, isoform A	GB10992
412843	phosphoenolpyruvate carboxykinase	GB16196
725522	hypothetical protein LOC725522	GB16168
551423	st 15-hydroxyprostaglandin dehydrogenase	GB18737
550686	st ATP citrate lyase CG8322-PA, isoform A	GB10992
412569	st CG1544-PA, isoform A	GB15468
411140	st Putative aldehyde dehydrogenase family 7 member A1 homolog	GB13401
551533	st pyridoxine 5-phosphate oxidase	GB13619
727598	st Probable cytochrome P450 6a13	
552418	st Probable cytochrome P450 6a14	GB14612
725159	st Probable cytochrome P450 6a14	GB14594
551626	st Probable cytochrome P450 6a17	GB10668
410492	st Probable cytochrome P450 9f2	GB19820
411893	st Cytochrome P450 315a1, mitochondrial precursor	GB16447
413833	st Cytochrome P450 4c3	GB10905
552679	st Cytochrome P450 4c3	GB18743
725087	st Cytochrome P450 6a22	GB12885
727290	st Probable cytochrome P450 303a1	GB18872

551632	st Probable cytochrome P450 305a1	GB14915
412467	st CG11089-PA	GB14677
409759	st CG2989-PA	GB15345
552073	st CG8646-PA	GB13184
725646	st N-acetylneuraminate pyruvate lyase	GB17289,
409250	st beta-ureidopropionase	GB20148
carbohydrate:		
406114	alpha-amylase	GB18312
409889	alpha-glucosidase	GB12607
726818	st Beta-hexosaminidase beta chain precursor	
408788	st CG17323-PA	GB16747
413705	st CG9357-PA	GB15116
411484	st N-acetylgalactosamine kinase	GB10505
409814	st CG15117-PA, isoform A	GB14269
408871	st Sorbitol dehydrogenase-2 CG4649-PA	GB14284
Protein:		
410639	st Aromatic-L-amino-acid decarboxylase	GB14019
725400	st CG11796-PA, isoform A	GB18360
410530	st CG16771-PA	GB13388
727115	st CG8412-PA	
410627	st Dipeptidase B CG9285-PA, isoform A	GB19499
551465	st Homogentisate 1,2-dioxygenase (Homogentisicase)	GB11477
724239	st Kynurenine/alpha-aminoadipate aminotransferase mitochondrial precursor	GB10285
411288	st dunce CG32498-PO, isoform O	GB15311
726845	st homogentisate 1,2-dioxygenase CG4779-PA	GB15325
408930	tyrosine hydroxylase	GB15303
550932	arginine kinase	GB10973
406155	prophenoloxidase	GB18313
410550	st Aminomethyltransferase, mitochondrial precursor)	GB12854
551507	st CG11236-PA	GB10520
411796	st CG3011-PA	GB14485
727115	st CG8412-PA	
408587	st Histidine decarboxylase CG3454-PA	GB10303
412619	st Phosphoribosylamidotransferase CG2867-PA	GB16566
409582	st Punch CG9441-PB, isoform B	GB15785
725099	st spermidine synthase	GB12895
Lipid		
726445	glycerol-3-phosphate dehydrogenase	GB11613
727166	similar to CG15531-PA	GB12710

409628	st Ceramidase CG1471-PA, isoform A	GB12800
409709	st Y4C6B.6	GB13722
406066	juvenile hormone esterase	GB15327
408567	st CG12262-PA	GB16579
410254	st CG9547-PA	GB14051
408689	fatty acid binding protein	GB15299,
signal peptide:		
410337	venom dipeptidylpeptidase IV	GB14496
406093	apisimin	GB19468
biological regulation:		
406110	G-protein coupled receptor	GB17991
406066	juvenile hormone esterase	GB15327
406069	kruppel-like protein 1	
protein fate:		
411846	st F18A12.8a	GB13209
725380	chymotrypsin inhibitor	GB15018,
transcription:		
727085	st zinc finger protein 111	GB16262
725279	st zinc finger protein 617	
cytoskeleton:		
411894	st Dynein heavy chain at 93AB CG3723-PA	GB11916
cell communication/signal transduction:		
410228	st cAMP-dependent protein kinase CG6117-PA, isoform A	GB14368
412316	st solute carrier family 24, member 5	GB14667
409881	st Myosin regulatory light chain 2	GB13399
Colony communication		
725103	chemosensory protein 6	GB13325
677678	odorant binding protein 12	GB13299
406094	antennal-specific protein 3c	GB18819
725382	chemosensory protein 1	GB17875
677674	odorant binding protein 13	GB18363
677673	odorant binding protein 14	OBP14
552478	odorant binding protein 17	GB11092
677671	odorant binding protein 3	GB19454
406065	worker-enriched antennal transcript	Amwat
storage:		
551648	hexamerin 110	GB14361
406117	hexamerin 70b	GB10869
409354	hexamerin 70c	GB13613
726182	larval-specific very high density lipoprotein	GB15055

406088	vitellogenin	GB13999

The sorting into functional categories revealed differences between nurse bees and foragers. Nurse bees expressed five genes coding for storage proteins whereas the foragers expressed none in this category. In genes encoding nutrient metabolism the foragers had high expression in six genes and the nurse bees in two. For genes involved in protein and fat metabolism the two groups showed elevated expression in an equal number of genes.

In genes related two immunity eight showed high expression in foragers and two in nurse bees. Foragers are the only ones having heightened levels of mRNA encoding antibacterial proteins.

In genes involved in biological regulation, two are up in foragers and one in nurse bees. Foragers have high expression of *Kruppel-like protein* and nurse bees have a high expression of *Juvenile Hormone esterase.*

More genes encoding proteins involved in colony communication were upregulated in nurse bees than in foragers or more presicely; seven in nurse bees and two in foragers.

Genes related to transcription, cytoskeleton and signal peptides only showed heightened levels in foragers.

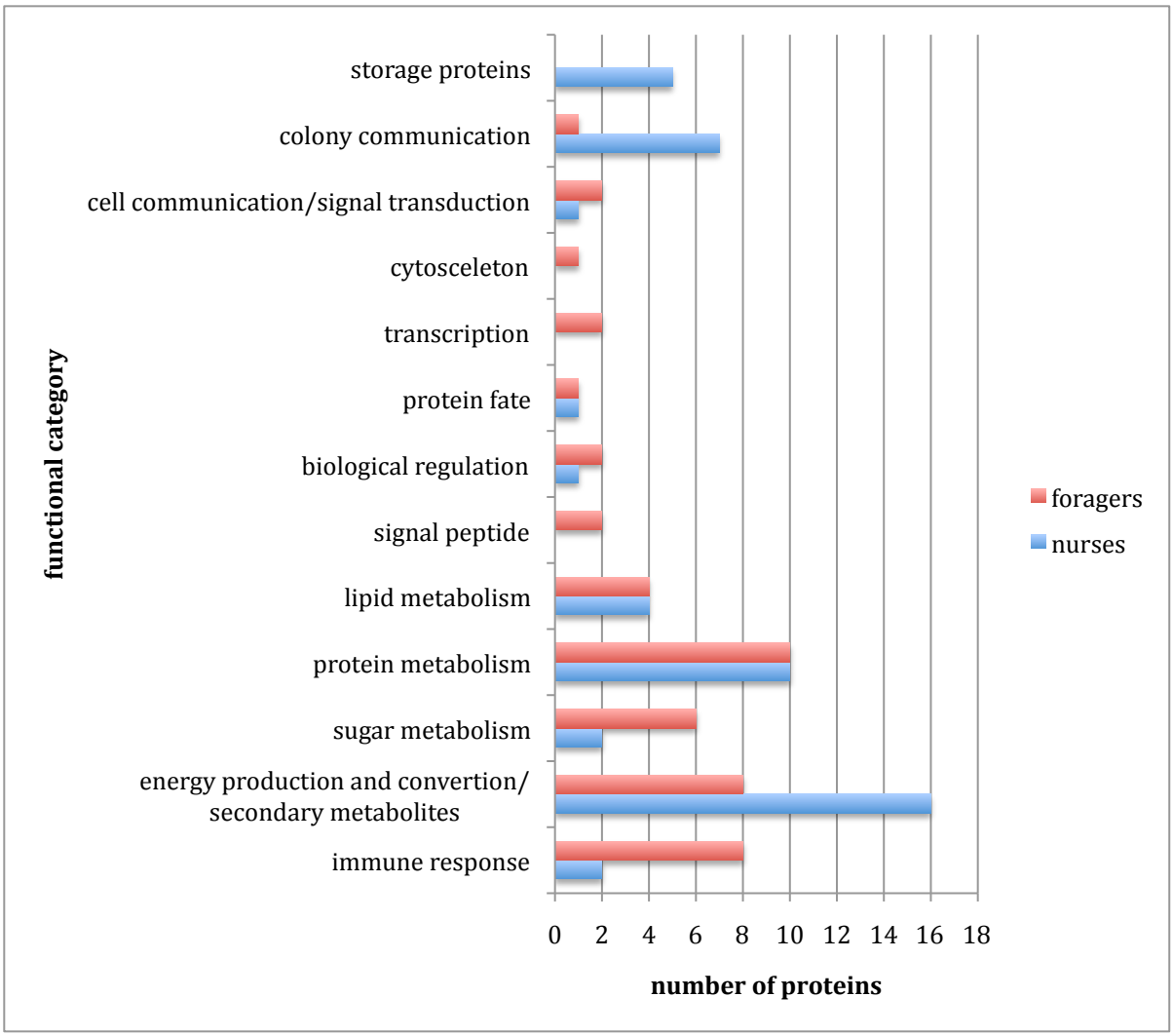

Figure1: *Overview of the differences in expression between nurses and foragers sorted by functional categories*.

Discussion

The division into functional groups (table 2, figure 1) reveal different biological patterns in nurses and foragers. The groups differ the most in expression of genes related to storage proteins, communication, secondary metabolites and immune response. The difference in storage proteins is perhaps not surprising as high protein and lipid reserves are a known characteristic of the nurse bee phenotype. The foragers are likewise known to have depleted nutrient reserves. One of the more interesting proteins highly expressed in nurse bees, is vitellogenin (*vg gene expression*). Vitellogenin is a yolk precursor protein that in most species is expressed in connection to egg formation or reproduction (*5*). The honey bee worker is a functionally sterile female that utilizes vitellogenin in novel ways and the protein is thought to have pleiotrophic functions (*12*). It has previously been reported that nurse bees are high in vitellogenin and that foragers are low in vitellogenin levels, the expression profiles of this study indicate the same. In addition, it has been shown by RNA interference studies (RNAi knockdowns) that depletion of vitellogenin from young bees leads to precocious foraging, supporting an idea of vitellogenin as important for the decision of phenotype transition (*25*).

Another group of known storage proteins for insects, the hexamerins (*26*), was also significantly upregulated in nurse bees compared to foragers. Hexamerins are involved in the dynamics of amino acid storage/exploitation in metamorphosis or development from larva to the adult stage, and may also function as juvenile hormone binding proteins (*27*). It has recently been documented by Martins et al. 2010, that adult worker bees express high levels of *Hex70a* and *Hex110* (*27*). They hypothesise that *Hex70a* is produced and stored in excess in nurse bees to later sustain basal metabolism during the forager phase. The same pattern could not be shown for *Hex110.* High expression levels did not result in high blood levels of the protein. Our results show that nurse bees have high expression levels of *Hex70b, 70c and Hex110*. We were not able to find a significant difference in *Hex70a*, in fact the gene was not part of the blast result for all 8640 contigs. The lack of *Hex70a* in our results is quite surprising. Likewise the significant higher expression of *Hex70b and Hex70c* in nurse bees is surprising, as these genes, to our knowledge, have not been reported expressed in adult bees. However, the *hex* genes are reported to have a motif for a *ultraspiracle* binding site (*27*) which is a nuclear receptor for juvenile hormone (*28*) and could possibly be involved in the physical changes preceding the transition to a foraging phase.

The elevated expression of a high number of genes involved in sugar metabolism in foragers is as expected. Foragers show the highest mass specific metabolic rate of flying insects and are dependent of a high turnover of sugar for energy production (*16*). However, more interestingly there does not seem to be a difference in protein and lipid metabolic activity. This result is interesting seeing that lipolytic activity has previously been reported to be high in nurses and low in foragers (*29*). Nurse bees are the colony's main lipid providers (*30*) and foragers are frequently fed proteins by nurse bees since they can't obtain amino acids on their own due to low levels of digestive endopeptidases (*16*). The seemingly equal metabolic activity level is probably due to the coarse division of the gene expression results into functional groups. By further fine division of the annotations it is clear that the real difference in activity levels are masked. The nurse bees express genes that are predominantly involved in fatty acid metabolism (i. e. juvenile hormone esterase, similar to CG12262-PA and similar to CG9547-PA), whereas foragers have high expression of catabolic genes like glycerophospholipids and sphingolipids; mainly components of cell membranes and mediators of signal transduction.

The functional category "energy production/convertion and secondary metabolites" has more genes upregulated in nurse bees. This is in contradiction to the results from a previous study done on protein level showing increased levels of proteins involved in energy production and metabolic signalling in foragers (*31*). The colliding results may be due to differences in the way functional groups were classified. However, Ament et al. (2008) describes the switch from nurse to forager as being associated with a drop in energy metabolism in honey bee brain (*32*). Ament et al. (2008) also hypothesise that high metabolism is a requirement for brain plasticity in nurses, and that this plasticity does not necessarily apply to other tissues. Our results suggest that it may in fact apply to other tissues. If this is the case a possible explanation could be the nurse bees having high metabolism in the fat body because of elevated activity to convert and produce metabolites for production of royal and worker jelly and to fill up own energy storage.

Down regulation of the expression and accumulation of storage proteins as a consequence of activation of the immune system is thought to be a strategy to redirect resources to combat injury or infection (*33*). We found that while storage proteins are missing, the immune response related genes *defensin*, *hymenoptaecin* and *abaecin* are highly expressed in foragers. Workers involved in foraging duties are more exposed to pathogens and toxins as they engage in numerous foraging trips outside the protected nest; flowers have rich faunas of bacteria and

fungi, and some pollens and nectars are poisonous to bees (*34, 35*). The gene *defensin* is shown to encode both royalisin, found in royal jelly and defensin found in the haemolymph of bacterially infected bees (*36*). The release of antimicrobial effectors is triggered by activation of one of the immune signalling cascades Toll or immune deficiency (IMD) pathway. Reducing the transcription factor Relish, part of the IMD pathway, has been shown to affect levels of abaecin and hymenoptaecin (*37*). In further analysis of the complete data set it would be interesting to see if Relish and genes involved in these pathways show elevated expression as well.

The lysosome genes are also highly upregulated in the forager phenotype, they are possibly involved in the breakdown of dead invading microbes or may be involved in degradation of old or unwanted components in the bee itself (*38*). Thus, this group of genes could either be an addition to the defence system of xenobiotic components, or an indication of the process of decay in the senescing worker bee (*4*) and may be interesting for further studies of the senescence process.

We found that foragers had elevated expression of kruppel-like protein 1 (*Kr-h1)*. The association of Kr-h1 with the transition to foraging is well established (*39, 40*), but its specific role remains unclear (*39*). In *Drosophila melongaster* it is a mediator of Juvenile hormone (JH) action (*40*), and even though JH treatment has been associated with upregulation of *Kr-h1* levels in bumble bees (*41*), studies on bees treated with the JH mimic methopren did not prove *Kruppel* to be directly linked to JH in honey bees (*39*). If a hive is emptied of it's nurses, some foragers will go back to nursing, regaining much of the physical characteristics of a nurse in a process called reversion, which is a much used way to uncouple social status and age in honey bee research (*42, 43*). *Kr-h1* levels have been proven unaffected by reversion, leading to the suspicion that it is associated with permanent physiological changes in the bees body (*43*). It would be interesting to do a study on *Kr-h1* expression in young bees treated with methopren before they made the transition to forager.

To maintain a functioning hive, communication between its inhabitants is essential and this is done for the larger part through odor/pheromone signaling (*15*). Both groups express genes involved in communication such as odorant binding and chemosensory proteins, but nurse bees have the highest number of genes upregulated compared to foragers. Both odorant binding and chemosensory proteins might be of particular significance for social insects that live in large colonies with no central control helping them to distribute numerous tasks among

thousands of individuals (*44, 45*). The nurse bees can be seen as a connection point of all communication in the hive. They feed and groom the colony and are therefore in close contact with the queen, developing larvae and foragers, able to spread pheromone signals to all members of the society. Cell communication, cytoskeleton, signal peptides and factors involved in biological regulation are higher in foragers. These findings are only represented by one or two proteins in each category, but are coherent with a previous study on age-associated changes in gene expression in honey bee brains (*46*). The high expression level of genes involved in these functional groups could be due to the foragers demanding foraging flights. One of them, *similar to Myosin regulatory light chain 2*, is responsible for producing muscle contractions (*47*), and the high expression of cytoskeleton genes could be related to rebuilding of cells damaged from the strain of foraging. Two genes encoding transcription related factors are higher in nurses than in foragers, both of them are reported as repressors of transcription, suggesting that repression of some or many genes may be associated with age or foraging state.

In general it can be said the genes necessary and sufficient for complex social behaviors are largely a mystery. The publication of the complete *Apis mellifera* genome sequence (Apimel4; The Honey bee Genome Sequencing Consortium, 2006), can be of great help in identifying and characterizing the underlying modulators of the behavioral repertoire displayed by the honey bee. Building up transcriptomal profiles for the different polyphenisms of the worker bees can be of great help in understanding the mechanisms behind honey bee behavior and plasticity of aging. This thesis has focused on describing some of the main differences in gene expression between two main behavioral groups/phenotypes of the worker honey bee. It has unfortunately not been possible to delve more deeply into the material and what is presented is merely a scratch on the surface. Despite the shallowness of the research, I believe that the results presented are interesting and showcase the need for a solid baseline to further the understanding of the complexity of the bee worker phenotype regulation process.

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Attachment 1

complete list of differentially expressed genes

contig no	Gene Name (David)	Entrez gene ID
15741	hypothetical LOC552685	552685
9992	similar to CG12045-PA	413115
22058	odorant binding protein 14	677673
47621	hypothetical protein LOC727165	727165
88346	similar to CG4409-PA	727522
37591	similar to CG14661-PA	552773
5317	similar to pericardin CG5700-PB	724749
38838	similar to CG32209-PB	408365
469	similar to CG33257-PA	412520
19106	similar to CG8927-PA, isoform A	552217
45577	similar to CG13676-PA	552724
9271	hexamerin 110	551648
16035	similar to Gasp CG10287-PA	725932
14396	similar to CG3777-PB, isoform B	408970
10110	similar to LDLa domain containing chitin binding protein 1 CG8756-PD, isoform D	551323
19024	larval-specific very high density lipoprotein	726182
45941	hypothetical protein LOC727136	727136
1506	odorant binding protein 13	677674
1239	hypothetical protein LOC724210	724210
1086	vitellogenin	406088
13016	similar to CG8927-PA, isoform A	413113
23765	similar to pawn CG11101-PA	409598
23418	similar to Activin Like Protein at 23B CG16987-PA, isoform A	726478
12008	similar to CG15884-PA	725509
7654	similar to UDP-glycosyltransferase 35b CG6649-PA	725017
4134	similar to CG32499-PA	725813
22796	similar to CG3609-PA	552024
31453	hypothetical LOC552190	552190
1084	similar to CG4778-PA	408726
8959	similar to dihydroxyacetone kinase 2	413697
3068	similar to spermidine synthase	725099
31805	similar to knickkopf CG6217-PA	410606
3920	similar to CG5326-PA, isoform A	724552
8964	similar to Location Of Vulva defective family member (lov-1)	725164
2597	similar to CG17052-PA	724382
1552	troponin C type IIb	494501
6139	similar to Peritrophin A CG17058-PA, isoform A	410065
11346	similar to CG8646-PA	411657
77609	similar to CG32972-PB, isoform B	412222

14034	similar to Cytochrome P450 4c3 (CYPIVC3)	552679
399	similar to leucine rich repeat containing 40	724187
31105	similar to CG9134-PB, isoform B	410293
1306	similar to CG8630-PA	724226
12051	similar to CG15269-PA	725520
30410	similar to CG32250-PA	551241
3498	odorant binding protein 17	552478
20768	similar to CG9796-PA	552600
1523	similar to Flavin-containing monooxygenase 1 CG3006-PA	410687
6560	similar to CG12492-PA	410021
13380	similar to CG7365-PA	725668
23742	similar to CG6180-PA	726495
12344	similar to CG6131-PA	725547
4415	prophenoloxidase	406155
8604	similar to Cad74A CG6445-PA, isoform A	410368
2881	yellow	408273
30870	similar to BE10.2	413590
17311	similar to Cadherin-related tumor suppressor precursor (Protein fat)	410009
8614	similar to CG6216-PA, isoform A	550958
12320	similar to CG13607-PA	551991
17421	similar to Organic cation transporter CG6331-PA	412399
9306	similar to CG11236-PA	551507
12299	hypothetical protein LOC727567	727567
2035	similar to 15-hydroxyprostaglandin dehydrogenase	551423
7318	similar to Ugt86Dd CG6633-PA	414050
22086	similar to CG4797-PB, isoform B	726395
2864	similar to Drop CG1897-PA	724412
642	similar to desat1 CG5887-PA, isoform A	552417
7244	similar to CG3812-PA, isoform A	724951
14314	similar to CG1213-PA, isoform A	412797
42553	similar to CG8561-PA	410555
898	similar to CG1887-PA	413408
21413	similar to CG5873-PA	412013
10989	similar to CG32645-PB	410976
3975	similar to CG15080-PA	724569
26144	similar to 5-hydroxytryptamine 1A receptor (5-HT-1A) (Serotonin receptor 1A) (5-HT1A)	727590
4598	similar to Punch CG9441-PB, isoform B	409582
45605	hypothetical protein LOC727131	727131
10586	similar to CG15279-PA, isoform A	725346
38166	similar to CG10026-PA, isoform A	552408
11407	similar to ade5 CG3989-PA	551966
3277	similar to CG8412-PA	727115
11623	similar to CG9864-PA	725462
9337	hypothetical protein LOC725217	725217

20786	similar to F55A4.8a	552783
12645	similar to CG1869-PA	413481
16118	similar to GST-containing FLYWCH zinc-finger protein CG33546-PC, isoform C	725942
3265	similar to Cytochrome P450 315a1, mitochondrial precursor (CYPCCCXVA1) (Shadow protein)	411893
23825	hypothetical protein LOC726502	726502
59794	similar to CG7365-PA	552829
4256	similar to CG31522-PA, isoform A	550828
51042	similar to CG17292-PA, isoform A	727193
370	similar to Myosin regulatory light chain 2 (MLC-2)	409881
32240	hexamerin 70c	409354
371	similar to CG31997-PA	411622
5071	troponin C type I	411659
11348	similar to Actin-87E	551176
32479	similar to CG2837-PB, isoform B	726864
7905	similar to CG3690-PA	410705
2454	similar to Sorbitol dehydrogenase-2 CG4649-PA	408871
1753	similar to failed axon connections CG4609-PA, isoform A	409643
12030	similar to CG40109-PA.3	412279
7344	similar to CG7802-PA, isoform A	413256
3306	similar to CG9918-PD	551571
6457	juvenile hormone esterase	406066
15074	similar to CG5867-PA	409602
11702	similar to CG10936-PA, isoform A	552048
45269		
3762	similar to mitochondrial ribosomal protein L19 CG8039-PA	552593
15748	hypothetical protein LOC725903	725903
1059	arginine kinase	550932
18722	similar to Neurogenic locus Notch protein precursor	551873
29088	similar to dusky-like CG15013-PA, isoform A	408277
6924	laccase 2	410365
71227	similar to BE10.2	727403
20757	troponin T	411184
2592	similar to dumpy CG33196-PB	724381
8365	similar to CG30420-PA, isoform A	552002
52729	similar to retinol dehydrogenase 11	552758
2249	similar to CG8646-PA	552073
8348	similar to CG13643-PA	411273
7580	similar to CG3812-PA, isoform A	724995
26177	similar to CG32036-PB	726623
8935	troponin C type IIa	494503
2977	similar to CG14301-PA	411343
15655	similar to CG9547-PA	410254
10592	similar to Histidine decarboxylase CG3454-PA	408587

112876	similar to CG1136-PA	727578
3823	similar to ATP citrate lyase CG8322-PA, isoform A	550686
13609	similar to CG15786-PA	725682
4369	similar to CG12262-PA	408567
47709	hypothetical protein LOC727170	727170
25080	similar to CG6879-PA	410515
2270	similar to CG33515-PA	412858
59133	similar to Probable cytochrome P450 303a1 (CYPCCCIIIA1)	727290
12622	hypothetical protein LOC725578	725578
22392	similar to CG4090-PA	410509
2504	hexamerin 70b	406117
14372	similar to Synaptic vesicle membrane protein VAT-1 homolog	409207
843	similar to CG9514-PA	410734
7782	similar to CG6180-PA	408516
456	similar to Karl CG4139-PA, isoform A	409025
3818	similar to CG1124-PA	412768
8999	similar to CG32405-PA	412202
1803	hypothetical protein LOC724583	724583
6694	similar to Deoxyribonuclease II CG7780-PA	413489
11615	similar to CG31973-PA, isoform A	552276
2741	chymotrypsin inhibitor	725380
13640	similar to CG31937-PA	411863
1071	fatty acid binding protein	408689
117240	similar to CG10672-PA	552390
28496	similar to CG12075-PA, isoform A	552005
11646	hypothetical LOC552770	552770
1795	chemosensory protein 1	725382
5597	similar to SP1070 CG9138-PA	412825
25497	similar to Probable cytochrome P450 305a1 (CYPCCCVA1)	551632
8284	similar to CG6969-PA	412774
14000	similar to CG17360-PA	413802
34573	similar to krotzkopf verkehrt CG2666-PA, isoform A	411645
14052	similar to CG7365-PA	551805
12057	hypothetical protein LOC725522	725522
38623	similar to CG31684-PA	413723
14313	similar to CG2989-PA	409759
2256	similar to dumpy CG33196-PB	724342
32230	similar to Farnesyl pyrophosphate synthase CG12389-PA	726859
2768	similar to N-acetylneuraminate pyruvate lyase	725646
11700	similar to CG10050-PA	411215
18366	similar to CG9372-PA	726126
22421	similar to CG13889-PA	726420
11743	similar to Phosphoribosylamidotransferase CG2867-PA	412619
1664	similar to ion transport peptide CG13586-PA	724270

51739	similar to CG3244-PA	552154
4807	similar to Putative aldehyde dehydrogenase family 7 member A1 homolog (ALH-9)	411140
13050	similar to Scavenger receptor class C, type I CG4099-PA	411253
1515	similar to CG9518-PA	410733
2069	worker-enriched antennal transcript	406065
791	similar to CG15117-PA, isoform A	409814
36846	similar to miniature CG9369-PA	408508
2928	similar to dumpy CG33196-PB	724426
26430	hypothetical protein LOC726632	726632
14395	similar to N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase precursor (Glycosylasparaginase) (Aspartylglucosaminidase) (N4-(N-acetyl-beta-glucosaminyl)-L-asparagine amidase) (AGA)	411392
775	similar to beat-VII CG14249-PA, isoform A	551098
8276	similar to Cytochrome P450 6a22 (CYPVIA22)	725087
18027	similar to lymphocyte cytosolic protein 2	551213
15740	similar to b6 CG3100-PA	725902
1982	similar to peptidylprolyl isomerase (cyclophilin)-like 6	724323
14665	hypothetical protein LOC725784	725784
43660	similar to CG1544-PA, isoform A	412569
6703	similar to CG5278-PA	551938
19342	similar to Paired box pox-meso protein (Paired box mesodermal protein)	726200
1806	odorant binding protein 3	677671
39149	similar to CG14439-PA	412925
3985	similar to CG11089-PA	412467
20361	similar to CG7120-PA	408329
17692	similar to Osiris 14 CG1155-PA	408538
77928	similar to Cytochrome P450 4c3 (CYPIVC3)	413833
39529	similar to Aminomethyltransferase, mitochondrial precursor (Glycine cleavage system T protein) (GCVT)	410550
59118	similar to krotzkopf verkehrt CG2666-PA, isoform A	727291
757	antennal-specific protein 3c	406094
24026	similar to Tenascin major CG5723-PB	410739
14291	similar to PTK7 protein tyrosine kinase 7 isoform a precursor	410685
1611	similar to CG31559-PA	411159
2884	similar to CG3011-PA	411796
18338	similar to CG10632-PA	726122
3137	similar to beta-ureidopropionase	409250
22345	similar to CG6767-PB, isoform B	551448
5624	hypothetical LOC408807	408807
5624	similar to slit homolog 1 [Apis mellifera]	
1525	similar to Kynurenine/alpha-aminoadipate aminotransferase mitochondrial precursor	724239
2314	similar to Dynein heavy chain at 93AB CG3723-PA	411894
19059	similar to CG30437-PA, isoform A	552811

29177	similar to CG17739-PA	413185
6343	similar to Homogentisate 1,2-dioxygenase (Homogentisicase) (Homogentisate oxygenase) (Homogentisic acid oxidase)	551465
4628	similar to CG5157-PA	724654
1681	similar to CG2663-PB, isoform B	551761
7575	hypothetical protein LOC724993	724993
3848	hymenoptaecin	406142
434	defensin 1	406143
15784	similar to homogentisate 1,2-dioxygenase CG4779-PA	725911
31885	homogentisate 1,2-dioxygenase	726845
3977	similar to CG32263-PA	724570
19014	similar to Sox21b CG32139-PA	727672
404	hypothetical protein LOC724275	724275
21395	similar to CG32791-PA	410888
46333	kruppel-like protein 1	406069
16633	kekkon-1	552187
8946	peptidoglycan recognition protein S1	725158
27884	glycerol-3-phosphate dehydrogenase	726445
8948	cytochrome P450 6AS10	725159
1352	tyramine receptor	406110
9298	similar to CG1461-PA	551404
7282	similar to Aromatic-L-amino-acid decarboxylase (AADC) (DOPA decarboxylase) (DDC)	410639
21446	similar to CG12164-PA	551089
3250	similar to branchless CG4608-PA	724469
16311	kelch-like 10 (Drosophila)	411968
10994	similar to CG11796-PA, isoform A	725400
6556	similar to CG10960-PB, isoform B	727668
2894	similar to CG9895-PA	552397
27548	similar to Probable cytochrome P450 6a13 (CYPVIA13)	727598
25392	hypothetical LOC551496	551496
6349	cytochrome P450 6BE1	552418
16333	similar to TD and POZ domain containing 5	550795
18650	GMC oxidoreductase 3	410747
3238	similar to Enhancer of split mbeta protein (E(spl)mbeta) (HLH-mbeta) (Split locus enhancer protein mA)	724465
12640	cyclin-dependent kinase 4	408406
2291	hypothetical protein LOC724354	724354
26064	hypothetical protein LOC726611	726611
20380	similar to Dipeptidase B CG9285-PA, isoform A	410627
34901	serine protease homolog 50	726934
27869	similar to SET domain and mariner transposase fusion	726705
31203	similar to Beta-hexosaminidase beta chain precursor (N-acetyl-beta-glucosaminidase) (Beta-N-acetylhexosaminidase) (Hexosaminidas	726818
2268	similar to CG4406-PA	411058

560	similar to CG13759-PA	724126
444	tyrosine hydroxylase	408930
621	similar to CG18678-PA	413568
1103	alpha glucosidase 2	409889
11741	similar to Na-Ca exchanger 5	411781
27174	cuticular protein 5	552350
34908	cardioacceleratory peptide receptor	726935
20752	similar to CG40500-PA.3	726309
18359	similar to Carboxypeptidase B precursor (Pancreas-specific protein) (PASP)	551327
44346	similar to CG8412-PA	727115
21713	similar to CG7142-PA	726372
5625	similar to CG10513-PA	724781
36822	venom dipeptidylpeptidase IV	410337
26400	hypothetical LOC408280	408280
22355	similar to CG10960-PB, isoform B	413576
31801	similar to CG4797-PB, isoform B	726839
23103	chitinase	413705
23779	similar to Caspase precursor (drICE)	411381
2481	chemosensory protein 6	725103
20108	similar to CG31871-PA	411353
92060	chitinase	727535
17017	similar to CG8083-PA, isoform A	410532
2629	similar to CG14516-PA, isoform A	551224
20716	similar to CG7720-PB, isoform B	410626
11969	similar to protein tyrosine phosphatase domain containing 1 protein isoform 2	410442
5883	similar to Na pump subunit CG5670-PH, isoform H	413471
26518	malvolio	494509
130008	similar to CG9452-PA	727614
2607	similar to radial spokehead-like 3	551802
24048	similar to Organic anion transporting polypeptide 33Ea CG5427-PA	409649
3049	abaecin	406144
10007	cuticular protein 13	725300
26867	similar to CG8389-PA, isoform A	552202
3585	similar to myosin, heavy polypeptide 9, non-muscle isoform 1	551252
36919	similar to CG14661-PA	726981
2278	serine protease 22	413645
8657	kinesin 3E	552512
19043	serine protease 36	410894
18986	similar to Glucose dehydrogenase	726177
20147	similar to Adenylyl cyclase 78C CG10564-PA	726262
6565	similar to CG11093-PA	410761
7264	similar to CG14446-PA	724960
10294	maternal protein exuperantia	551582
3487	ATP citrate lyase	550686

4333	similar to Multiple inositol polyphosphate phosphatase 1 CG4123-PA, isoform A	724619
15032	beta-1,3-glucan recognition protein 2	725832
23844	similar to CG17633-PA	551524
9322	glucuronosyltransferase	408788
1188	similar to faint sausage CG17716-PA	724195
8918	serine protease 14	725154
379	alpha-amylase	406114
11731	serine protease 35	552301
20085	similar to CG31666-PA, isoform A	551086
9630	similar to CG31954-PA	725250
8620	similar to CG13842-PA	409603
20087	serine protease 40	409626
2576	similar to CG17119-PA, isoform A	411429
21407	sulfonylurea receptor	410944
1052	cytochrome P450 6AS17	551626
6553	dihydrolipamide dehydrogenase 2	412340
21377	serine protease 17	726352
11269	similar to CG4025-PA	551941
11941	similar to CG10019-PA	408646
5336	similar to CG5155-PA	412162
5279	similar to CG7888-PB, isoform B	552193
25450	similar to C25A1.5	726585
13733	similar to F18A12.8a	411846
2293	similar to CG14516-PA, isoform A	551180
13344	Cdk5 activator-like protein	411494
28769	similar to CG13424-PA	726730
18352	similar to SoxNeuro CG18024-PA	408411
26415	similar to pyridoxine 5-phosphate oxidase	551533
42581	POU domain, class 2, transcription factor 1	727085
10733	similar to solute carrier family 24, member 5	412316
30867	hexosaminidase 2	409014
3709	similar to CG10830-PA	725415
2725	similar to CG3630-PA	410370
2608	hypothetical protein LOC724386	724386
3642	neutral ceramidase	409628
15039	hypothetical protein LOC725833	725833
13991	cytochrome P450 9Q1	410492
13635	similar to CG30269-PA	725688
7631	phosphoenolpyruvate carboxykinase	412843
48039	similar to mitochondrial ribosomal protein L50 CG8612-PA	727172
17093	similar to Niemann-Pick Type C-1 CG5722-PA	408992
20369	similar to Peptidyl-prolyl cis-trans isomerase, rhodopsin-specific isozyme precursor (PPIase) (Rotamase)	408428
20793	similar to CG15533-PA	726315

64820	similar to CG4409-PA	727344
16968	developmental protein eyes absent	412528
38959	similar to CG7367-PA	727032
1782	glucocerebrosidase	409709
6171	hypothetical protein LOC726629	726629
9125	similar to homeobox only domain	726978
5089	similar to CG16771-PA	410530
1192	similar to CG10154-PA	724199
19656	similar to Vesicular glutamate transporter CG9887-PA	410752
23376	organic anion transporting polypeptide 33Eb ortholog	726470
3195	similar to N-acetylgalactosamine kinase (GalNAc kinase) (Galactokinase 2)	411484
2378	similar to CG5630-PA	725773
17697	similar to lethal (3) 87Df CG7620-PA	726070
438	apisimin	406093
27516	thrombospondin	413782
9917	similar to zinc finger protein 617	725279
10025	hypothetical LOC551717	551717
3650	similar to no mechanoreceptor potential A CG13207-PB, isoform B	724615
19022	similar to popeye protein 3	410381
23363	similar to serine/threonine kinase 32B	413577
47679	similar to CG15531-PA	727166
959	similar to Suppressor of cytokine signaling 2 (SOCS-2) (Cytokine-inducible SH2 protein 2) (CIS-2) (STAT-induced STAT inhibitor 2	411290
28765	tolkin	410386
2638	cAMP-specific 3',5'-cyclic phosphodiesterase	411288
3917	serine protease 44	408534
44261	similar to CG40263-PA.3	411052
39295	similar to CG17292-PA, isoform A	727037
2120	sarcoplasmic calcium-binding protein 2	410013
19687	similar to CG16708-PA, isoform A	408315
25181	similar to CG9451-PA	726571
29187	hypothetical protein LOC726750	726750
13959	hypothetical protein LOC725725	725725
68193	heparan sulfate N-deacetylase/ N-sulfotransferase	413242
6296	similar to CG11071-PA	724854
3060	odorant binding protein 12	677678
16664	similar to CG9380-PB, isoform B	725984
10339	similar to CG33515-PA	408857
18333	cAMP-dependent protein kinase 3	410228
23753	similar to Bestrophin 1 CG6264-PA	411764
23471	similar to CG9699-PA, isoform A	412647
2627	similar to CG6330-PA, isoform A	411599