

SPATIOTEMPORAL MEMORIES IN HONEY BEES: MICROARRAY ANALYSIS OF TIME-
TRAINED FORAGERS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Entomology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2010

Urbana, Illinois

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ABSTRACT

Honey bees feed on flowers, many of which produce nectar during certain restricted, but consistent, time windows in the day. Previous research has shown that honey bees have the ability to form distinct spatiotemporal memories that allow them to return repeatedly to different attractive food sources at different times of day. As a first step toward understanding the molecular basis of this striking flower fidelity, this study was designed to determine whether distinct spatiotemporal memories of a food source are associated with distinct patterns of gene expression in the brain. Different groups of foragers from the same colony were trained to collect sucrose solution from an artificial feeder at one of two different times of day, one in the morning and the other in the afternoon. Bees from both training groups were collected at both training times, thus yielding a set of bees that was undergoing stereotypical food anticipatory behavior and another set that was inactive for each collection. Microarray analysis revealed that hundreds of genes were differentially expressed in the brain based on either time of day or activity state. A subset of these genes showed unique patterns of expression for each of the spatiotemporal foraging memories. Surprisingly, some of the genes involved in the regulation of circadian rhythms, such as *period* and *cryptochrome*, appear to change in expression with activity state rather than time of day. Overall, these results indicate that distinct spatiotemporal foraging memories in honey bees are associated with distinct neurogenomic states involving genes that regulate temporal activity and genes associated with appetitive behavior.

ACKNOWLEDGMENTS

I gratefully thank the many people who helped make this project a reality. I especially thank my adviser Dr. Gene Robinson and collaborators Byron Van Nest, Jennifer N. Johnson, Sam D. Boyd, Dr. Sandra Rodriguez-Zas, and Dr. Darrell Moore. I also thank H. Trang Vu, Dr. Thomas Newman, and Karen Pruiett for their technical support. Finally, and most of all, I thank my parents who have always been there for me.

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INTRODUCTION

Honey bees (*Apis mellifera*) have the remarkable ability to form and utilize spatiotemporal memories in order to direct their foraging behavior. Like virtually all of the approximately 16,000 species of bees found worldwide, honey bees rely exclusively on flowers as their sole source of nutrition (Michener, 2000). As part of their long and intricate coevolution with flowering plants, bees have evolved many behaviors that increase their foraging efficiency. Because different species of flowering plants bloom at different times of day (Linnaeus, 1751), nectar and pollen are available for pollinators only during discrete yet consistent windows of time (Gimenes et al., 1993; Doorn and Meeteren, 2003; Matile, 2006). Honey bees, accordingly, have evolved the ability to alter their foraging behavior in a circadian manner based on experience of when food has been available on previous days (Beling, 1929; Wahl, 1932). In other words, bees can learn not only where to fly to collect food, but also when to do so.

Studies in the early twentieth century demonstrated that honey bee foraging rhythmicity is entrained by the timing of food rewards (Buttel-Reepen, 1900; Forel, 1910). Beling (1929) was able to train bees to show up to a feeder at multiple distinct windows of time by limiting the food availability to only those times on previous days. The resulting temporal foraging pattern persisted even when the feeder was left empty on subsequent days, indicating that bees were learning and anticipating a feeding event. Additionally, Beling (1929) showed by using an indoor flight chamber that this circadian food anticipatory behavior could occur under constant lighting conditions and was likely the result of an endogenous time-keeping mechanism. Renner (1957) demonstrated conclusively that bees have an internal clock with his classic transatlantic transplantation

experiments. He trained bees in an indoor flight room in Paris and then flew the bees to an identical flight room in New York where they were kept under constant conditions. The bees exhibited foraging patterns based on Parisian time, thus revealing that they orchestrate their rhythmicity based on an internal time sense and not a response to local cues.

Honey bees, like most animals, have an internal clock composed of genes situated in transcriptional feedback loops that regulate their own expression, other clock genes, and other downstream genes (Ruben et al., 2006). These feedback loops, composed of both positively and negatively regulating elements, create oscillations in the level of gene expression which are necessary for circadian behavioral rhythms. In most animals specific nuclei in the brain function as “master” clocks which help to coordinate gene expression rhythms across multiple tissues both within the brain and outside of it (reviewed Dibner et al., 2010). These master clocks (lateral neurons in *Drosophila melanogaster*, Park and Griffith, 2006; suprachiasmatic nucleus in mammals, Welsh et al., 1995) are typically entrained to light/dark cycles and alter their ability to fire action potentials and their spontaneous firing rate in a circadian manner, even under introduced constant environmental conditions. The increased firing of action potentials in such master clocks coincides with the onset of behavioral activity and regulates sleep/wake cycles.

Although much is known about the molecular basis of basic clock function, much less is known about the molecular mechanisms underlying activity rhythms associated with feeding. Like bees, mice and other animals can be trained to forage at specific times of day resulting in rhythmic anticipatory appetitive behavior (reviewed Mistleberger,

2009). Although progress in understanding the neural and molecular basis of this behavior has been slow (Mistleberger, 2009), evidence is mounting that clocks in tissues outside of the master clock nuclei, including areas of the brain (Angeles-Castellanos et al., 2007) and the liver (Vollmers et al., 2009), are necessary for circadian food anticipatory activity. The molecular basis of food anticipatory states has yet to be elucidated in insects, perhaps due to the inability of the primary genetic model species, *Drosophila melanogaster*, to anticipate circadian food availability (Oishi et al., 2004),

We hypothesized that distinct spatiotemporal memories of a food source are associated with distinct patterns of brain gene expression. Transcriptional profiles in honey bee brains have been shown to be strongly correlated with certain behaviors such as nursing (brood care) and foraging (Whitfield et al., 2003, Alaux et al., 2009b). The duration of each of these behaviors is each a week or longer, and comparison between the brains of nurses and foragers revealed large gene expression differences (~20-40% of transcripts tested in Alaux et al., 2009b and Whitfield et al., 2003, respectively), indicating that different behaviors can be associated with different brain neurogenomic states. Other behaviors that are more transient in duration such as guarding the hive, comb building, and undertaking (the removal of dead bees from the colony) each last for only a day or two and are associated with few or no differences in neurogenomic state (Cash et al., 2005). Given the persistence of honey bee foraging rhythms over periods of days (Beling, 1929) as well as the known influences of gene expression on other forms of rhythmic behavior in other animals, we hypothesized that transcriptional differences exist between bees trained to foraging in different spatiotemporal patterns. If so, this

information could then contribute to an understanding of the molecular basis of food anticipatory states.

METHODS

We trained different bees from the same hive to forage at different times of day, at different locations associated with different scents. Our experimental design enabled us to search separately for gene expression patterns associated with differences in activity state, time of day, or in association with specific spatiotemporal memories.

Bees, Training and Collection:

Two unrelated, typical colonies of European honey bees were each placed into an eight-frame, clear plastic-walled observation hive (97cm x 96cm). Each colony was headed by a naturally mated queen and composed of ~8,000 workers at the start of the study. The hives were kept at the former Marine Corps Armory site, warrant of East Tennessee State University, in Johnson City, TN. The hives were enclosed within a small wooden shed to allow for observations without exposing the colony to direct sunlight, and the bees were allowed to adjust to the new environment for at least one week before training commenced. The sides of the hive were constructed out of 16 clear plastic windows that could be opened to allow for the collection of bees with minimal disturbance to the hive. Two replicates of the experiment were performed, one for each colony, from Aug 14-19 and Aug 21-28, 2007. Over the duration of the experiment, a moderate amount of several different species of wildflowers were in bloom at the study site.

The field component of this study consisted of three phases: orientation, training, and collection. The orientation phase lasted one or two days, during which the bees learned the location of the artificial feeder. Filter paper soaked with sucrose solution was

used to lure bees out of the hive and to transfer them from the hive entrance to a feeder located a meter away. The feeder consisted of a 96-well tissue culture plate placed on a piece of scented filter paper, situated on top of a short (40cm) table. The wells contained 2M sucrose solution. Once a steady number of bees (about 5/min) were observed flying to the feeder on their own, the filter paper transfer was stopped, and the feeder was moved several meters away from the hive. When the foragers again found the feeder at a steady rate, the feeder was moved again several more meters away. This was repeated until the feeder reached the desired training location, ~120m away from the hive. For each replicate training was performed at two different times of day, once in the morning from 0900-1015 and again in the afternoon from 1700-1815. At the end of each training period, the feeder was emptied and washed, and the filter paper was replaced with a new unscented sheet. Different locations and different scents were used for each of the two training times, morning and afternoon. Essential oil extracts of lilac and lavender flowers were used for scents in the morning and afternoon respectively.

The training phase consisted of adding scent and sucrose solution to the feeder during the training time, and marking foragers that were recruited to the training location by the trained bees from the orientation phase. These new recruits were marked with an individual paint mark code, as in von Frisch (1967). Their time of arrival was recorded for each foraging trip. Although most bees displayed fidelity to a single training time, some did not, and any bee bearing the paint code indicating that she had obtained a food reward at the other training time was culled to prevent cross-recruitment based on residual smells. Training continued for 4-7 days until there were a sufficient number ($N \approx 40$) of trained bees at both training times.

On collection day, the feeder was left empty and unscented to insure that any gene expression differences were a result of memory and not recent experience with the food source. All bees collected for molecular analysis were sampled from the hive, 15-45 min before the onset of the training time. The bees were collected directly off combs to help prevent confounding effects associated with the energy expenditure from flying to the feeder. Focal bees were located and identified by their paint code, the windows of the observation hive were flipped up, and soft forceps were used to collect the bee directly into liquid nitrogen to flash-freeze the transcriptional profile. Both “anticipating bees” (bees whose training time was soon approaching) and “inactive bees” (bees trained to the other time) were collected simultaneously out of the hive at both times, resulting in four experimental groups (Fig. 1). Anticipating bees were generally found near the entrance of the hive, as described by von Frisch (1967). These bees monitor returning foragers for scents or dances that may indicate that the food source to which they are trained has already become available. If left undisturbed, these bees would have eventually flown to the feeding location even without provocation by other cues (von Frisch, 1967). Inactive bees were typically found farther away from the entrance in areas associated with honey storage. These bees were not observed performing other behaviors and appeared to be in a sleep-like state, as described by previous investigators (von Frisch, 1967; Kaiser and Steiner-Kaiser, 1983). The four behavioral groups analyzed in this study were: Morning-Trained, Morning-Collected (tAM-cAM); Morning-Trained, Afternoon-Collected (tAM-cPM); Afternoon-Trained, Afternoon-Collected (tPM-cPM); and Afternoon-Trained, Morning-Collected (tPM-cAM).

Bees representing these four groups were collected for molecular analysis if they received at least three days of training. Furthermore, each bee included in the study (N=76) flew to the feeder and received a food reward at least 2, 6, and 6 times on the last three days of training, respectively. This criterion identifies the most highly trained and motivated bees that would have flown to the feeder at the correct time had they not been collected. Experiments performed on the same study site in previous years (combined data from Moore and Doherty, 2009 and Van Nest et al., unpublished) suggests that >90% of bees with this level of training will fly to the empty, unscented feeder at the appropriate time on the following day. Of the 76 bees collected according to this criterion, we selected the 12 bees from each of the four experimental groups (6 from each replicate, 2 replicates) with the most visits to the feeder during the training phase for the microarray analysis (N=48). Table 1 shows the distribution of visits by bees during the training phase and which bees were selected for microarray analysis.

Microarray analysis:

The heads of the bees were freeze-dried (-80°C and 300mTorr) and the brains dissected out while submerged in dry ice/ethanol (as in Schulz and Robinson, 1999). The brains were homogenized and nucleic acids extracted using phenol/chloroform and RNeasy (Qiagen, Valencia, California) kits. While bound to the columns, the samples were treated with DNase (also Qiagen). From the resulting RNA, 500 ng were amplified using MessageAmpII (Ambion / Applied Biosystems, Austin, Texas) kits. Amplified RNA was labeled with Cy3 or Cy5 dyes using Kreatech (Amsterdam, The Netherlands) ULS labeling system. The 48 individuals were hybridized in pairs with a loop design that

used 113 microarrays (printed by W.M. Keck Center for Comparative and Functional Genomics, University of Illinois). The microarrays contained 13,440 distinct 70-mer oligonucleotides based on information from the honey bee genome (Honey Bee Genome Sequencing Consortium, 2006). The oligonucleotide sequences were chosen based on predicted gene models and expressed sequence tags, and each was spotted twice on the array. This “second generation” honey bee microarray has been characterized by Alaux et al. (2009b), and from this point on, each representative sequence will be referred to as a “gene.” These gene oligonucleotides as well as positive and negative control oligonucleotides added up to 28,800 features (“spots”) on the array. Hybridized arrays were scanned and analyzed using GenePix software. As in Whitfield et al. (2006), only features having a median intensity of >300 were included in the analysis. Also, genes that did not have a representing feature above threshold for all of the individuals were excluded from subsequent analyses. Genes that are highly expressed in the hypopharyngeal glands were also excluded due to the risk of contamination during brain dissection (Alaux et al., 2009a). A total of 8,209 genes remained after these exclusions, representing the number of genes in the brain expressed above threshold for all individuals.

Statistical analysis of microarray results:

A linear mixed effects model was used to analyze the normalized log₂-transformed fluorescent intensities for each gene (Alaux et al., 2009b). The model accounted for the effects of dye, microarray, individual sample, and source colony. Two-

way ANOVA with post-hoc contrasts was used to determine significant differences based on the time of collection, the training group, and the activity state.

Principal component and hierarchical clustering analyses of the gene expression values showed that 11 individuals, relatively evenly distributed across experimental groups, were clustered together. Investigation revealed that the RNA amplification of all 11 of these individuals took place on two sequential days, and the affected 11 were the lowest yielding in final quantity of RNA on those days. For this reason, these 11 individuals and the 37 arrays they were measured with were dropped and the ANOVA rerun. Only results of this reanalysis are reported here. Genes showing differential expression at a false discover rate (FDR) corrected p-value of < 0.01 were considered significant.

Genes identified as differentially expressed in this experiment were compared with those from previously published studies to probe for similarities and differences across different behavioral states. Gene list overlap analysis was performed using an exact hypergeometric probability test (one-tailed) in Microsoft Excel (Redmond, Washington).

Functional insights into differentially expressed genes were obtained by conducting a Gene Ontology term enrichment analysis. This analysis was performed using the FlyBase identification number representing the best BLAST hit for each honey bee gene (Honey Bee Genome Sequencing Consortium, 2006) and the DAVID Bioinformatics Resources Functional Annotation tool (Huang et al., 2009).

Quantitative RT-PCR analysis and array validation:

Quantitative reverse transcriptase polymerase chain reaction was used to confirm some of the results obtained from microarray analysis. Twenty-four genes were selected for validation based on significance, fold change, and functions of interest (Fig. 6). Two hundred nanograms of the original unamplified RNA from all original 76 individuals in the study were used to synthesize cDNAs using ArrayScript (Ambion) reverse transcriptase. qPCR was performed in triplicate 10 μ L reactions in 384 well plates using PerfeCTa SYBR Green Fastmix (Quanta Biosystems, Gaithersburg, MD). Reactions not within 0.5CT agreement with the others were discounted. The results were analyzed by standard weighted means 2-way ANOVA with post-hoc contrasts using SAS (SAS Institute Inc., Cary, NC). Agreement of gene expression results between microarray and qPCR was tested using standardized data (“standardize” procedure in SAS, mean=0, standard deviation=1) which were then analyzed using linear discriminant analysis (“lda” function) in MASS package (Venables and Ripley, 2002) for R statistical software (R Development Core Team, 2006).

RESULTS

Behavioral evidence for spatiotemporal memories:

Over the course of the two replicates, 184 foragers were marked individually and followed throughout the course of the experiment. Of these bees, 28 were culled upon their arrival at the wrong feeding station, i.e., the one to which they were *not* initially trained. This means that the majority (85%) of the bees specialized and foraged exclusively at one training time or the other. Temporal specialization has been observed in previous studies (Kleber, 1935; Moore et al., 1989), and this specialization indicates that bees formed strong foraging-related spatiotemporal memories in this study. Twenty-five bees (14%) that were marked and trained did not appear on the final training day and were not found during the collections from the hive. These disappearances represent natural mortality expected among forager honey bees (Winston, 1987).

Honey bees clearly exhibited food anticipatory behavior in the course of the experiment. Outside of the training time, trained bees were typically found scattered in areas of the hive away from the entrance; however, as the training time approached, the trained bees began to cluster near the entrance and dance floor. Furthermore, on training days, bees frequently flew to the feeder before food or scent had been applied for that day. The frequency of these reconnaissance flights increased as the training time approached (Fig. 2). As had been previously reported (Moore and Rankin 1983), afternoon-trained bees for some reason exhibit anticipatory behavior for a longer period of time before the onset of the training time than did morning-trained bees.

Brain Gene Expression Differences Associated with Time of Collection:

To determine whether there were genes showing brain expression differences associated with time of collection, independent of time of training, we used two-way ANOVA to contrast the gene expression values for tAM-cAM and tPM-cAM with tAM-cPM and tPM-cPM bees (Fig. 1). There were 798 genes showing differences in brain gene expression between morning- and afternoon-collected bees (FDR <0.01). This represents ~10% of the genes on the microarray analyzed in this study.

Gene Ontology (GO) analysis revealed 80 categories significantly enriched (Modified Fisher Exact $p < 0.05$) for genes with differences in brain gene expression associated with time of day (Tables 2-4). Among enriched terms for genes with higher expression in the morning-collected bees were “organelle ATP synthesis coupled electron transport” and numerous mitochondria associated terms. There were also multiple terms associated with ribosomes and translation as well as proteasomes and catabolic processes. Enriched terms for genes more highly expressed in afternoon-collected bees included “cell adhesion,” “lipid metabolic process” and “lipid transport,” and “protein localization.” Terms associated with circadian behavior such as “circadian rhythm,” “rhythmic behavior,” “locomotory behavior,” and “sleep” all appear exclusively for the list of genes with higher expression in the afternoon.

Brain Gene Expression Differences Associated with Time of Training:

To determine whether there were genes showing brain expression differences associated with time of training, independent of time of collection, we contrasted tAM-cAM and tAM-cPM with tPM-cAM and tPM-cPM bees. Relative to other categories,

fewer genes, 229, were found to be differentially expressed between the two groups of bees trained to forage either in the morning or afternoon, regardless of whether they were collected in an anticipatory or inactive state ($FDR < 0.01$). GO analysis showed an enrichment of upregulated genes involved with “carbohydrate catabolic processes” and “kinase regulator activity” in the morning training groups (Tables 5-7). Afternoon-trained groups showed enrichment for genes associated with “response to heat” and “oxidoreductase activity, acting on the CH-CH group of donors.” Among the genes showing time of training differences were *foraging* and *clock*, both of which had higher expression in morning-trained bees.

Brain Gene Expression Differences Associated with State of Food Anticipation:

To determine whether there were genes showing brain expression differences associated with food anticipatory state, independent of time of training or collection, we contrasted tAM-cAM and tPM-cPM with tAM-cPM and tPM-cAM bees. Bees collected 15-45 min prior to their time of training were actively anticipating food availability and preparing for flight while bees collected during the opposite training period were inactive. This difference in activity state was associated with a very large number of genes, 2028 differentially expressed brain transcripts, or about 25% of all genes on the microarray analyzed in this study. Genes upregulated in anticipating bees showed significant GO enrichment for many categories associated with nervous system function including “cation transport,” “transmission of nerve impulse,” “neurotransmitter secretion,” and “synaptic transmission” (Tables 8-10). Behavioral categories were also present including “rhythmic process” and “circadian behavior,” as well as “response to

chemical stimulus,” “response to light stimulus,” and “memory” even though the bees had not recently encountered the environment outside the hive. *ankyrin*, *GB16541*, the heat shock proteins *hsp8* and *hsp90a*, and the insulin receptor *inR* were all confirmed via q-PCR as correlated with the state of food anticipation.

Inactive bees showed a remarkably strong signature of ribosome-related genes in their transcriptional profile. In all, 99 genes annotated as being associated with the ribonucleoprotein complex were significantly upregulated in inactive bees, with the GO term “structural constituent of the ribosome” having the strongest enrichment found in the entire experiment (Modified Fisher Exact $p = 1.07E-46$). Other significantly enriched terms include “mitochondrial part,” “mitochondrial ribosome,” “proteasome complex,” “DNA-directed RNA polymerase activity,” and “spliceosome.” Some of the genes upregulated in inactive bees and confirmed by qRT-PCR include the histone H1 and the duo of canonical clock genes *period* and *cryptochrome* (Ruben et al., 2006).

The list of genes differentially expressed between anticipating and inactive groups was compared to a list of genes generated by Alaux et al. (2009b) for differences between (non-foraging) nurse and forager honey bees. There was significant overlap for these lists ($r = 1.2$, $p < 0.0001$), with 66% of genes either up- or down-regulated in a concordant way for both anticipating bees in this study and foragers in Alaux et al. (2009b).

Brain Gene Expression Differences Associated with Distinct Spatiotemporal Memories:

To determine whether there were genes showing brain expression differences associated with distinct spatiotemporal memories, we contrasted tAM-cAM with tPM-

cPM bees. These two groups were both anticipating food availability, but at a different time of day and for a different location. A total of 1329 genes were differentially expressed in the brains between these two groups (t-test $p < 0.01$). This large number of genes included many genes found to be differentially expressed in the analyses described above. Of the 1329 differentially expressed genes, 624 also varied significantly ($p < 0.01$) with time of day, 180 also varied significantly with time of training, and 618 also varied significantly with state of food anticipation (Fig. 4). The same pattern of decomposition is reflected in the GO enrichment analysis (Tables 11-13). For example, terms such as “cell adhesion” and “carbohydrate catabolic processes” appeared in the analysis of tAM-cAM vs. tPM-cPM bees as well as in the time of day analysis. GO enrichment also revealed other differences including “lipid metabolic process,” “carbohydrate metabolic process,” and “chemosensory behavior.”

Removing all the genes that varied with time of day, time of training, and/or anticipatory state still left 352 genes that showed significant differences in brain expression between tAM-cAM vs. tPM-cPM bees. These expression differences are associated with other unknown aspects that contribute to the formation of the two unique spatiotemporal memories. GO enriched terms for these spatiotemporal memory-related genes include “extracellular structure organization and biogenesis” and “synaptogenesis.”

Effects of Time of Day, Time of Training and Anticipatory State on Brain

Expression of Canonical Clock Genes:

cycle (*cyc*), a gene comprising part of the positive element of the feedback loop in the bee’s circadian clock, was robustly differentially expressed based on time of day of

collection (ANOVA $p < 0.0001$) but was not affected by time of training or activity state. Another canonical clock gene in the positive element of the clock, *clock (clk)*, showed a significant difference based on time of training ($p = 0.0035$) but no effect based on anticipatory state or time of collection; there was higher expression among morning-trained bees, both active and inactive. By contrast, *period (per)*, a gene associated with the negative element of the feedback loop of the clock, was differentially expressed as a function of both time of day of collection ($p = 0.03$) and activity state ($p < 0.0001$), but not time of training. *cryptochrome (cry)*, which is also involved in the negative element of the clock, was differentially expressed based on both time of day of collection ($p < 0.0001$) and activity state ($p < 0.0001$), and not time of training.

Validation of microarray results with qPCR:

Because each gene could show significant expression differences for each of the time of collection, time of training, activity state, and/or unique memory analyses, Linear Discriminant Analysis (LDA) was used to examine the gestalt of similarities and differences between the four experimental groups and the two different expression measurement platforms. Expression values for each of the genes in this analysis are found in Figure 6. Overall expression patterns were similar between microarray and qPCR analysis, as revealed by LDA (Fig. 3). This analysis also revealed strong clustering of the four experimental groups with only the data from these 24 genes. This further reflects the robustness of the differences between the four groups and supports the hypothesis that spatiotemporal foraging rhythms are associated with unique neurogenomic states.

DISCUSSION

The principal significance of these results is that they demonstrate that the ability of honey bees to form distinct spatiotemporal foraging memories is associated with the formation of distinct neurogenomic profiles of gene expression in the brain. Our experimental design allowed us to determine that the neurogenomic signature of a spatiotemporal memory is actually composed of some genes that are influenced by the time of training, some that are influenced by the time of day they were collected, some genes that are associated with the bee's activity state, and still others that are not influenced by any of these three variables. This finding provides a vivid example of the modular composition of a complex neurogenomic phenotype.

This study identified a relatively large number of genes that showed differences in expression as a function of time of collection, i.e., genes whose expression varied in the morning vs. the afternoon. A similar number of genes varied in expression as a function of light-dark conditions in *Drosophila melanogaster* heads (712 genes under light-dark conditions, 115 genes under constant darkness, Ueda et al., 2002). In our study, genes upregulated in the morning included those associated with ATP synthesis, mitochondria, and NADH oxireductase activity as well as the canonical clock gene *cycle* (*bmal1* in mammals). These findings are consistent with previous reports of a link between the positive element of the molecular clock and the regulation of basic metabolic processes (Eckel-Mahan and Sassone-Corsi, 2009; Ramsey et al., 2009). Genes upregulated in the afternoon included cell adhesion molecules, which have been implicated in the synchronization of circadian oscillators (Miche and Colwell, 2001; Shen et al., 1997) and

in the regulation of learning and memory (Welzl and Stork, 2003). Perhaps this finding reflects the occurrence of memory consolidation in foragers during the night.

Relatively few genes showed differences associated with the time at which the bee was trained, regardless of whether it was then collected in the morning or afternoon, either anticipating or inactive. These gene expression differences may reflect either a natural proclivity to respond to training at one time versus another or differences in the environment encountered as a result of the different training times. Among the genes differentially expressed were genes associated with metabolism as well as *foraging* and *clock*. Upregulation of *foraging* has been implicated in increased phototactic behavior in honey bees (Ben-Shahar et al., 2003), and increased phototaxis could make the foragers more likely to respond to the morning feeder (which coincides more closely with sunrise) than the afternoon feeder. Other genetic differences for similar phenomena include polymorphisms in the human ortholog of *clock* associated with differences in the times at which people prefer to either be active or sleeping (Katzenberg et al., 1998).

The largest differences in brain gene expression detected in this study were related to differences in the activity state of the bees, either anticipating flight to a specific food reward or inactive. Bees collected just before their time of training were observed displaying the stereotypical anticipatory behavior that precedes exiting the hive to fly to the feeder. Renner's (1957) classic experiments demonstrate that anticipatory behavior in bees arises as a function of an internal circadian clock that allows the bees to time the availability of food. Results of GO analyses suggest that mechanisms involved in stimulus perception and response are reactivated in a circadian manner during the anticipatory state, perhaps "priming" the brain for subsequent activity. In addition, GO

categories such as “cation transport,” “transmission of nerve impulse,” and “neurotransmitter secretion” point toward possible mechanisms that the brain would utilize in the initiation of behavior. Mammalian pacemaker neurons found in the suprachiasmatic nucleus generate autonomous circadian activity patterns when isolated (Green and Gillette, 1982), and cation channels play a role in this autoactivation (Meredith et al., 2006; Wang and Huang, 2006; Kononenko and Berezetskaya, 2010). The differential regulation of cation channels and other genes associated with neural signaling in the bee illuminate how, after over 22 hours of inactivity, the brain state changes in a way to initiate activity. There was also a significant enrichment of genes encoding heat shock proteins upregulated in anticipating bees. Williams et al. (2008) has shown an upregulation in heat shock proteins in flight muscle associated with foraging activity. These genes could play a role in preparing foragers for the stress of encountering the environment outside the hive as well as the natural oxidative stress that occurs during metabolically demanding flight. It is also possible that heat shock proteins play more of a direct role in behavior. Knock-down of heat shock proteins has been shown to affect behavioral rhythmicity in flies even though the core molecular oscillators appear unaffected (Hung et al., 2009).

Genes upregulated in inactive bees were highlighted by a large number associated with transcription, translation and proteasome function. Inactive foragers have long been thought to enter into sleep-like states (von Frisch, 1967; Kaiser and Steiner-Kaiser, 1983; Kaiser, 1988; Eban-Rothschild and Bloch, 2008; Klein et al., 2008); this speculation is strengthened by work in *Drosophila* rigorously demonstrating that insects sleep (Hendricks et al., 2000; Shaw et al., 2000; reviewed Harbison et al., 2009). Perhaps

inactive bees are showing evidence for the kind of purging of old proteins and building of new proteins that has been associated with sleep in other organisms (reviewed Mackiewicz et al., 2008).

Comparing the differences in brain gene expression in this study for active anticipating bees and inactive bees with the differences in Alaux et al. (2009b) for nurse and forager bees revealed that anticipating bees have a brain expression profile that resembles the forager profile, while inactive bees more closely resemble the nurse profile. Among the genes differentially expressed in both contexts is the insulin receptor (*inR*), which shows higher expression in both foragers compared to nurses and in anticipating foragers compared to inactive foragers. Insulin and other nutritional signaling pathways play a part in honey bee worker division of labor (Ament et al., 2008), and RNAi knock-down of the insulin receptor alters honey bee foraging behavior (Wang et al., 2010). These results imply that some of the same genes involved in the regulation of foraging over the lifetime of the bee are also involved in the regulation of foraging within a given day.

Our results indicate that spatiotemporal foraging in honey bees is a composite phenotype involving genes that are expressed differently with time of day, time of training, or activity state, as well as other genes that vary with spatiotemporal memory and not with any of these three other factors. Among those in the latter category was a significant overrepresentation of genes involved in synaptogenesis, consistent with the idea that different spatiotemporal memories have unique representations in the brain. In addition, the overrepresentation of genes previously shown to be involved in chemosensory behavior perhaps reflects the possibility that some aspects of the olfactory

system are differentially utilized in the learning of the unique scents used in the spatiotemporal training.

Some of the genes well known to be involved in the regulation of circadian rhythms in a variety of organisms showed interesting patterns of expression in this study. Expression of *per* and *cry* in bees trained in the morning was consistent with patterns reported by Ruben et al. (2006) under *ad libitum* feeding conditions; however, this was not the case for the afternoon-trained bees which showed an altered expression pattern. This is perhaps because the time-training procedures utilized in this experiment uncoupled activity state from time of day, and food acted as a zeitgeber to set elements of the internal clock which then dictated foraging rhythms on subsequent days. Intriguingly, another clock gene, *cyc*, did not show this altered expression pattern. The regulation of circadian rhythms is thought to be based on two interacting sets of transcription factors, the “positive” element, composed of *clk* and *cyc* which positively regulate expression of other clock genes, and the “negative” element, composed of *per* and *cry* which negatively regulate expression of other clock genes (reviewed Allada and Chung, 2010). The differences observed in the time-trained bees suggest that in the honey bee these two sets of regulatory loops have become uncoupled, with the positive element of the clock synchronizing their gene expression oscillations to day/night cycles while the negative element synchronizes their oscillations to the time of food availability. This is supported by the recent finding that genes involved in insulin signaling are involved in setting the pace of the negative element of the clock in *Drosophila* (Zheng and Seghal, 2010).

It should be noted that this study was performed using whole brains. The possibility exists that the altered pattern of gene expression due to spatiotemporal training

occurred only in a subset of neurons, and different neural circuits could have different expression profiles. Previous studies in mice have found that restricted feeding schedules shifted expression of *per*, but only in specific brain regions (Angeles-Costellanos et al., 2007; reviewed Webb et al., 2009). Furthermore, peripheral oscillators in other tissues such as the insect fat body (a metabolic tissue analogous to vertebrate livers) could play a role in behavioral rhythmicity as it relates to feeding (Xu et al., 2008; Vollmers et al., 2009; reviewed Escobar et al., 2009).

Our study indicates that the spatiotemporal foraging ability of bees is reflected by a composite molecular phenotype involving thousands of genes that can be further described in their associations with the time of day, the training group, or the activity state. This study is the first to separate genes involved with activity rhythms from genes influenced by time of day in an insect, without the confounding effects of sleep deprivation. We identified genes associated with motivation in the form of food anticipation, and we have demonstrated that spatiotemporal training creates neurogenomic profiles distinct for each training regime. These findings highlight the surprisingly close relationship between changes in brain gene expression and naturally occurring behavioral plasticity (Robinson et al. 2005; Robinson et al. 2008).

FIGURES

Figure 1. Experimental design and the four behavioral groups analyzed in this study. Two groups of bees from the same colony were trained to forage at an artificial feeder either in the morning or in the afternoon. Bees from each training group were collected just prior to the onset each training time, in the morning or afternoon. Bees collected near to their training time were undergoing stereotypical anticipatory behavior that precedes flying to a food source, while bees collected outside of their training time were inactive. (add abbreviations, e.g. tAM-cAM, for group names)

		Time of collection	
		AM	PM
Training group	AM	tAMcAM Active / anticipating	tAMcPM Inactive
	PM	tPMcAM Inactive	tPMcPM Active / anticipating

Figure 2. Anticipatory behavior. Trained bees frequently fly to the feeder in anticipation of food availability. The frequency of these flights increases as the training time approaches. These data are from Aug. 18, 2007, the day prior to the day of collection for the first replicate of the experiment.

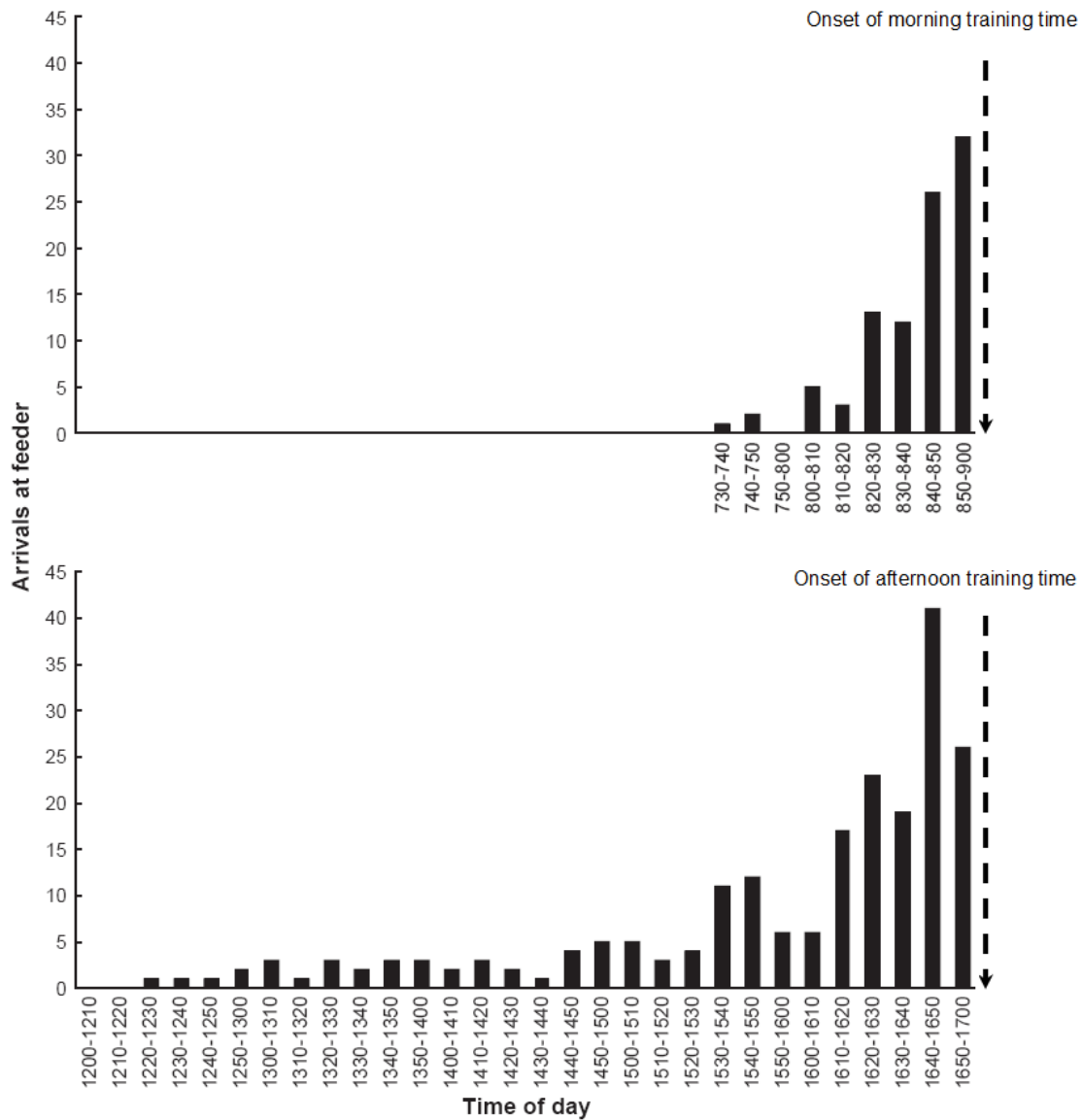


Figure 3. Linear Discriminant Analysis indicates strong similarities between results obtained with microarrays and qPCR and supports the hypothesis that distinct neurogenomic states exist for each of the four groups in the experiment. Microarray and qRT-PCR data for the same 37 individuals is shown here. AA = AM (morning) trained, AM collected; AP = AM trained, PM (afternoon) collected; PA = PM trained, AM collected; and PP = PM trained, PM collected. Black, orange, yellow, and sky blue represent the microarray data while red, pink, green, and blue represent the qPCR data.

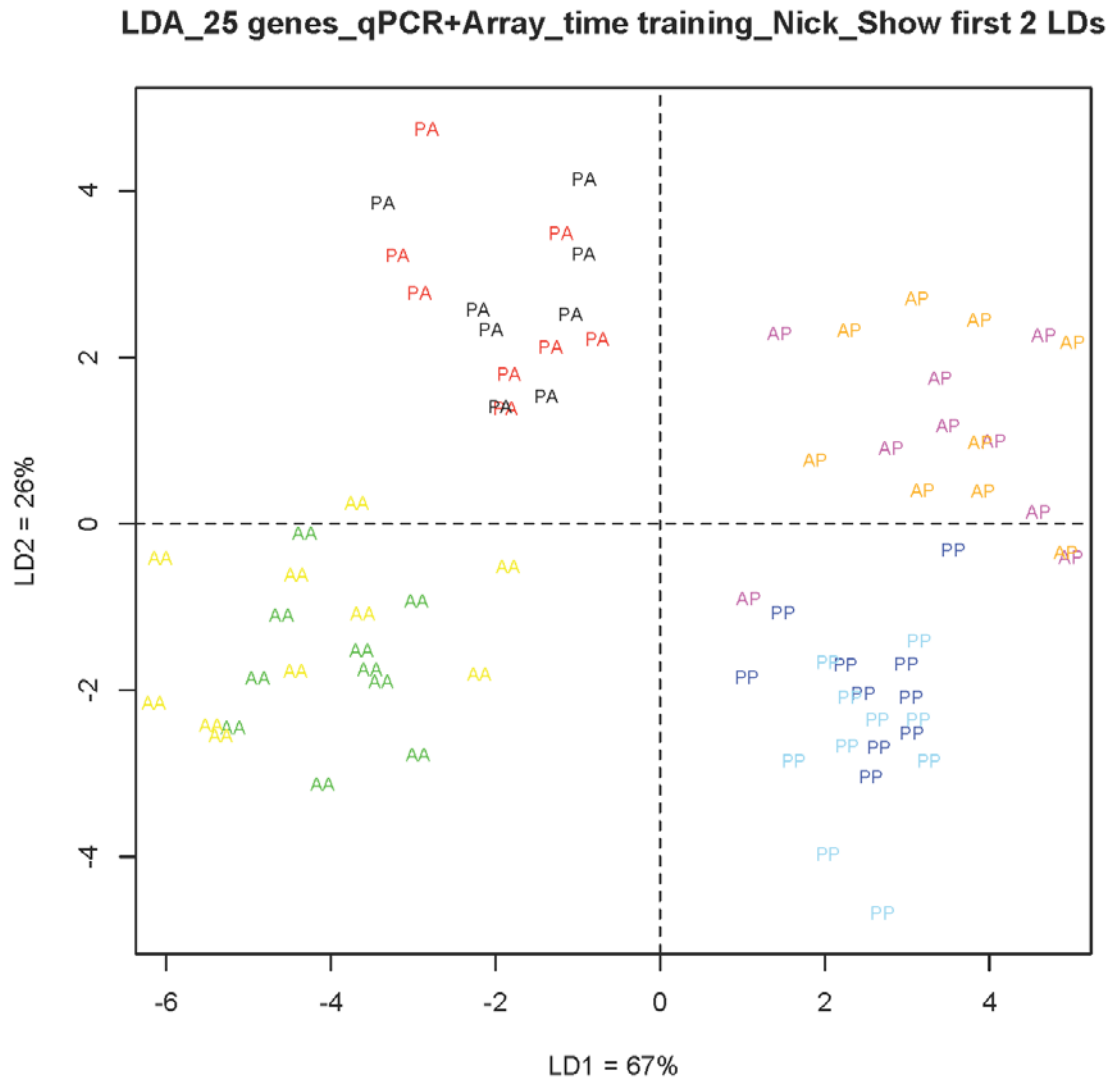


Figure 4. Decomposition of brain gene expression differences associated with distinct spatiotemporal memories: effects of time of collection, time of training and activity state. A total of 1329 genes were differentially expressed between the two active groups with distinct spatiotemporal memories. Expression of a majority of these genes was also influenced by time of day, time of training, and/or activity state. 352 of the 1329 genes did not have significant differences in expression in these other analyses and represent unique transcriptional signatures associated with a unique spatiotemporal memory.

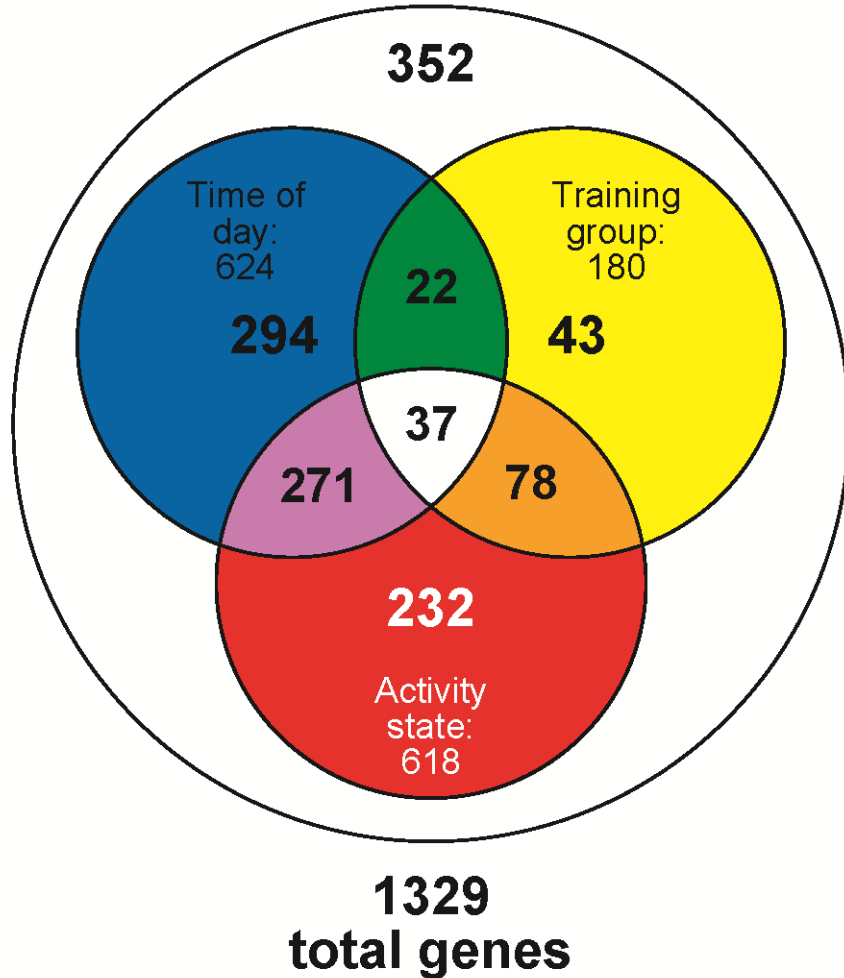


Figure 5. Expression of canonical clock genes. Genes representing the set of transcription factors comprising the negative element of the molecular clock (*per*, *cry*, and *tim2*) have expression patterns that are more closely associated with the activity state of the bee rather than time of day. Genes representing the positive element of the molecular clock (*cyc* and *clk*) do not show any significant differences based on activity state, although *cyc* shows clear differences based on the time of day.

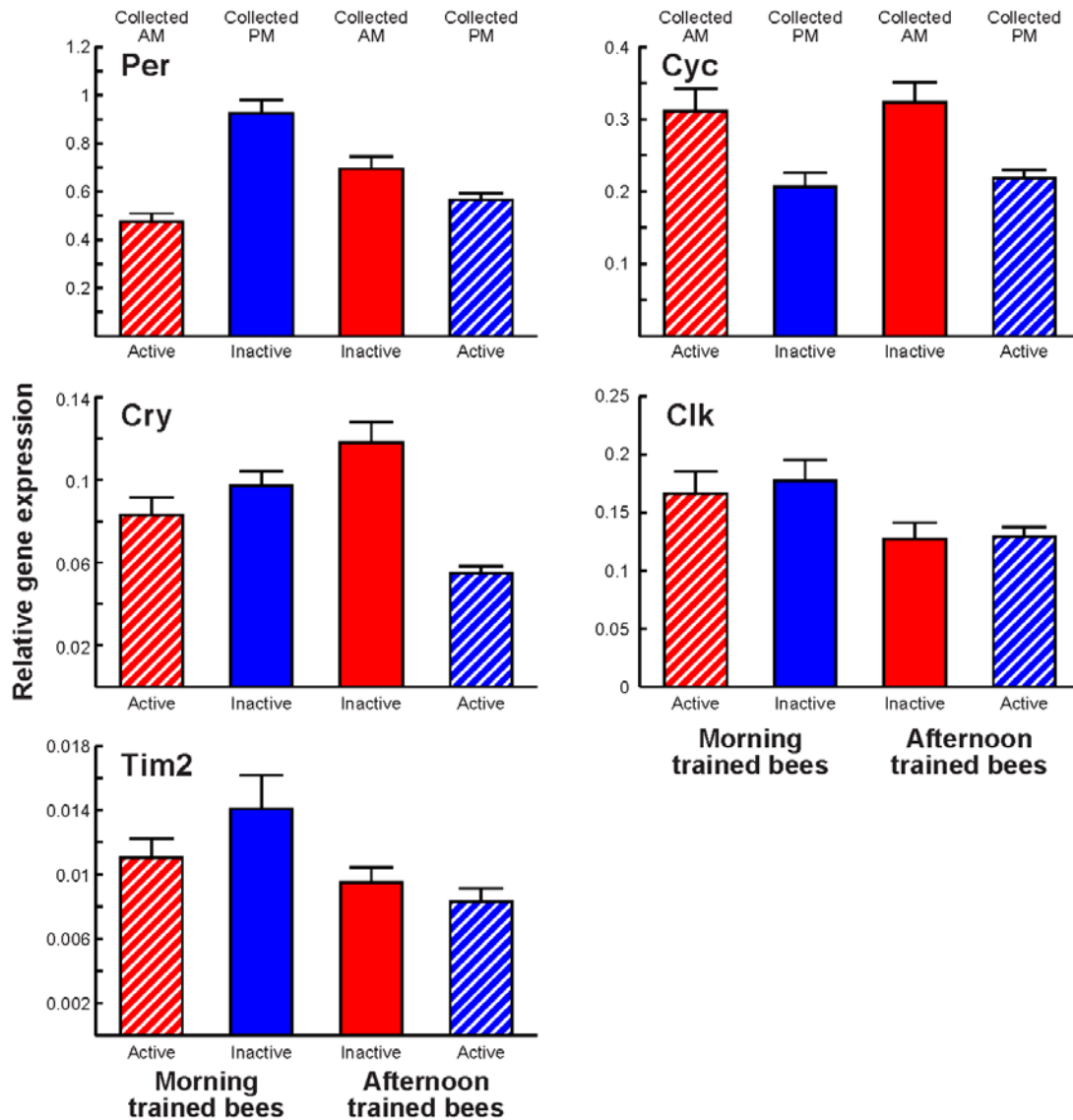
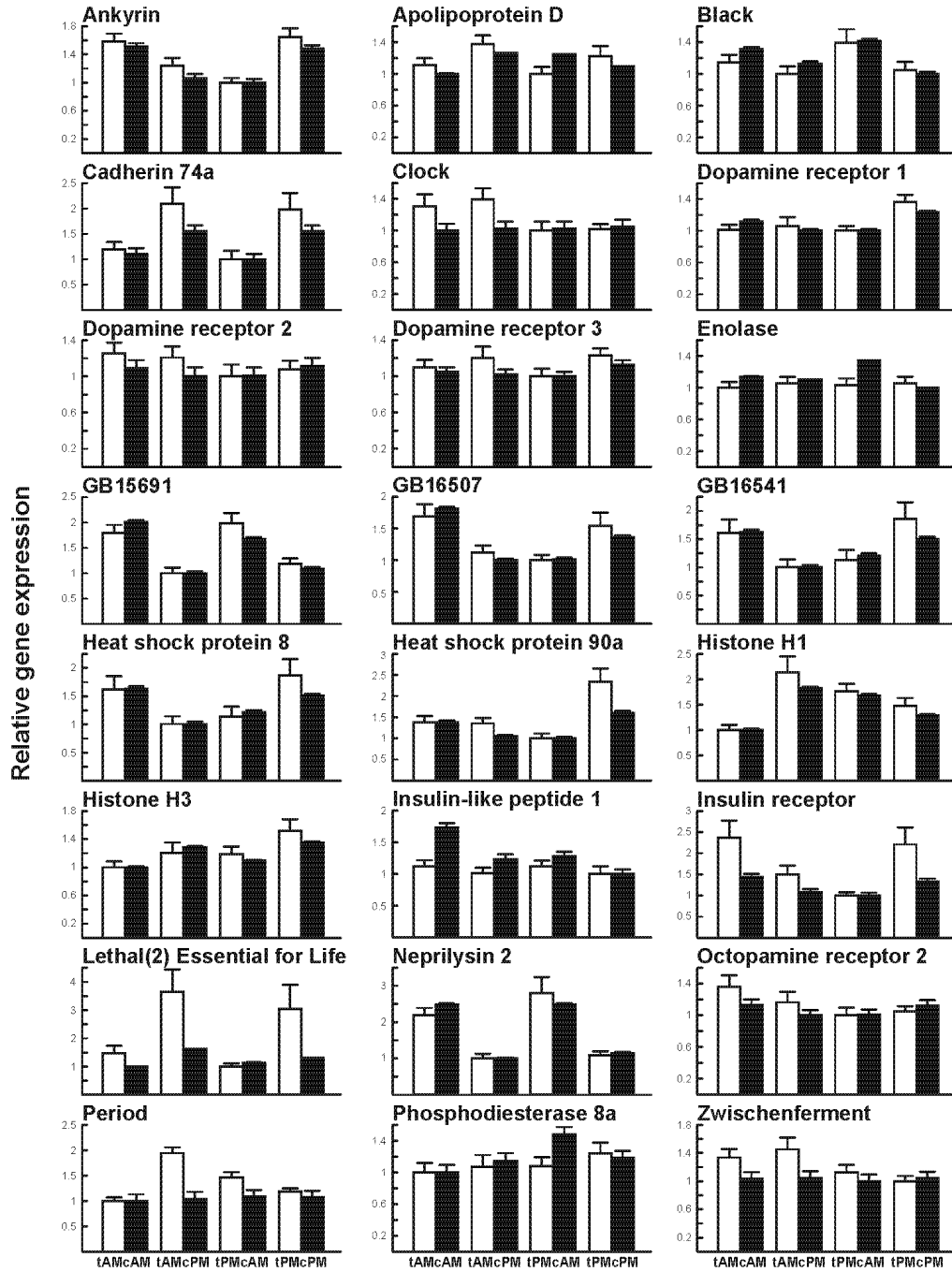


Figure 6: Comparison between microarray and qPCR gene expression. Microarray data are based off of the best 37 performing foragers while qPCR data include all 76 bees that passed the behavioral threshold for inclusion in the study.



TABLES

Table 1. Number of visits to the feeders by day. Bees visited the artificial feeder multiple times over the duration of the training time. The performance of the 37 bees used for the microarray analysis is shown here.

Bee ID	Training group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
BWP	AM				6	6	9	13
PWY	AM			1	5	10	8	8
PYW	AM			13	17	13	8	12
YBB	AM					9	12	14
YPB	AM					7	8	12
YYW	AM				6	11	10	11
BYY	AM					3	12	13
WcPR	AM					12	13	8
WcWP	AM					13	11	14
WcWY	AM			12		14	13	13
BBW	AM				5	10	10	9
BPW	AM				6	13	14	15
YWW	AM				10	12	11	12
YYG	AM				5	4	10	10
BBY	AM					2	11	8
RWP	AM					5	7	10
WRR	AM					2	6	6
WcPW	AM					14	13	10
WcYA	AM			10		13	15	16
RBW	PM				7	5	13	20
RWP	PM		1	3	4	7	9	12
WPP	PM			5	18	15	14	18
WRP	PM		1	6	6	6	7	12
YcGG	PM			6	4	12	13	16
YcGW	PM			7	6	10	16	20
YcWB	PM				10	12	17	23
YcWY	PM			15	15	8	14	18
GGG	PM	1	2	2	14	15	18	20
RGG	PM	4	7	8	12	9	12	19
RWR	PM	4	8	22	21	10	17	17
WPRC	PM			10	10	10	10	18
WWW	PM	3	2	2	10	13	14	11
YcBY	PM			10	14	12	15	16
YcWP	PM			4	13	9	18	22
YcYR	PM			10	12	14	8	17
YcYW	PM			10	6	12	7	16
YcYY	PM			19	17	16	15	19

Table 2. Significantly enriched GO categories for genes differentially expressed between morning- and afternoon-collected foragers.

GO category	Term	Count	%	P-value
Cell Component level 1	extracellular region	20	4.55%	6.23E-04
Mol Function level 2	structural constituent of ribosome	25	5.68%	0.0015191
Mol Function level 2	isomerase activity	13	2.95%	0.0018495
Cell Component level 3	cytoplasm	132	30.00%	0.0019373
Biol Process level 3	cellular catabolic process	29	6.59%	0.0023235
Biol Process level 2	catabolic process	31	7.05%	0.0027466
Cell Component level 5	cytoplasm	132	30.00%	0.0038595
Biol Process level 3	cellular biosynthetic process	62	14.09%	0.0040918
Cell Component level 4	cytoplasm	132	30.00%	0.0041929
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	15	3.41%	0.0042346
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	15	3.41%	0.0042463
Cell Component level 3	proteasome core complex (sensu Eukaryota)	6	1.36%	0.0053134
Cell Component level 4	proteasome core complex (sensu Eukaryota)	6	1.36%	0.0058954
Cell Component level 5	proteasome core complex (sensu Eukaryota)	6	1.36%	0.0059093
Cell Component level 4	cytosol	27	6.14%	0.0073611
Cell Component level 5	cytosol	27	6.14%	0.0073719
Cell Component level 3	ribosome	25	5.68%	0.007872
Biol Process level 2	cell adhesion	19	4.32%	0.0083836
Biol Process level 1	biological adhesion	19	4.32%	0.0098868
Cell Component level 4	ribosome	25	5.68%	0.0103702
Cell Component level 5	ribosome	25	5.68%	0.0103915
Cell Component level 4	cytosolic part	17	3.86%	0.0107386
Cell Component level 3	ribosomal subunit	22	5.00%	0.010746
Cell Component level 3	cytoplasmic part	108	24.55%	0.0107505
Cell Component level 5	cytosolic part	17	3.86%	0.0107693
Biol Process level 2	biosynthetic process	68	15.45%	0.0124358
Cell Component level 4	ribosomal subunit	22	5.00%	0.0137762
Cell Component level 5	ribosomal subunit	22	5.00%	0.0138119
Cell Component level 3	proteasome complex (sensu Eukaryota)	10	2.27%	0.0145621
Biol Process level 2	maintenance of protein localization	4	0.91%	0.0164169
Biol Process level 3	maintenance of protein localization	4	0.91%	0.0166999
Cell Component level 4	proteasome complex (sensu Eukaryota)	10	2.27%	0.0168338
Cell Component level 5	proteasome complex (sensu Eukaryota)	10	2.27%	0.0168818
Biol Process level 4	maintenance of protein localization	4	0.91%	0.0172851
Mol Function level 4	magnesium ion binding	7	1.59%	0.0192516
Biol Process level 2	maintenance of localization	5	1.14%	0.0193023
Cell Component level 5	cytoplasmic part	108	24.55%	0.0197579
Cell Component level 4	cytoplasmic part	108	24.55%	0.0202964
Biol Process level 1	maintenance of localization	5	1.14%	0.020394
Mol Function level 1	antioxidant activity	5	1.14%	0.0208064
Cell Component level 3	small ribosomal subunit	11	2.50%	0.0215521
Biol Process level 4	cellular macromolecule catabolic process	17	3.86%	0.0220579
Mol Function level 5	nucleoside diphosphate kinase activity	3	0.68%	0.0234745
Biol Process level 3	cell redox homeostasis	7	1.59%	0.0243666
Biol Process level 4	oocyte microtubule cytoskeleton organization	6	1.36%	0.0249095
Biol Process level 5	establishment and/or maintenance of cytoskeleton polarity	6	1.36%	0.024991
Cell Component level 4	small ribosomal subunit	11	2.50%	0.0250229

Table 2 continued.

GO category	Term	Count	%	P-value
Cell Component level 5	small ribosomal subunit	11	2.50%	0.0250959
Biol Process level 3	macromolecule catabolic process	19	4.32%	0.0254762
Biol Process level 5	cell redox homeostasis	7	1.59%	0.0258417
Mol Function level 2	oxidoreductase activity	38	8.64%	0.026437
Biol Process level 2	translation	34	7.73%	0.0265895
Cell Component level 3	germline ring canal	4	0.91%	0.0293654
Cell Component level 3	intercellular bridge	4	0.91%	0.0293654
Cell Component level 2	intercellular bridge	4	0.91%	0.0296506
Biol Process level 2	cell fate commitment	17	3.86%	0.0300359
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	7	1.59%	0.0309689
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	7	1.59%	0.0310442
Cell Component level 4	germline ring canal	4	0.91%	0.0313204
Mol Function level 2	peroxidase activity	4	0.91%	0.0326794
Mol Function level 2	transmembrane transporter activity	41	9.32%	0.0327761
Biol Process level 4	translation	34	7.73%	0.0334721
Biol Process level 5	translation	34	7.73%	0.0336353
Cell Component level 4	lipid particle	17	3.86%	0.0339202
Cell Component level 5	lipid particle	17	3.86%	0.0340264
Biol Process level 3	cell-cell adhesion	9	2.05%	0.0345628
Biol Process level 4	cell fate commitment	17	3.86%	0.0348558
Mol Function level 4	peroxidase activity	4	0.91%	0.0349835
Mol Function level 3	oxidoreductase activity, acting on peroxide as acceptor	4	0.91%	0.0366343
Mol Function level 2	substrate-specific transporter activity	40	9.09%	0.0374497
Biol Process level 3	macromolecule biosynthetic process	42	9.55%	0.0377369
Biol Process level 3	lipid metabolic process	25	5.68%	0.0390406
Cell Component level 3	proton-transporting two-sector ATPase complex, proton-transporting	5	1.14%	0.0400385
Mol Function level 3	substrate-specific transmembrane transporter activity	37	8.41%	0.0432041
Cell Component level 4	proton-transporting two-sector ATPase complex, proton-transporting	5	1.14%	0.043255
Cell Component level 5	proton-transporting two-sector ATPase complex, proton-transporting	5	1.14%	0.0433397
Mol Function level 4	endopeptidase activity	19	4.32%	0.0437712
Mol Function level 5	glutathione peroxidase activity	3	0.68%	0.044135
Biol Process level 2	behavior	21	4.77%	0.044499
Mol Function level 4	rRNA binding	4	0.91%	0.0487927

Table 3. Significantly enriched GO categories for genes more highly expressed in morning-collected foragers.

Category	Term	Count	%	PValue
Cell Component level 4	cytosol	49	11.29%	2.80E-14
Cell Component level 5	cytosol	49	11.29%	4.56E-14
Mol Function level 2	structural constituent of ribosome	44	10.14%	5.34E-14
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	30	6.91%	7.15E-14
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	30	6.91%	1.00E-13
Cell Component level 3	ribosomal subunit	41	9.45%	2.61E-13
Cell Component level 3	ribosome	44	10.14%	5.96E-13
Cell Component level 4	ribosomal subunit	41	9.45%	1.22E-12
Cell Component level 5	ribosomal subunit	41	9.45%	1.86E-12
Cell Component level 4	cytosolic part	33	7.60%	1.93E-12
Cell Component level 5	cytosolic part	33	7.60%	2.75E-12
Cell Component level 4	ribosome	44	10.14%	2.99E-12
Cell Component level 5	ribosome	44	10.14%	4.62E-12
Biol Process level 2	translation	54	12.44%	9.42E-11
Biol Process level 4	translation	54	12.44%	2.54E-10
Biol Process level 5	translation	54	12.44%	2.86E-10
Cell Component level 3	cytoplasm	149	34.33%	3.14E-10
Biol Process level 3	cellular biosynthetic process	80	18.43%	3.40E-10
Biol Process level 3	macromolecule biosynthetic process	62	14.29%	1.55E-09
Biol Process level 2	biosynthetic process	86	19.82%	4.92E-09
Cell Component level 4	cytoplasm	149	34.33%	8.49E-09
Cell Component level 5	cytoplasm	149	34.33%	2.14E-08
Cell Component level 2	ribonucleoprotein complex	49	11.29%	2.51E-08
Cell Component level 3	ribonucleoprotein complex	49	11.29%	2.55E-08
Cell Component level 3	cytoplasmic part	125	28.80%	2.57E-08
Cell Component level 5	cytosolic large ribosomal subunit (sensu Eukaryota)	18	4.15%	5.69E-08
Cell Component level 4	ribonucleoprotein complex	49	11.29%	1.16E-07
Cell Component level 3	small ribosomal subunit	19	4.38%	1.57E-07
Cell Component level 5	ribonucleoprotein complex	49	11.29%	1.73E-07
Mol Function level 1	structural molecule activity	48	11.06%	1.75E-07
Biol Process level 2	cellular metabolic process	217	50.00%	1.78E-07
Biol Process level 1	metabolic process	235	54.15%	2.30E-07
Cell Component level 4	small ribosomal subunit	19	4.38%	3.33E-07
Cell Component level 5	small ribosomal subunit	19	4.38%	4.07E-07
Cell Component level 4	cytoplasmic part	125	28.80%	4.24E-07
Cell Component level 4	large ribosomal subunit	24	5.53%	7.97E-07
Cell Component level 5	cytoplasmic part	125	28.80%	9.10E-07
Cell Component level 5	large ribosomal subunit	24	5.53%	1.01E-06
Cell Component level 1	macromolecular complex	120	27.65%	1.87E-06
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	12	2.76%	5.63E-06
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	12	2.76%	6.43E-06
Cell Component level 3	mitochondrial membrane part	24	5.53%	7.30E-06
Cell Component level 4	mitochondrial membrane part	24	5.53%	1.66E-05
Cell Component level 3	organelle inner membrane	29	6.68%	1.81E-05
Cell Component level 5	mitochondrial membrane part	24	5.53%	2.07E-05
Biol Process level 3	oxidative phosphorylation	22	5.07%	2.50E-05
Cell Component level 3	mitochondrial part	46	10.60%	3.21E-05
Cell Component level 3	mitochondrial envelope	32	7.37%	3.50E-05

Table 3 continued

Category	Term	Count	%	PValue
Mol Function level 4	rRNA binding	7	1.61%	3.69E-05
Cell Component level 4	mitochondrial inner membrane	29	6.68%	4.50E-05
Cell Component level 4	organelle inner membrane	29	6.68%	4.50E-05
Cell Component level 5	mitochondrial inner membrane	29	6.68%	5.74E-05
Cell Component level 5	organelle inner membrane	29	6.68%	5.74E-05
Cell Component level 2	non-membrane-bound organelle	60	13.82%	8.70E-05
Cell Component level 3	intracellular non-membrane-bound organelle	60	13.82%	8.82E-05
Cell Component level 4	mitochondrial envelope	32	7.37%	9.04E-05
Cell Component level 3	mitochondrial membrane	29	6.68%	9.32E-05
Cell Component level 4	mitochondrial part	46	10.60%	1.07E-04
Cell Component level 2	intracellular part	193	44.47%	1.14E-04
Cell Component level 5	mitochondrial envelope	32	7.37%	1.16E-04
Cell Component level 3	intracellular part	193	44.47%	1.19E-04
Cell Component level 5	mitochondrial part	46	10.60%	1.47E-04
Cell Component level 3	proteasome complex (sensu Eukaryota)	13	3.00%	1.82E-04
Biol Process level 3	electron transport	25	5.76%	2.11E-04
Cell Component level 4	mitochondrial membrane	29	6.68%	2.20E-04
Cell Component level 4	mitochondrial respiratory chain	16	3.69%	2.56E-04
Cell Component level 5	mitochondrial membrane	29	6.68%	2.76E-04
Biol Process level 2	generation of precursor metabolites and energy	31	7.14%	2.80E-04
Cell Component level 4	proteasome complex (sensu Eukaryota)	13	3.00%	2.93E-04
Cell Component level 5	mitochondrial respiratory chain	16	3.69%	2.98E-04
Cell Component level 5	proteasome complex (sensu Eukaryota)	13	3.00%	3.33E-04
Cell Component level 4	intracellular non-membrane-bound organelle	60	13.82%	3.49E-04
Biol Process level 4	ATP synthesis coupled electron transport	14	3.23%	4.66E-04
Biol Process level 5	organelle ATP synthesis coupled electron transport	14	3.23%	4.89E-04
Cell Component level 5	intracellular non-membrane-bound organelle	60	13.82%	5.00E-04
Cell Component level 3	proteasome core complex (sensu Eukaryota)	7	1.61%	5.01E-04
Cell Component level 4	mitochondrion	51	11.75%	5.91E-04
Biol Process level 3	cellular catabolic process	29	6.68%	5.95E-04
Biol Process level 2	catabolic process	31	7.14%	6.39E-04
Cell Component level 4	proteasome core complex (sensu Eukaryota)	7	1.61%	6.61E-04
Biol Process level 4	cellular protein metabolic process	113	26.04%	6.93E-04
Cell Component level 5	proteasome core complex (sensu Eukaryota)	7	1.61%	7.12E-04
Cell Component level 2	intracellular organelle part	106	24.42%	7.80E-04
Cell Component level 2	organelle envelope	33	7.60%	7.88E-04
Cell Component level 3	intracellular organelle part	106	24.42%	7.93E-04
Cell Component level 3	organelle envelope	33	7.60%	7.94E-04
Cell Component level 5	mitochondrion	51	11.75%	8.09E-04
Biol Process level 3	cellular macromolecule metabolic process	113	26.04%	8.58E-04
Cell Component level 2	organelle part	106	24.42%	8.62E-04
Biol Process level 3	macromolecule catabolic process	22	5.07%	8.90E-04
Cell Component level 4	lipid particle	21	4.84%	8.96E-04
Cell Component level 1	envelope	33	7.60%	9.25E-04
Cell Component level 5	lipid particle	21	4.84%	0.0010636
Biol Process level 3	protein metabolic process	114	26.27%	0.0010652
Cell Component level 2	protein complex	90	20.74%	0.0011932
Mol Function level 4	endopeptidase activity	23	5.30%	0.0012034
Cell Component level 1	organelle part	106	24.42%	0.0013123
Cell Component level 4	mitochondrial proton-transporting ATP synthase complex	7	1.61%	0.0017934
Cell Component level 2	intracellular	201	46.31%	0.001805

Table 3 continued

Category	Term	Count	%	PValue
Cell Component level 4	organelle envelope	33	7.60%	0.00184
Cell Component level 3	intracellular	201	46.31%	0.0018776
Cell Component level 5	mitochondrial proton-transporting ATP synthase complex	7	1.61%	0.0019271
Cell Component level 3	proton-transporting ATP synthase complex	7	1.61%	0.002099
Cell Component level 5	organelle envelope	33	7.60%	0.0022954
Cell Component level 4	proton-transporting ATP synthase complex	7	1.61%	0.0027311
Cell Component level 5	proton-transporting ATP synthase complex	7	1.61%	0.0029309
Cell Component level 4	intracellular part	193	44.47%	0.0038914
Biol Process level 2	macromolecule metabolic process	168	38.71%	0.0039313
Biol Process level 2	primary metabolic process	195	44.93%	0.0040656
Biol Process level 4	cellular macromolecule catabolic process	18	4.15%	0.0046933
Cell Component level 4	intracellular organelle part	106	24.42%	0.0048768
Biol Process level 5	cellular protein catabolic process	11	2.53%	0.0051776
Biol Process level 5	modification-dependent macromolecule catabolic process	11	2.53%	0.0051776
Mol Function level 4	NADH dehydrogenase activity	9	2.07%	0.0053976
Mol Function level 3	oxidoreductase activity, acting on NADH or NADPH	10	2.30%	0.0056099
Biol Process level 4	protein catabolic process	12	2.76%	0.0062543
Biol Process level 5	protein catabolic process	12	2.76%	0.0064999
Cell Component level 5	intracellular organelle part	106	24.42%	0.0077543
Cell Component level 3	proteasome regulatory particle, base subcomplex (sensu Eukaryota)	5	1.15%	0.0080292
Biol Process level 4	mitochondrial electron transport, NADH to ubiquinone	9	2.07%	0.0086152
Cell Component level 3	respiratory chain complex I	9	2.07%	0.0088171
Cell Component level 3	NADH dehydrogenase complex (quinone)	9	2.07%	0.0088171
Cell Component level 4	proteasome regulatory particle, base subcomplex (sensu Eukaryota)	5	1.15%	0.0096349
Cell Component level 4	proteasome regulatory particle, base subcomplex (sensu Eukaryota)	5	1.15%	0.0096349
Cell Component level 5	proteasome regulatory particle, base subcomplex (sensu Eukaryota)	5	1.15%	0.0101182
Biol Process level 1	gene expression	86	19.82%	0.0102501
Biol Process level 4	biopolymer catabolic process	15	3.46%	0.0112687
Cell Component level 4	NADH dehydrogenase complex (quinone)	9	2.07%	0.0118661
Cell Component level 4	respiratory chain complex I	9	2.07%	0.0118661
Cell Component level 4	mitochondrial respiratory chain complex I	9	2.07%	0.0118661
Mol Function level 3	peptidase activity	27	6.22%	0.0122024
Cell Component level 5	respiratory chain complex I	9	2.07%	0.0128434
Cell Component level 5	NADH dehydrogenase complex (quinone)	9	2.07%	0.0128434
Cell Component level 5	mitochondrial respiratory chain complex I	9	2.07%	0.0128434
Mol Function level 2	oxidoreductase activity	39	8.99%	0.0132598
Mol Function level 4	magnesium ion binding	7	1.61%	0.0147554
Mol Function level 1	antioxidant activity	5	1.15%	0.0194061
Mol Function level 5	nucleoside diphosphate kinase activity	3	0.69%	0.0216029
Biol Process level 3	translational initiation	8	1.84%	0.0216292
Cell Component level 3	proton-transporting two-sector ATPase complex	9	2.07%	0.0218842
Biol Process level 4	alcohol biosynthetic process	4	0.92%	0.0226078
Mol Function level 3	translation factor activity, nucleic acid binding	10	2.30%	0.0227962
Biol Process level 5	monosaccharide biosynthetic process	4	0.92%	0.0229404
Mol Function level 5	ATPase activity, coupled to transmembrane movement of substances	12	2.76%	0.023083
Biol Process level 5	translational initiation	8	1.84%	0.0245098
Mol Function level 5	threonine endopeptidase activity	4	0.92%	0.026654
Mol Function level 2	translation factor activity, nucleic acid binding	10	2.30%	0.0267606
Cell Component level 4	mitochondrial proton-transporting ATP synthase complex, coupling factor F(o)	4	0.92%	0.0287939

Table 3 continued.

Category	Term	Count	%	PValue
Cell Component level 4	proton-transporting two-sector ATPase complex	9	2.07%	0.0287981
Cell Component level 5	mitochondrial proton-transporting ATP synthase complex, coupling factor F(o)	4	0.92%	0.0298729
Biol Process level 3	cofactor metabolic process	19	4.38%	0.0304676
Cell Component level 5	proton-transporting two-sector ATPase complex	9	2.07%	0.030975
Mol Function level 3	electron carrier activity	14	3.23%	0.0310238
Biol Process level 4	cellular carbohydrate metabolic process	18	4.15%	0.032086
Cell Component level 4	organellar small ribosomal subunit	7	1.61%	0.032882
Cell Component level 4	mitochondrial small ribosomal subunit	7	1.61%	0.032882
Cell Component level 3	proton-transporting two-sector ATPase complex, proton-transporting domain	5	1.15%	0.0331262
Cell Component level 2	organelle membrane	35	8.06%	0.0334203
Cell Component level 3	organelle membrane	35	8.06%	0.033605
Mol Function level 4	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	12	2.76%	0.0347517
Cell Component level 5	organellar small ribosomal subunit	7	1.61%	0.0348972
Cell Component level 5	mitochondrial small ribosomal subunit	7	1.61%	0.0348972
Mol Function level 1	translation regulator activity	10	2.30%	0.0366818
Cell Component level 3	proton-transporting ATP synthase complex, coupling factor F(o)	4	0.92%	0.0372693
Cell Component level 4	proton-transporting two-sector ATPase complex, proton-transporting domain	5	1.15%	0.0390894
Mol Function level 5	glutathione peroxidase activity	3	0.69%	0.0407249
Cell Component level 5	proton-transporting two-sector ATPase complex, proton-transporting domain	5	1.15%	0.0408587
Cell Component level 4	proton-transporting ATP synthase complex, coupling factor F(o)	4	0.92%	0.0425747
Mol Function level 3	glutathione peroxidase activity	3	0.69%	0.0434953
Cell Component level 5	proton-transporting ATP synthase complex, coupling factor F(o)	4	0.92%	0.0441213
Biol Process level 3	carbohydrate metabolic process	24	5.53%	0.0449514
Biol Process level 5	nucleoside triphosphate biosynthetic process	8	1.84%	0.0450824

Table 4. Significantly enriched GO categories for genes more highly expressed in afternoon-collected foragers.

Category	Term	Count	%	PValue
Biol Process level 3	lipid metabolic process	31	7.51%	4.03E-05
Biol Process level 2	behavior	26	6.30%	1.21E-04
Biol Process level 1	localization	101	24.46%	1.29E-04
Mol Function level 5	peptide-methionine-(S)-S-oxide reductase activity	5	1.21%	2.09E-04
Mol Function level 4	oxidoreductase activity, acting on sulfur group of donors, disulfide as acceptor	5	1.21%	2.42E-04
Biol Process level 1	rhythmic process	10	2.42%	2.74E-04
Cell Component level 4	endoplasmic reticulum	22	5.33%	5.30E-04
Cell Component level 5	endoplasmic reticulum	22	5.33%	6.17E-04
Mol Function level 2	isomerase activity	13	3.15%	6.27E-04
Biol Process level 2	circadian rhythm	9	2.18%	0.0011106
Biol Process level 1	maintenance of localization	6	1.45%	0.0013915
Biol Process level 2	maintenance of localization	6	1.45%	0.0013967
Biol Process level 4	cellular lipid metabolic process	23	5.57%	0.0014662
Biol Process level 3	cellular lipid metabolic process	23	5.57%	0.0018197
Biol Process level 2	rhythmic behavior	8	1.94%	0.0018381
Biol Process level 3	rhythmic behavior	8	1.94%	0.0018859
Biol Process level 1	response to stimulus	48	11.62%	0.0044975
Mol Function level 3	oxidoreductase activity, acting on sulfur group of donors	5	1.21%	0.0047919
Mol Function level 3	protein kinase regulator activity	7	1.69%	0.005311
Mol Function level 2	kinase regulator activity	8	1.94%	0.0054863
Biol Process level 4	circadian behavior	7	1.69%	0.0066873
Biol Process level 3	circadian behavior	7	1.69%	0.0072193
Biol Process level 1	biological adhesion	17	4.12%	0.0087802
Biol Process level 2	cell adhesion	17	4.12%	0.0088381
Biol Process level 4	maintenance of protein localization	4	0.97%	0.0102032
Biol Process level 5	prosthetic group metabolic process	5	1.21%	0.0102412
Biol Process level 3	chemosensory behavior	9	2.18%	0.010311
Biol Process level 2	maintenance of protein localization	4	0.97%	0.0105234
Biol Process level 3	maintenance of protein localization	4	0.97%	0.0106556
Biol Process level 4	learning	7	1.69%	0.0107586
Biol Process level 5	establishment and/or maintenance of cytoskeleton polarity	6	1.45%	0.0110295
Biol Process level 3	prosthetic group metabolic process	5	1.21%	0.0112753
Biol Process level 4	oocyte microtubule cytoskeleton organization	6	1.45%	0.0115858
Cell Component level 4	endoplasmic reticulum part	10	2.42%	0.0117409
Cell Component level 3	endoplasmic reticulum part	10	2.42%	0.0118183
Cell Component level 5	endoplasmic reticulum part	10	2.42%	0.0126273
Biol Process level 3	learning and/or memory	9	2.18%	0.0143174
Mol Function level 3	lipid transporter activity	5	1.21%	0.0153015
Biol Process level 2	response to abiotic stimulus	14	3.39%	0.0164695
Biol Process level 5	oocyte axis determination	11	2.66%	0.0170473
Biol Process level 5	olfactory learning	6	1.45%	0.0180273
Biol Process level 4	oocyte axis determination	11	2.66%	0.0184252
Mol Function level 5	protein-methionine-R-oxide reductase activity	3	0.73%	0.0191484
Cell Component level 4	germline ring canal	4	0.97%	0.0201784
Cell Component level 3	germline ring canal	4	0.97%	0.0201915
Cell Component level 3	intercellular bridge	4	0.97%	0.0201915
Cell Component level 2	intercellular bridge	4	0.97%	0.0204245
Biol Process level 4	sequestering of lipid	3	0.73%	0.0206687

Table 4 continued.

Category	Term	Count	%	PValue
Biol Process level 5	oocyte construction	11	2.66%	0.0215182
Biol Process level 2	response to chemical stimulus	17	4.12%	0.0215598
Biol Process level 3	locomotory behavior	11	2.66%	0.0228915
Biol Process level 1	establishment of localization	79	19.13%	0.023452
Biol Process level 2	transport	78	18.89%	0.0235355
Biol Process level 2	establishment of localization	79	19.13%	0.0236624
Biol Process level 5	oocyte development	11	2.66%	0.0240492
Biol Process level 2	cell motility	17	4.12%	0.0252634
Biol Process level 3	oocyte construction	11	2.66%	0.0256152
Biol Process level 3	transport	78	18.89%	0.0258479
Biol Process level 2	sleep	4	0.97%	0.0258566
Biol Process level 4	oocyte development	11	2.66%	0.025918
Biol Process level 3	cell motility	17	4.12%	0.0261964
Biol Process level 4	lipid transport	6	1.45%	0.0287432
Biol Process level 3	lipid transport	6	1.45%	0.0305132
Biol Process level 1	multicellular organismal process	90	21.79%	0.0305133
Biol Process level 5	germ cell development	13	3.15%	0.0313704
Mol Function level 2	oxidoreductase activity	34	8.23%	0.0323092
Cell Component level 1	extracellular region	14	3.39%	0.032869
Biol Process level 4	germ cell development	13	3.15%	0.0340403
Biol Process level 2	localization of cell	17	4.12%	0.0340801
Biol Process level 1	developmental process	85	20.58%	0.0341666
Cell Component level 4	integral to membrane	53	12.83%	0.0349069
Biol Process level 3	response to wounding	4	0.97%	0.0367741
Cell Component level 4	intrinsic to membrane	53	12.83%	0.0376841
Biol Process level 3	germ cell development	13	3.15%	0.0378387
Biol Process level 2	response to external stimulus	12	2.91%	0.0392604
Biol Process level 5	cell redox homeostasis	6	1.45%	0.0395485
Cell Component level 3	intrinsic to membrane	53	12.83%	0.0398333
Biol Process level 4	axis specification	13	3.15%	0.0403711
Cell Component level 5	integral to membrane	53	12.83%	0.0423426
Biol Process level 4	oocyte differentiation	11	2.66%	0.0428938
Biol Process level 3	cell redox homeostasis	6	1.45%	0.0437733
Biol Process level 3	axis specification	13	3.15%	0.0447613
Biol Process level 5	membrane lipid metabolic process	9	2.18%	0.0454009
Cell Component level 5	intrinsic to membrane	53	12.83%	0.0456156
Biol Process level 5	nerve-nerve synaptic transmission	4	0.97%	0.045949
Biol Process level 4	membrane lipid metabolic process	9	2.18%	0.0481834

Table 5. Significantly enriched GO categories for genes differentially expressed between morning- and afternoon-training groups.

GO Category	Term	Count	%	PValue
Biol Process level 5	mesoderm development	6	8.70%	0.00125
Biol Process level 4	mesoderm development	6	8.70%	0.00156
Biol Process level 2	catabolic process	10	14.49%	0.0037
Mol Function level 5	phosphoric diester hydrolase activity	4	5.80%	0.00408
Mol Function level 2	kinase regulator activity	4	5.80%	0.00884
Biol Process level 3	macromolecule catabolic process	7	10.14%	0.01226
Biol Process level 3	cellular catabolic process	8	11.59%	0.02177
Biol Process level 5	tissue development	6	8.70%	0.02218
Biol Process level 4	tissue development	6	8.70%	0.02661
Mol Function level 3	oxidoreductase activity, acting on CH-CH group of donors	3	4.35%	0.02812
Biol Process level 3	tissue development	6	8.70%	0.02948
Biol Process level 4	carbohydrate catabolic process	4	5.80%	0.03153
Mol Function level 2	two-component response regulator activity	2	2.90%	0.03198
Mol Function level 5	fructose-bisphosphate aldolase activity	2	2.90%	0.03513
Mol Function level 3	two-component response regulator activity	2	2.90%	0.03528
Biol Process level 4	response to heat	3	4.35%	0.04237
Cell Component level 3	organelle inner membrane	6	8.70%	0.0441
Biol Process level 3	response to heat	3	4.35%	0.0444
Mol Function level 3	protein kinase regulator activity	3	4.35%	0.0481

Table 6. Significantly enriched GO categories for genes more highly expressed in the morning training group.

Category	Term	Count	%	PValue
Mol Function level 2	kinase regulator activity	3	12.00%	0.011422
Mol Function level 5	fructose-bisphosphate aldolase activity	2	8.00%	0.0152706
Biol Process level 2	catabolic process	5	20.00%	0.0268475
Biol Process level 4	carbohydrate catabolic process	3	12.00%	0.0291361
Mol Function level 4	aldehyde-lyase activity	2	8.00%	0.0344794
Biol Process level 3	macromolecule catabolic process	4	16.00%	0.0352098
Mol Function level 3	kinase inhibitor activity	2	8.00%	0.0411818
Biol Process level 4	mesoderm development	3	12.00%	0.0439602
Biol Process level 5	mesoderm development	3	12.00%	0.0472205

Table 7. Significantly enriched GO categories for genes more highly expressed in the afternoon training group.

Category	Term	Count	%	PValue
Biol Process level 5	nucleotide metabolic process	5	11.11%	0.0081195
Mol Function level 3	oxidoreductase activity, acting on the CH-CH group of donors	3	6.67%	0.0108935
Biol Process level 2	response to abiotic stimulus	5	11.11%	0.0112865
Biol Process level 4	response to heat	3	6.67%	0.0169156
Biol Process level 3	response to heat	3	6.67%	0.0183582
Biol Process level 4	nucleobase, nucleoside and nucleotide metabolic process	5	11.11%	0.0191574
Mol Function level 2	two-component response regulator activity	2	4.44%	0.0199138
Mol Function level 3	two-component response regulator activity	2	4.44%	0.0215197
Biol Process level 3	response to temperature stimulus	3	6.67%	0.0242887
Biol Process level 5	establishment and/or maintenance of chromatin architecture	4	8.89%	0.0254058
Biol Process level 5	DNA packaging	4	8.89%	0.0254058
Cell Component level 3	organelle inner membrane	5	11.11%	0.0408723
Biol Process level 4	two-component signal transduction system (phosphorelay)	2	4.44%	0.0421511
Cell Component level 4	organelle inner membrane	5	11.11%	0.0444113
Cell Component level 4	mitochondrial inner membrane	5	11.11%	0.0444113
Cell Component level 2	organelle membrane	7	15.56%	0.0444657
Cell Component level 3	organelle membrane	7	15.56%	0.0445454
Cell Component level 3	mitochondrial part	7	15.56%	0.0452947
Cell Component level 5	mitochondrial inner membrane	5	11.11%	0.0483786
Cell Component level 5	organelle inner membrane	5	11.11%	0.0483786
Cell Component level 4	organelle membrane	7	15.56%	0.0494455

Table 8. Significantly enriched GO categories for genes differentially expressed between anticipating bees and their inactive nestmates.

GO Category	Term	Count	%	PValue
Mol Function level 2	structural constituent of ribosome	91	7.97%	5.70E-28
Cell Component level 3	ribosome	90	7.88%	3.53E-26
Cell Component level 4	ribosome	90	7.88%	6.54E-25
Cell Component level 3	ribosomal subunit	80	7.01%	1.82E-24
Cell Component level 5	ribosome	90	7.88%	2.45E-24
Cell Component level 4	ribosomal subunit	80	7.01%	2.57E-23
Cell Component level 5	ribosomal subunit	80	7.01%	8.46E-23
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	51	4.47%	1.36E-21
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	51	4.47%	3.11E-21
Cell Component level 2	ribonucleoprotein complex	109	9.54%	2.08E-18
Cell Component level 3	ribonucleoprotein complex	109	9.54%	2.15E-18
Cell Component level 4	cytosolic part	57	4.99%	6.49E-18
Cell Component level 5	cytosolic part	57	4.99%	1.53E-17
Cell Component level 4	ribonucleoprotein complex	109	9.54%	4.26E-17
Biol Process level 2	translation	111	9.72%	1.08E-16
Cell Component level 5	ribonucleoprotein complex	109	9.54%	2.75E-16
Cell Component level 1	macromolecular complex	285	24.96%	2.80E-16
Biol Process level 5	translation	111	9.72%	3.17E-16
Biol Process level 4	translation	111	9.72%	4.11E-16
Biol Process level 3	cellular biosynthetic process	174	15.24%	5.28E-16
Mol Function level 1	structural molecule activity	109	9.54%	5.67E-15
Cell Component level 3	cytoplasmic part	276	24.17%	1.34E-13
Cell Component level 3	cytoplasm	319	27.93%	4.17E-13
Cell Component level 3	small ribosomal subunit	35	3.06%	1.23E-12
Biol Process level 3	macromolecule biosynthetic process	126	11.03%	1.99E-12
Cell Component level 5	cytosolic large ribosomal subunit (sensu Eukaryota)	31	2.71%	2.88E-12
Cell Component level 4	small ribosomal subunit	35	3.06%	4.24E-12
Cell Component level 5	small ribosomal subunit	35	3.06%	7.25E-12
Biol Process level 2	biosynthetic process	184	16.11%	8.12E-12
Cell Component level 4	cytoplasmic part	276	24.17%	1.78E-11
Cell Component level 4	large ribosomal subunit	46	4.03%	3.27E-11
Cell Component level 5	large ribosomal subunit	46	4.03%	6.20E-11
Cell Component level 4	cytoplasm	319	27.93%	8.49E-11
Cell Component level 5	cytoplasmic part	276	24.17%	1.86E-10
Cell Component level 3	mitochondrial part	102	8.93%	6.03E-10
Cell Component level 5	cytoplasm	319	27.93%	1.18E-09
Cell Component level 2	non-membrane-bound organelle	137	12.00%	1.43E-09
Cell Component level 3	intracellular non-membrane-bound organelle	137	12.00%	1.47E-09
Cell Component level 2	organelle part	256	22.42%	1.81E-09
Cell Component level 2	intracellular organelle part	255	22.33%	2.42E-09
Cell Component level 3	intracellular organelle part	255	22.33%	2.57E-09
Cell Component level 1	organelle part	256	22.42%	3.86E-09
Cell Component level 4	cytosol	69	6.04%	4.98E-09
Cell Component level 4	mitochondrial part	102	8.93%	6.20E-09
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	20	1.75%	6.65E-09
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	20	1.75%	9.13E-09
Cell Component level 5	cytosol	69	6.04%	1.09E-08
Cell Component level 5	mitochondrial part	102	8.93%	1.71E-08
Biol Process level 3	oxidative phosphorylation	45	3.94%	2.04E-08
Cell Component level 4	intracellular non-membrane-bound organelle	137	12.00%	2.32E-08
Cell Component level 3	mitochondrial membrane part	45	3.94%	3.56E-08

Table 8 continued.

GO Category	Term	Count	%	PValue
Cell Component level 2	protein complex	214	18.74%	4.29E-08
Cell Component level 5	intracellular non-membrane-bound organelle	137	12.00%	7.73E-08
Cell Component level 4	mitochondrial membrane part	45	3.94%	1.28E-07
Cell Component level 4	intracellular organelle part	255	22.33%	1.52E-07
Cell Component level 4	mitochondrion	115	10.07%	1.77E-07
Cell Component level 5	mitochondrial membrane part	45	3.94%	2.22E-07
Cell Component level 5	mitochondrion	115	10.07%	4.89E-07
Cell Component level 5	intracellular organelle part	255	22.33%	9.48E-07
Mol Function level 3	substrate-specific transmembrane transporter activity	94	8.23%	1.83E-06
Biol Process level 2	generation of precursor metabolites and energy	67	5.87%	2.82E-06
Cell Component level 4	mitochondrial respiratory chain	29	2.54%	1.15E-05
Cell Component level 4	lipid particle	42	3.68%	1.50E-05
Cell Component level 5	mitochondrial respiratory chain	29	2.54%	1.65E-05
Cell Component level 3	mitochondrial lumen	45	3.94%	2.17E-05
Cell Component level 4	mitochondrial ribosome	31	2.71%	2.18E-05
Cell Component level 4	organellar ribosome	31	2.71%	2.18E-05
Cell Component level 5	lipid particle	42	3.68%	2.38E-05
Mol Function level 5	cation transmembrane transporter activity	64	5.60%	2.89E-05
Cell Component level 3	mitochondrial envelope	58	5.08%	3.15E-05
Cell Component level 5	mitochondrial ribosome	31	2.71%	3.15E-05
Cell Component level 5	organellar ribosome	31	2.71%	3.15E-05
Cell Component level 3	mitochondrial membrane	53	4.64%	5.91E-05
Cell Component level 4	mitochondrial matrix	45	3.94%	6.35E-05
Cell Component level 4	mitochondrial lumen	45	3.94%	6.35E-05
Cell Component level 5	mitochondrial matrix	45	3.94%	9.99E-05
Cell Component level 5	mitochondrial lumen	45	3.94%	9.99E-05
Cell Component level 3	organelle inner membrane	49	4.29%	1.02E-04
Biol Process level 5	organelle ATP synthesis coupled electron transport	25	2.19%	1.02E-04
Biol Process level 4	ATP synthesis coupled electron transport	25	2.19%	1.08E-04
Cell Component level 4	mitochondrial envelope	58	5.08%	1.09E-04
Mol Function level 2	substrate-specific transporter activity	101	8.84%	1.20E-04
Cell Component level 2	intracellular organelle	367	32.14%	1.41E-04
Cell Component level 3	intracellular organelle	367	32.14%	1.49E-04
Cell Component level 2	intracellular part	434	38.00%	1.57E-04
Mol Function level 4	ion transmembrane transporter activity	72	6.30%	1.60E-04
Cell Component level 3	intracellular part	434	38.00%	1.68E-04
Cell Component level 5	mitochondrial envelope	58	5.08%	1.83E-04
Cell Component level 4	mitochondrial membrane	53	4.64%	1.85E-04
Biol Process level 3	electron transport	47	4.12%	1.91E-04
Cell Component level 1	organelle	368	32.22%	2.48E-04
Cell Component level 4	mitochondrial inner membrane	49	4.29%	2.94E-04
Cell Component level 4	organelle inner membrane	49	4.29%	2.94E-04
Cell Component level 5	mitochondrial membrane	53	4.64%	2.98E-04
Mol Function level 2	oxidoreductase activity	92	8.06%	3.30E-04
Cell Component level 4	mitochondrial small ribosomal subunit	15	1.31%	4.07E-04
Cell Component level 4	organellar small ribosomal subunit	15	1.31%	4.07E-04
Cell Component level 5	mitochondrial inner membrane	49	4.29%	4.59E-04
Cell Component level 5	organelle inner membrane	49	4.29%	4.59E-04
Mol Function level 4	primary active transmembrane transporter activity	33	2.89%	4.92E-04
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transport	33	2.89%	4.92E-04
Cell Component level 5	mitochondrial small ribosomal subunit	15	1.31%	4.96E-04
Cell Component level 5	organellar small ribosomal subunit	15	1.31%	4.96E-04
Mol Function level 2	transmembrane transporter activity	99	8.67%	6.91E-04
Biol Process level 3	protein metabolic process	261	22.85%	0.001267

Table 8 continued.

GO Category	Term	Count	%	PValue
Biol Process level 5	phosphorylation	78	6.83%	0.001786
Mol Function level 3	active transmembrane transporter activity	51	4.47%	0.001917
Mol Function level 1	transporter activity	115	10.07%	0.002074
Mol Function level 5	ATPase activity, coupled to transmembrane movement	22	1.93%	0.002118
Biol Process level 2	behavior	47	4.12%	0.00223
Cell Component level 2	organelle envelope	61	5.34%	0.002349
Cell Component level 3	organelle envelope	61	5.34%	0.002374
Biol Process level 1	metabolic process	526	46.06%	0.002606
Cell Component level 1	envelope	61	5.34%	0.002656
Cell Component level 2	organelle membrane	79	6.92%	0.002811
Cell Component level 3	organelle membrane	79	6.92%	0.002846
Biol Process level 5	protein import into mitochondrion	7	0.61%	0.003261
Biol Process level 5	inner mitochondrial membrane organization and biog	6	0.53%	0.003712
Biol Process level 2	cellular metabolic process	483	42.29%	0.003747
Cell Component level 3	proton-transporting two-sector ATPase complex	17	1.49%	0.004454
Biol Process level 4	cellular protein metabolic process	249	21.80%	0.005056
Mol Function level 3	heme-copper terminal oxidase activity	7	0.61%	0.0051
Mol Function level 3	oxidoreductase activity, acting on heme group of donors	7	0.61%	0.0051
Biol Process level 5	ATP biosynthetic process	15	1.31%	0.005125
Mol Function level 4	cytochrome-c oxidase activity	7	0.61%	0.005794
Mol Function level 4	oxidoreductase activity, acting on diphenols	7	0.61%	0.005794
Mol Function level 4	oxidoreductase activity, acting on heme group of donors	7	0.61%	0.005794
Mol Function level 5	cytochrome-c oxidase activity	7	0.61%	0.005856
Mol Function level 5	ubiquinol-cytochrome-c reductase activity	7	0.61%	0.005856
Mol Function level 4	P-P-bond-hydrolysis-driven protein transmembrane	10	0.88%	0.006009
Mol Function level 4	macromolecule transmembrane transporter activity	10	0.88%	0.006009
Mol Function level 5	P-P-bond-hydrolysis-driven protein transmembrane	10	0.88%	0.006085
Cell Component level 4	organelle envelope	61	5.34%	0.006439
Cell Component level 4	proton-transporting two-sector ATPase complex	17	1.49%	0.006884
Biol Process level 4	ATP synthesis coupled proton transport	14	1.23%	0.007512
Biol Process level 4	ATP metabolic process	15	1.31%	0.007551
Cell Component level 5	proton-transporting two-sector ATPase complex	17	1.49%	0.008261
Cell Component level 4	intracellular organelle	367	32.14%	0.008338
Cell Component level 4	organelle membrane	79	6.92%	0.008917
Biol Process level 3	cellular macromolecule metabolic process	250	21.89%	0.008971
Cell Component level 2	intracellular	455	39.84%	0.009311
Cell Component level 5	organelle envelope	61	5.34%	0.009669
Cell Component level 3	receptor complex	6	0.53%	0.009838
Cell Component level 3	intracellular	455	39.84%	0.009852
Mol Function level 3	oxidoreductase activity, acting on diphenols	7	0.61%	0.010391
Biol Process level 5	mitochondrial membrane organization and biogenesis	6	0.53%	0.010437
Biol Process level 4	mitochondrial membrane organization and biogenesis	6	0.53%	0.010578
Mol Function level 4	oxidoreductase activity, acting on sulfur group	5	0.44%	0.010601
Mol Function level 5	glycerol kinase activity	5	0.44%	0.010691
Mol Function level 5	peptide-methionine-(S)-S-oxide reductase activity	5	0.44%	0.010691
Cell Component level 3	proton-transporting two-sector ATPase complex	9	0.79%	0.011909
Mol Function level 3	protein transmembrane transporter activity	11	0.96%	0.012167
Mol Function level 4	NADH dehydrogenase activity	14	1.23%	0.012224
Cell Component level 3	mitochondrial intermembrane space protein transporter	5	0.44%	0.012498
Biol Process level 3	chemosensory behavior	16	1.40%	0.012661
Biol Process level 1	rhythmic process	13	1.14%	0.013624
Biol Process level 3	phosphorus metabolic process	88	7.71%	0.014416
Mol Function level 4	protein transmembrane transporter activity	11	0.96%	0.01442
Biol Process level 5	second-messenger-mediated signaling	14	1.23%	0.014463

Table 8 continued.

GO Category	Term	Count	%	PValue
Cell Component level 3	respiratory chain complex III	7	0.61%	0.014628
Cell Component level 4	mitochondrial intermembrane space protein transpor	5	0.44%	0.014639
Mol Function level 4	hydrolase activity, acting on acid anhydrides	24	2.10%	0.014643
Biol Process level 5	purine nucleotide biosynthetic process	19	1.66%	0.014738
Cell Component level 4	proton-transporting two-sector ATPase complex	9	0.79%	0.015422
Cell Component level 5	mitochondrial intermembrane space protein transport	5	0.44%	0.015654
Cell Component level 5	proton-transporting two-sector ATPase complex	9	0.79%	0.017196
Cell Component level 3	respiratory chain complex I	14	1.23%	0.017575
Cell Component level 3	NADH dehydrogenase complex (quinone)	14	1.23%	0.017575
Cell Component level 4	respiratory chain complex III	7	0.61%	0.018036
Cell Component level 4	mitochondrial respiratory chain complex III	7	0.61%	0.018036
Biol Process level 3	response to organic substance	8	0.70%	0.01816
Biol Process level 4	phosphate metabolic process	88	7.71%	0.018179
Biol Process level 3	cofactor metabolic process	40	3.50%	0.018848
Biol Process level 2	rhythmic behavior	11	0.96%	0.019637
Cell Component level 5	respiratory chain complex III	7	0.61%	0.019703
Cell Component level 5	mitochondrial respiratory chain complex III	7	0.61%	0.019703
Cell Component level 2	membrane part	177	15.50%	0.020378
Biol Process level 3	rhythmic behavior	11	0.96%	0.020485
Cell Component level 3	membrane part	177	15.50%	0.020595
Cell Component level 3	respiratory chain complex IV	6	0.53%	0.021179
Biol Process level 3	hydrogen transport	16	1.40%	0.021778
Biol Process level 4	mitochondrial electron transport, ubiquinol to cyt	6	0.53%	0.022682
Biol Process level 5	proton transport	16	1.40%	0.023001
Cell Component level 4	intracellular part	434	38.00%	0.023121
Cell Component level 3	nuclear envelope-endoplasmic reticulum network	14	1.23%	0.023402
Cell Component level 3	endoplasmic reticulum membrane	14	1.23%	0.023402
Biol Process level 4	hydrogen transport	16	1.40%	0.023625
Biol Process level 4	proton transport	16	1.40%	0.023625
Mol Function level 5	ATPase activity, coupled to transmembrane movement	23	2.01%	0.024124
Biol Process level 4	cofactor biosynthetic process	24	2.10%	0.024266
Biol Process level 5	nucleoside triphosphate biosynthetic process	15	1.31%	0.024509
Cell Component level 4	respiratory chain complex I	14	1.23%	0.024666
Cell Component level 4	mitochondrial respiratory chain complex I	14	1.23%	0.024666
Cell Component level 4	NADH dehydrogenase complex (quinone)	14	1.23%	0.024666
Cell Component level 4	respiratory chain complex IV	6	0.53%	0.025334
Cell Component level 4	mitochondrial respiratory chain complex IV	6	0.53%	0.025334
Biol Process level 2	maintenance of localization	7	0.61%	0.025924
Biol Process level 1	maintenance of localization	7	0.61%	0.026176
Biol Process level 2	circadian rhythm	12	1.05%	0.026402
Mol Function level 3	oxidoreductase activity, acting on NADH or NADPH	15	1.31%	0.026467
Biol Process level 4	mitochondrial electron transport, NADH to ubiquinone	14	1.23%	0.02665
Cell Component level 5	respiratory chain complex IV	6	0.53%	0.027326
Cell Component level 5	mitochondrial respiratory chain complex IV	6	0.53%	0.027326
Biol Process level 1	gene expression	192	16.81%	0.027374
Biol Process level 5	male mating behavior	7	0.61%	0.027527
Biol Process level 5	male courtship behavior	7	0.61%	0.027527
Biol Process level 4	male mating behavior	7	0.61%	0.02792
Cell Component level 5	respiratory chain complex I	14	1.23%	0.028412
Cell Component level 5	mitochondrial respiratory chain complex I	14	1.23%	0.028412
Cell Component level 5	NADH dehydrogenase complex (quinone)	14	1.23%	0.028412
Mol Function level 3	oxidoreductase activity, acting on sulfur group of	6	0.53%	0.02921
Biol Process level 2	maintenance of protein localization	5	0.44%	0.030557
Biol Process level 5	protein targeting to mitochondrion	10	0.88%	0.030724

Table 8 continued.

GO Category	Term	Count	%	PValue
Biol Process level 5	fatty acid biosynthetic process	9	0.79%	0.030869
Biol Process level 3	maintenance of protein localization	5	0.44%	0.031233
Biol Process level 4	fatty acid biosynthetic process	9	0.79%	0.0314
Mol Function level 4	rRNA binding	6	0.53%	0.032258
Biol Process level 4	maintenance of protein localization	5	0.44%	0.032291
Cell Component level 4	endoplasmic reticulum membrane	14	1.23%	0.032497
Cell Component level 4	nuclear envelope-endoplasmic reticulum network	14	1.23%	0.032497
Biol Process level 4	coenzyme metabolic process	36	3.15%	0.03479
Biol Process level 3	ion transport	49	4.29%	0.037092
Cell Component level 5	endoplasmic reticulum membrane	14	1.23%	0.037255
Cell Component level 5	nuclear envelope-endoplasmic reticulum network	14	1.23%	0.037255
Mol Function level 4	oxidoreductase activity, acting on NADH or NADPH	11	0.96%	0.037803
Mol Function level 4	hydrolase activity, acting on carbon-nitrogen	4	0.35%	0.038192
Mol Function level 5	NADH dehydrogenase (quinone) activity	11	0.96%	0.038271
Cell Component level 5	intracellular organelle	367	32.14%	0.039331
Mol Function level 3	transferase activity, transferring alkyl or aryl	9	0.79%	0.03961
Biol Process level 2	behavioral interaction between organisms	10	0.88%	0.039653
Biol Process level 3	behavioral interaction between organisms	10	0.88%	0.041129
Biol Process level 3	circadian behavior	10	0.88%	0.041129
Biol Process level 3	mating behavior	10	0.88%	0.041129
Cell Component level 3	mitochondrial inner membrane presequence translocation	7	0.61%	0.041624
Biol Process level 5	cation transport	42	3.68%	0.041817
Biol Process level 4	ion transport	49	4.29%	0.042978
Biol Process level 4	mating behavior	10	0.88%	0.043467
Biol Process level 4	circadian behavior	10	0.88%	0.043467
Biol Process level 5	coenzyme biosynthetic process	21	1.84%	0.043554
Biol Process level 4	cation transport	42	3.68%	0.043792
Biol Process level 5	carboxylic acid biosynthetic process	9	0.79%	0.044252
Biol Process level 5	polyamine metabolic process	4	0.35%	0.044721
Biol Process level 4	organic acid biosynthetic process	9	0.79%	0.044979
Mol Function level 3	electron carrier activity	26	2.28%	0.04503
Biol Process level 5	ribonucleotide biosynthetic process	18	1.58%	0.047223
Cell Component level 4	mitochondrial large ribosomal subunit	15	1.31%	0.049846

Table 9. Significantly enriched GO categories for genes more highly expressed in active anticipating bees, as compared to inactive bees.

Category	Term	Count	%	PValue
Biol Process level 2	behavior	39	7.20%	1.04E-07
Biol Process level 3	neurological system process	55	10.15%	2.86E-07
Mol Function level 2	substrate-specific transporter activity	65	11.99%	9.26E-07
Biol Process level 2	system process	55	10.15%	9.78E-07
Cell Component level 4	plasma membrane part	40	7.38%	1.22E-06
Cell Component level 3	plasma membrane part	40	7.38%	1.54E-06
Biol Process level 5	synaptic transmission	36	6.64%	1.57E-06
Cell Component level 5	plasma membrane part	40	7.38%	1.58E-06
Biol Process level 4	synaptic transmission	36	6.64%	2.89E-06
Biol Process level 1	multicellular organismal process	143	26.38%	3.08E-06
Mol Function level 2	transmembrane transporter activity	64	11.81%	3.99E-06
Mol Function level 3	substrate-specific transmembrane transporter activity	61	11.25%	5.18E-06
Biol Process level 1	response to stimulus	74	13.65%	5.71E-06
Mol Function level 1	transporter activity	74	13.65%	6.20E-06
Biol Process level 4	transmission of nerve impulse	41	7.56%	8.74E-06
Cell Component level 2	membrane	140	25.83%	1.21E-05
Cell Component level 3	membrane	140	25.83%	1.24E-05
Biol Process level 2	cell communication	113	20.85%	1.33E-05
Cell Component level 4	membrane part	107	19.74%	1.38E-05
Biol Process level 3	ion transport	39	7.20%	1.51E-05
Biol Process level 4	ion transport	39	7.20%	1.85E-05
Cell Component level 4	intrinsic to plasma membrane	24	4.43%	1.89E-05
Cell Component level 5	intrinsic to plasma membrane	24	4.43%	2.24E-05
Cell Component level 2	membrane part	107	19.74%	2.75E-05
Cell Component level 3	membrane part	107	19.74%	2.80E-05
Biol Process level 3	chemosensory behavior	15	2.77%	3.18E-05
Mol Function level 4	ion transmembrane transporter activity	50	9.23%	3.48E-05
Biol Process level 3	cell-cell signaling	42	7.75%	3.53E-05
Biol Process level 4	intracellular signaling cascade	52	9.59%	6.91E-05
Biol Process level 2	response to chemical stimulus	28	5.17%	1.11E-04
Cell Component level 4	plasma membrane	51	9.41%	1.13E-04
Biol Process level 5	cation transport	32	5.90%	1.20E-04
Biol Process level 1	establishment of localization	119	21.96%	1.23E-04
Cell Component level 3	plasma membrane	51	9.41%	1.44E-04
Mol Function level 5	cation transmembrane transporter activity	42	7.75%	1.48E-04
Cell Component level 5	integral to plasma membrane	22	4.06%	1.50E-04
Biol Process level 2	establishment of localization	119	21.96%	1.51E-04
Biol Process level 3	transport	117	21.59%	1.83E-04
Biol Process level 4	cation transport	32	5.90%	1.92E-04
Biol Process level 2	transport	117	21.59%	1.96E-04
Biol Process level 4	olfactory behavior	13	2.40%	2.75E-04
Biol Process level 3	learning and/or memory	14	2.58%	2.81E-04
Mol Function level 3	active transmembrane transporter activity	36	6.64%	3.08E-04
Cell Component level 4	intrinsic to membrane	75	13.84%	3.27E-04
Biol Process level 5	second-messenger-mediated signaling	12	2.21%	3.68E-04
Cell Component level 5	intrinsic to membrane	75	13.84%	4.51E-04
Cell Component level 3	intrinsic to membrane	75	13.84%	4.58E-04
Cell Component level 4	integral to membrane	74	13.65%	4.95E-04
Biol Process level 2	anatomical structure development	98	18.08%	6.02E-04
Cell Component level 5	integral to membrane	74	13.65%	6.77E-04
Biol Process level 1	rhythmic process	11	2.03%	6.79E-04
Biol Process level 1	localization	129	23.80%	7.38E-04

Table 9 continued.

Category	Term	Count	%	PValue
Biol Process level 5	olfactory learning	9	1.66%	8.24E-04
Biol Process level 3	system development	82	15.13%	8.33E-04
Biol Process level 2	multicellular organismal development	108	19.93%	0.001386
Biol Process level 1	developmental process	123	22.69%	0.001756
Biol Process level 3	signal transduction	87	16.05%	0.001841
Biol Process level 2	response to abiotic stimulus	20	3.69%	0.001917
Mol Function level 5	ATPase activity, coupled to transmembrane movement of ions	16	2.95%	0.001918
Biol Process level 2	circadian rhythm	10	1.85%	0.002271
Biol Process level 3	response to organic substance	7	1.29%	0.002358
Biol Process level 3	cytoplasm organization and biogenesis	15	2.77%	0.002425
Biol Process level 3	rhythmic behavior	9	1.66%	0.002784
Biol Process level 2	rhythmic behavior	9	1.66%	0.002797
Biol Process level 5	monovalent inorganic cation transport	21	3.87%	0.002878
Biol Process level 5	regulation of tube size, open tracheal system	7	1.29%	0.003446
Biol Process level 4	regulation of tube size, open tracheal system	7	1.29%	0.003905
	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	18	3.32%	0.003918
Mol Function level 4	learning	9	1.66%	0.004071
Biol Process level 4	tissue development	22	4.06%	0.004183
Biol Process level 5	ATP synthesis coupled proton transport	10	1.85%	0.004285
Biol Process level 3	organ development	63	11.62%	0.004891
Biol Process level 3	tissue development	22	4.06%	0.005023
Biol Process level 2	cell development	63	11.62%	0.005558
Biol Process level 4	tissue development	22	4.06%	0.005607
Cell Component level 4	proton-transporting two-sector ATPase complex	11	2.03%	0.005709
Cell Component level 3	proton-transporting two-sector ATPase complex	11	2.03%	0.005925
Biol Process level 5	ATP biosynthetic process	10	1.85%	0.006057
Biol Process level 4	organ development	63	11.62%	0.006078
Cell Component level 5	proton-transporting two-sector ATPase complex	11	2.03%	0.006163
Mol Function level 2	vitamin binding	7	1.29%	0.006451
Biol Process level 4	cell development	63	11.62%	0.006518
Cell Component level 4	lipid particle	20	3.69%	0.006848
Biol Process level 3	cell differentiation	71	13.10%	0.007448
Cell Component level 5	lipid particle	20	3.69%	0.007672
Biol Process level 5	phosphorylation	42	7.75%	0.008107
Biol Process level 5	intercellular junction assembly	8	1.48%	0.008123
	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in cyclic amides	4	0.74%	0.00816
Mol Function level 4	circadian behavior	8	1.48%	0.0088
Biol Process level 3	ATP metabolic process	10	1.85%	0.008991
Biol Process level 4	circadian behavior	8	1.48%	0.009278
	ATPase activity, coupled to transmembrane movement of substances	17	3.14%	0.009528
Mol Function level 5	adult behavior	11	2.03%	0.009635
Biol Process level 3	protein complex assembly	16	2.95%	0.009745
Biol Process level 5	cellular developmental process	71	13.10%	0.010013
Biol Process level 2	regulation of tube size	7	1.29%	0.010428
Biol Process level 5	carbohydrate metabolic process	32	5.90%	0.010492
Mol Function level 1	catalytic activity	220	40.59%	0.010505
Biol Process level 5	regulation of tube architecture, open tracheal system	8	1.48%	0.010732
Biol Process level 3	regulation of tube size	7	1.29%	0.011186
Biol Process level 5	synaptic vesicle transport	16	2.95%	0.011197
Biol Process level 3	regulation of tube architecture, open tracheal system	8	1.48%	0.011607
Biol Process level 4	regulation of tube size	7	1.29%	0.011722
Biol Process level 4	protein complex assembly	16	2.95%	0.012129

Table 9 continued.

Category	Term	Count	%	PValue
Biol Process level 5	regulation of neurotransmitter levels	19	3.51%	0.012717
Biol Process level 5	regulation of tube length, open tracheal system	4	0.74%	0.013701
Biol Process level 5	regulation of tube diameter, open tracheal system	4	0.74%	0.013701
Biol Process level 4	synaptic vesicle transport	16	2.95%	0.013895
Biol Process level 3	response to drug	4	0.74%	0.014264
Biol Process level 3	regulation of tube length, open tracheal system	4	0.74%	0.014264
Biol Process level 4	regulation of tube diameter, open tracheal system	4	0.74%	0.014665
Biol Process level 3	regulation of neurotransmitter levels	19	3.51%	0.014741
Biol Process level 5	endothelial cell development	5	0.92%	0.014961
Biol Process level 4	response to light stimulus	11	2.03%	0.015068
Biol Process level 3	locomotory behavior	14	2.58%	0.0151
Mol Function level 2	transferase activity	75	13.84%	0.015383
Biol Process level 3	endothelial cell development	5	0.92%	0.015735
Biol Process level 4	intercellular junction assembly and maintenance	8	1.48%	0.015793
Biol Process level 1	biological regulation	119	21.96%	0.016158
Biol Process level 4	endothelial cell differentiation	5	0.92%	0.016284
Biol Process level 3	secretion	27	4.98%	0.016288
Biol Process level 2	secretion	27	4.98%	0.016455
Biol Process level 3	phosphorus metabolic process	49	9.04%	0.017167
Mol Function level 4	primary active transmembrane transporter activity	19	3.51%	0.017378
Biol Process level 5	secretory pathway	26	4.80%	0.017394
Biol Process level 5	nucleoside triphosphate biosynthetic process	10	1.85%	0.017531
Biol Process level 5	lipid biosynthetic process	14	2.58%	0.017829
Cell Component level 5	microtubule cytoskeleton	16	2.95%	0.017944
Mol Function level 4	ARF guanyl-nucleotide exchange factor activity	4	0.74%	0.018417
Biol Process level 5	courtship behavior	7	1.29%	0.018632
Biol Process level 5	secretion by cell	26	4.80%	0.018958
Biol Process level 3	lipid biosynthetic process	14	2.58%	0.019991
Biol Process level 5	regulated secretory pathway	17	3.14%	0.020046
Biol Process level 5	neurotransmitter secretion	17	3.14%	0.020046
Biol Process level 4	phosphate metabolic process	49	9.04%	0.020146
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transporter activity	19	3.51%	0.020371
Biol Process level 3	secretory pathway	26	4.80%	0.020759
Biol Process level 4	courtship behavior	7	1.29%	0.020836
Biol Process level 4	memory	7	1.29%	0.020836
Biol Process level 4	cofactor biosynthetic process	15	2.77%	0.021031
Biol Process level 4	lipid biosynthetic process	14	2.58%	0.021509
Biol Process level 3	instar larval or pupal development	38	7.01%	0.021839
Biol Process level 5	small GTPase mediated signal transduction	19	3.51%	0.021984
Cell Component level 4	proton-transporting two-sector ATPase complex, catalytic domain	6	1.11%	0.022107
Biol Process level 5	metal ion transport	17	3.14%	0.022442
Cell Component level 3	proton-transporting two-sector ATPase complex, catalytic domain	6	1.11%	0.022513
Biol Process level 1	reproduction	44	8.12%	0.022517
Biol Process level 3	secretion by cell	26	4.80%	0.022579
Biol Process level 5	nerve-nerve synaptic transmission	5	0.92%	0.022648
Biol Process level 3	neurotransmitter secretion	17	3.14%	0.022817
Mol Function level 4	kinase activity	40	7.38%	0.022971
Biol Process level 4	cellular carbohydrate metabolic process	22	4.06%	0.023002
Cell Component level 5	proton-transporting two-sector ATPase complex, catalytic domain	6	1.11%	0.02308
Biol Process level 4	secretory pathway	26	4.80%	0.0231
Biol Process level 3	tube morphogenesis	12	2.21%	0.023674
Mol Function level 4	secondary active transmembrane transporter activity	17	3.14%	0.024556
	transferase activity, transferring alkyl or aryl (other than methyl) groups	7	1.29%	0.02475
Biol Process level 4	regulated secretory pathway	17	3.14%	0.024759

Table 9 continued.

Category	Term	Count	%	PValue
Biol Process level 4	neurotransmitter secretion	17	3.14%	0.024759
Biol Process level 4	instar larval or pupal development	38	7.01%	0.024935
Biol Process level 5	behavioral response to ethanol	4	0.74%	0.025018
Biol Process level 4	secretion by cell	26	4.80%	0.025099
Biol Process level 4	tube morphogenesis	12	2.21%	0.025263
Biol Process level 2	generation of a signal involved in cell-cell signaling	17	3.14%	0.025667
Mol Function level 3	hydrolase activity, acting on acid anhydrides	45	8.30%	0.025958
Biol Process level 3	maintenance of protein localization	4	0.74%	0.026011
Biol Process level 2	maintenance of protein localization	4	0.74%	0.026059
Biol Process level 4	response to ethanol	4	0.74%	0.026717
Biol Process level 4	maintenance of protein localization	4	0.74%	0.026717
Biol Process level 4	behavioral response to ethanol	4	0.74%	0.026717
Biol Process level 4	generation of a signal involved in cell-cell signaling	17	3.14%	0.027638
Biol Process level 3	cofactor metabolic process	23	4.24%	0.028008
Biol Process level 3	post-embryonic development	38	7.01%	0.028207
Biol Process level 2	post-embryonic development	38	7.01%	0.028553
Biol Process level 5	autophagic cell death	9	1.66%	0.028938
Biol Process level 5	salivary gland histolysis	9	1.66%	0.028938
Biol Process level 5	proton transport	10	1.85%	0.029445
Mol Function level 2	lipid binding	12	2.21%	0.030789
Biol Process level 3	hydrogen transport	10	1.85%	0.032037
Biol Process level 5	synaptic vesicle priming	5	0.92%	0.032341
Biol Process level 5	male mating behavior	5	0.92%	0.032341
Biol Process level 5	male courtship behavior	5	0.92%	0.032341
Biol Process level 3	behavioral interaction between organisms	7	1.29%	0.03241
Biol Process level 3	mating behavior	7	1.29%	0.03241
Biol Process level 2	behavioral interaction between organisms	7	1.29%	0.032512
Mol Function level 5	manganese ion binding	7	1.29%	0.032564
Biol Process level 1	maintenance of localization	5	0.92%	0.033421
Biol Process level 4	mating behavior	7	1.29%	0.033829
Biol Process level 4	hydrogen transport	10	1.85%	0.033866
Biol Process level 4	proton transport	10	1.85%	0.033866
Biol Process level 2	maintenance of localization	5	0.92%	0.033989
Biol Process level 5	histolysis	9	1.66%	0.034407
Biol Process level 3	regulation of catalytic activity	13	2.40%	0.034671
Biol Process level 4	male mating behavior	5	0.92%	0.035028
Cell Component level 4	sodium:potassium-exchanging ATPase complex	3	0.55%	0.036643
Mol Function level 2	molecular adaptor activity	4	0.74%	0.036905
Cell Component level 4	cytoskeleton	23	4.24%	0.036905
Biol Process level 3	response to radiation	11	2.03%	0.036922
Cell Component level 3	sodium:potassium-exchanging ATPase complex	3	0.55%	0.036939
Biol Process level 4	nervous system development	40	7.38%	0.037207
Cell Component level 5	sodium:potassium-exchanging ATPase complex	3	0.55%	0.037414
Cell Component level 3	receptor complex	4	0.74%	0.03757
Biol Process level 5	tricarboxylic acid cycle	7	1.29%	0.037787
Biol Process level 5	aerobic respiration	7	1.29%	0.037787
Biol Process level 4	open tracheal system development	15	2.77%	0.038025
Biol Process level 5	maintenance of imaginal disc-derived wing hair orientation	3	0.55%	0.038654
Biol Process level 5	purine nucleotide biosynthetic process	11	2.03%	0.038807
Biol Process level 4	histolysis	9	1.66%	0.039073
Biol Process level 2	response to external stimulus	15	2.77%	0.039687
Mol Function level 3	carbon-carbon lyase activity	7	1.29%	0.039855
Biol Process level 4	sensory perception	14	2.58%	0.039946
Biol Process level 5	ectoderm development	13	2.40%	0.039968

Table 9 continued.

Category	Term	Count	%	PValue
Biol Process level 5	regulation of tube diameter	4	0.74%	0.039999
Cell Component level 5	cytoskeleton	23	4.24%	0.040936
Biol Process level 3	regulation of tube diameter	4	0.74%	0.041528
Biol Process level 5	fatty acid biosynthetic process	6	1.11%	0.041899
Biol Process level 4	cellular respiration	7	1.29%	0.04193
Biol Process level 4	regulation of tube diameter	4	0.74%	0.042616
Biol Process level 1	reproductive process	13	2.40%	0.042828
Mol Function level 3	transferase activity, transferring phosphorus-containing groups	46	8.49%	0.043077
Mol Function level 5	gated channel activity	13	2.40%	0.043161
Biol Process level 2	reproductive process	13	2.40%	0.044287
Mol Function level 4	prenyltransferase activity	5	0.92%	0.045639
Biol Process level 4	fatty acid biosynthetic process	6	1.11%	0.045885
Biol Process level 5	acetyl-CoA metabolic process	7	1.29%	0.046217
Biol Process level 5	coenzyme catabolic process	7	1.29%	0.046217
Biol Process level 3	tube development	12	2.21%	0.046274
Biol Process level 2	regulation of a molecular function	14	2.58%	0.04684
Biol Process level 4	ectoderm development	13	2.40%	0.04696
Biol Process level 5	monosaccharide catabolic process	8	1.48%	0.047619
Biol Process level 4	female gamete generation	31	5.72%	0.04762

Table 10. Significantly enriched GO categories for genes more highly expressed in inactive bees.

Category	Term	Count	%	PValue
Mol Function level 2	structural constituent of ribosome	85	14.14%	1.07E-46
Cell Component level 3	ribosome	84	13.98%	3.34E-45
Cell Component level 3	ribosomal subunit	76	12.65%	1.12E-42
Cell Component level 4	ribosome	84	13.98%	1.86E-42
Cell Component level 5	ribosome	84	13.98%	1.17E-41
Cell Component level 4	ribosomal subunit	76	12.65%	3.61E-40
Cell Component level 5	ribosomal subunit	76	12.65%	1.92E-39
Cell Component level 2	ribonucleoprotein complex	99	16.47%	1.99E-38
Cell Component level 3	ribonucleoprotein complex	99	16.47%	2.07E-38
Cell Component level 4	ribonucleoprotein complex	99	16.47%	2.23E-35
Cell Component level 5	ribonucleoprotein complex	99	16.47%	1.70E-34
Biol Process level 2	translation	93	15.47%	3.68E-31
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	48	7.99%	4.24E-31
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	48	7.99%	1.25E-30
Biol Process level 4	translation	93	15.47%	1.36E-30
Biol Process level 5	translation	93	15.47%	4.69E-30
Cell Component level 1	macromolecular complex	192	31.95%	1.26E-29
Cell Component level 2	intracellular organelle part	188	31.28%	1.67E-29
Cell Component level 3	intracellular organelle part	188	31.28%	1.78E-29
Cell Component level 2	organelle part	188	31.28%	2.44E-29
Cell Component level 1	organelle part	188	31.28%	6.37E-29
Cell Component level 3	cytoplasmic part	188	31.28%	1.83E-27
Cell Component level 4	cytosolic part	52	8.65%	6.27E-27
Mol Function level 1	structural molecule activity	90	14.98%	7.06E-27
Cell Component level 5	cytosolic part	52	8.65%	1.94E-26
Cell Component level 3	cytoplasm	206	34.28%	7.56E-25
Cell Component level 4	intracellular organelle part	188	31.28%	9.90E-25
Biol Process level 3	cellular biosynthetic process	121	20.13%	1.28E-23
Biol Process level 3	macromolecule biosynthetic process	98	16.31%	1.46E-23
Cell Component level 5	intracellular organelle part	188	31.28%	2.56E-23
Cell Component level 4	cytoplasmic part	188	31.28%	8.61E-23
Cell Component level 5	cytoplasmic part	188	31.28%	2.08E-21
Cell Component level 4	large ribosomal subunit	44	7.32%	1.99E-20
Cell Component level 5	large ribosomal subunit	44	7.32%	5.05E-20
Cell Component level 4	cytoplasm	206	34.28%	6.94E-20
Cell Component level 3	small ribosomal subunit	33	5.49%	1.12E-19
Cell Component level 3	mitochondrial part	82	13.64%	1.17E-19
Biol Process level 2	biosynthetic process	124	20.63%	6.51E-19
Cell Component level 4	small ribosomal subunit	33	5.49%	1.39E-18
Cell Component level 5	cytoplasm	206	34.28%	2.09E-18
Cell Component level 5	small ribosomal subunit	33	5.49%	2.86E-18
Cell Component level 2	intracellular part	261	43.43%	3.81E-18
Cell Component level 3	intracellular part	261	43.43%	4.18E-18
Cell Component level 5	cytosolic large ribosomal subunit (sensu Eukaryota)	29	4.83%	1.53E-17
Cell Component level 4	mitochondrial part	82	13.64%	1.90E-17
Cell Component level 2	non-membrane-bound organelle	100	16.64%	2.50E-17
Cell Component level 3	intracellular non-membrane-bound organelle	100	16.64%	2.57E-17
Cell Component level 4	mitochondrion	92	15.31%	7.88E-17
Cell Component level 5	mitochondrial part	82	13.64%	8.08E-17
Cell Component level 2	intracellular organelle	230	38.27%	3.09E-16
Cell Component level 3	intracellular organelle	230	38.27%	3.23E-16
Cell Component level 5	mitochondrion	92	15.31%	4.86E-16

Table 10 continued.

Category	Term	Count	%	PValue
Cell Component level 4	cytosol	58	9.65%	5.39E-16
Cell Component level 1	organelle	230	38.27%	1.28E-15
Cell Component level 5	cytosol	58	9.65%	1.60E-15
Cell Component level 4	intracellular non-membrane-bound organelle	100	16.64%	1.08E-14
Cell Component level 2	intracellular	267	44.43%	3.07E-14
Cell Component level 3	intracellular	267	44.43%	3.37E-14
Cell Component level 5	intracellular non-membrane-bound organelle	100	16.64%	5.40E-14
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	19	3.16%	1.51E-12
Cell Component level 3	mitochondrial membrane part	37	6.16%	2.21E-12
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	19	3.16%	2.29E-12
Biol Process level 2	cellular metabolic process	279	46.42%	3.06E-12
Cell Component level 4	intracellular part	261	43.43%	6.11E-12
Cell Component level 4	mitochondrial membrane part	37	6.16%	2.63E-11
Cell Component level 4	organellar ribosome	30	4.99%	3.11E-11
Cell Component level 4	mitochondrial ribosome	30	4.99%	3.11E-11
Cell Component level 4	intracellular organelle	230	38.27%	3.83E-11
Cell Component level 5	mitochondrial membrane part	37	6.16%	5.29E-11
Cell Component level 5	organellar ribosome	30	4.99%	5.62E-11
Cell Component level 5	mitochondrial ribosome	30	4.99%	5.62E-11
Biol Process level 1	gene expression	137	22.80%	6.15E-11
Biol Process level 1	metabolic process	296	49.25%	1.02E-10
Biol Process level 3	electron transport	41	6.82%	1.37E-10
Cell Component level 3	mitochondrial lumen	38	6.32%	2.83E-10
Cell Component level 5	intracellular organelle	230	38.27%	1.17E-09
Cell Component level 2	protein complex	128	21.30%	1.71E-09
Cell Component level 4	mitochondrial lumen	38	6.32%	3.06E-09
Cell Component level 4	mitochondrial matrix	38	6.32%	3.06E-09
Cell Component level 5	mitochondrial lumen	38	6.32%	5.96E-09
Cell Component level 5	mitochondrial matrix	38	6.32%	5.96E-09
Cell Component level 3	mitochondrial envelope	44	7.32%	1.05E-08
Mol Function level 2	oxidoreductase activity	67	11.15%	1.41E-08
Cell Component level 3	mitochondrial membrane	41	6.82%	1.65E-08
Biol Process level 3	oxidative phosphorylation	30	4.99%	4.22E-08
Cell Component level 3	organelle inner membrane	38	6.32%	5.04E-08
Cell Component level 4	mitochondrial respiratory chain	24	3.99%	5.41E-08
Cell Component level 5	mitochondrial respiratory chain	24	3.99%	8.49E-08
Cell Component level 2	organelle lumen	64	10.65%	8.98E-08
Cell Component level 3	organelle lumen	64	10.65%	9.12E-08
Cell Component level 1	membrane-enclosed lumen	64	10.65%	9.50E-08
Cell Component level 4	mitochondrial envelope	44	7.32%	1.27E-07
Cell Component level 4	mitochondrial membrane	41	6.82%	1.73E-07
Biol Process level 4	ATP synthesis coupled electron transport	21	3.49%	1.88E-07
Biol Process level 5	organelle ATP synthesis coupled electron transport	21	3.49%	2.53E-07
Cell Component level 5	mitochondrial envelope	44	7.32%	2.54E-07
Cell Component level 5	mitochondrial membrane	41	6.82%	3.32E-07
Cell Component level 4	mitochondrial inner membrane	38	6.32%	4.46E-07
Cell Component level 4	organelle inner membrane	38	6.32%	4.46E-07
Biol Process level 2	generation of precursor metabolites and energy	43	7.15%	5.32E-07
Cell Component level 5	mitochondrial inner membrane	38	6.32%	8.17E-07
Cell Component level 5	organelle inner membrane	38	6.32%	8.17E-07
Biol Process level 4	cellular protein metabolic process	149	24.79%	1.10E-06
Cell Component level 4	organellar small ribosomal subunit	14	2.33%	1.73E-06
Cell Component level 4	mitochondrial small ribosomal subunit	14	2.33%	1.73E-06
Biol Process level 3	protein metabolic process	152	25.29%	1.97E-06

Table 10 continued.

Category	Term	Count	%	PValue
Cell Component level 2	organelle envelope	45	7.49%	2.25E-06
Cell Component level 3	organelle envelope	45	7.49%	2.28E-06
Cell Component level 5	organellar small ribosomal subunit	14	2.33%	2.28E-06
Cell Component level 5	mitochondrial small ribosomal subunit	14	2.33%	2.28E-06
Cell Component level 1	envelope	45	7.49%	2.33E-06
Mol Function level 3	electron carrier activity	23	3.83%	3.45E-06
Biol Process level 3	cellular macromolecule metabolic process	149	24.79%	3.67E-06
Cell Component level 2	organelle membrane	55	9.15%	5.45E-06
Cell Component level 3	organelle membrane	55	9.15%	5.52E-06
Cell Component level 4	organelle envelope	45	7.49%	2.10E-05
Mol Function level 5	cytochrome-c oxidase activity	7	1.16%	3.84E-05
Cell Component level 5	organelle envelope	45	7.49%	3.85E-05
Mol Function level 4	cytochrome-c oxidase activity	7	1.16%	4.37E-05
	oxidoreductase activity, acting on heme group of donors, oxygen as			
Mol Function level 4	aCell Componenteptor	7	1.16%	4.37E-05
Mol Function level 3	oxidoreductase activity, acting on heme group of donors	7	1.16%	4.46E-05
Mol Function level 3	heme-copper terminal oxidase activity	7	1.16%	4.46E-05
Mol Function level 4	NADH dehydrogenase activity	12	2.00%	6.45E-05
Cell Component level 4	organelle membrane	55	9.15%	6.48E-05
Biol Process level 5	protein import into mitochondrion	7	1.16%	6.69E-05
Mol Function level 3	oxidoreductase activity, acting on NADH or NADPH	13	2.16%	1.15E-04
Cell Component level 4	mitochondrial large ribosomal subunit	15	2.50%	1.20E-04
Biol Process level 5	inner mitochondrial membrane organization and biogenesis	6	1.00%	1.34E-04
Cell Component level 5	organellar large ribosomal subunit	15	2.50%	1.56E-04
Cell Component level 5	mitochondrial large ribosomal subunit	15	2.50%	1.56E-04
Biol Process level 4	mitochondrial membrane organization and biogenesis	6	1.00%	3.88E-04
Biol Process level 5	mitochondrial membrane organization and biogenesis	6	1.00%	4.24E-04
	P-P-bond-hydrolysis-driven protein transmembrane transporter			
Mol Function level 5	activity	8	1.33%	4.51E-04
	P-P-bond-hydrolysis-driven protein transmembrane transporter			
Mol Function level 4	activity	8	1.33%	5.19E-04
Mol Function level 4	macromolecule transmembrane transporter activity	8	1.33%	5.19E-04
Mol Function level 4	protein transmembrane transporter activity	9	1.50%	5.54E-04
Mol Function level 3	protein transmembrane transporter activity	9	1.50%	5.67E-04
Cell Component level 3	respiratory chain complex I	12	2.00%	6.00E-04
Cell Component level 3	NADH dehydrogenase complex (quinone)	12	2.00%	6.00E-04
Mol Function level 4	rRNA binding	6	1.00%	6.58E-04
Biol Process level 4	mitochondrial electron transport, NADH to ubiquinone	12	2.00%	7.01E-04
Cell Component level 3	mitochondrial intermembrane space protein transporter complex	5	0.83%	9.45E-04
Biol Process level 1	cellular process	332	55.24%	9.61E-04
Cell Component level 3	respiratory chain complex IV	6	1.00%	0.001021
Cell Component level 3	intracellular membrane-bound organelle	171	28.45%	0.001095
Cell Component level 2	membrane-bound organelle	171	28.45%	0.001181
Cell Component level 4	NADH dehydrogenase complex (quinone)	12	2.00%	0.001231
Cell Component level 4	respiratory chain complex I	12	2.00%	0.001231
Cell Component level 4	mitochondrial respiratory chain complex I	12	2.00%	0.001231
Mol Function level 5	NADH dehydrogenase (quinone) activity	9	1.50%	0.001254
Biol Process level 2	primary metabolic process	243	40.43%	0.001299
Cell Component level 4	mitochondrial intermembrane space protein transporter complex	5	0.83%	0.001313
Biol Process level 5	protein targeting to mitochondrion	9	1.50%	0.001398
Cell Component level 5	mitochondrial intermembrane space protein transporter complex	5	0.83%	0.001441
Cell Component level 3	mitochondrial inner membrane presequence translocase complex	7	1.16%	0.001445
	oxidoreductase activity, acting on NADH or NADPH, quinone or			
Mol Function level 4	similar compound as acceptor	9	1.50%	0.001457
Cell Component level 5	respiratory chain complex I	12	2.00%	0.001505
Cell Component level 5	NADH dehydrogenase complex (quinone)	12	2.00%	0.001505

Table 10 continued.

Category	Term	Count	%	PValue
Cell Component level 5	mitochondrial respiratory chain complex I	12	2.00%	0.001505
Cell Component level 4	respiratory chain complex IV	6	1.00%	0.001514
Cell Component level 4	mitochondrial respiratory chain complex IV	6	1.00%	0.001514
Cell Component level 5	respiratory chain complex IV	6	1.00%	0.001692
Cell Component level 5	mitochondrial respiratory chain complex IV	6	1.00%	0.001692
Cell Component level 4	mitochondrial inner membrane presequence translocase complex	7	1.16%	0.00226
Cell Component level 5	mitochondrial inner membrane presequence translocase complex	7	1.16%	0.002564
Biol Process level 2	macromolecule metabolic process	207	34.44%	0.002923
Mol Function level 3	protein transporter activity	11	1.83%	0.004613
Biol Process level 4	mitochondrial electron transport, cytochrome c to oxygen	5	0.83%	0.005052
Mol Function level 5	ubiquinol-cytochrome-c reductase activity	5	0.83%	0.006149
Cell Component level 4	lipid particle	22	3.66%	0.006364
Mol Function level 4	oxidoreductase activity, acting on diphenols and related substances as donors, cytochrome as accepto	5	0.83%	0.006646
Cell Component level 5	lipid particle	22	3.66%	0.008376
Mol Function level 3	oxidoreductase activity, acting on diphenols and related substances as donors	5	0.83%	0.01037
Cell Component level 3	endoplasmic reticulum membrane	10	1.66%	0.010823
Cell Component level 3	nuclear envelope-endoplasmic reticulum network	10	1.66%	0.010823
Cell Component level 3	organelle envelope lumen	5	0.83%	0.016011
Cell Component level 4	nuclear envelope-endoplasmic reticulum network	10	1.66%	0.018325
Cell Component level 4	endoplasmic reticulum membrane	10	1.66%	0.018325
Biol Process level 4	mitochondrial transport	9	1.50%	0.019916
Cell Component level 5	nuclear envelope-endoplasmic reticulum network	10	1.66%	0.021195
Cell Component level 5	endoplasmic reticulum membrane	10	1.66%	0.021195
Cell Component level 4	organelle envelope lumen	5	0.83%	0.02145
Cell Component level 4	mitochondrial intermembrane space	5	0.83%	0.02145
Biol Process level 5	mitochondrial transport	9	1.50%	0.022032
Cell Component level 5	organelle envelope lumen	5	0.83%	0.023286
Cell Component level 5	mitochondrial intermembrane space	5	0.83%	0.023286
Mol Function level 5	protein-methionine-R-oxide reductase activity	3	0.50%	0.023954
Cell Component level 3	respiratory chain complex III	5	0.83%	0.024193
Cell Component level 3	endoplasmic reticulum part	11	1.83%	0.024221
Mol Function level 5	DNA-directed RNA polymerase activity	6	1.00%	0.02543
Biol Process level 3	oxygen and reactive oxygen species metabolic process	5	0.83%	0.031452
Cell Component level 4	respiratory chain complex III	5	0.83%	0.032125
Cell Component level 4	mitochondrial respiratory chain complex III	5	0.83%	0.032125
Cell Component level 3	proteasome complex (sensu Eukaryota)	10	1.66%	0.032858
Cell Component level 4	snRNP U6	4	0.67%	0.033136
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transporter activity	14	2.33%	0.034637
Cell Component level 5	respiratory chain complex III	5	0.83%	0.034783
Cell Component level 5	mitochondrial respiratory chain complex III	5	0.83%	0.034783
Cell Component level 5	snRNP U6	4	0.67%	0.035333
Cell Component level 3	small nuclear ribonucleoprotein complex	10	1.66%	0.038359
Mol Function level 4	primary active transmembrane transporter activity	14	2.33%	0.040635
Cell Component level 4	endoplasmic reticulum part	11	1.83%	0.040712
Mol Function level 3	oxidoreductase activity, acting on sulfur group of donors	4	0.67%	0.045678
Mol Function level 3	oxidoreductase activity, acting on the CH-CH group of donors	5	0.83%	0.046226
Cell Component level 5	endoplasmic reticulum part	11	1.83%	0.04692
Cell Component level 3	spliceosome	12	2.00%	0.047535

Table 11. Significantly enriched GO categories for genes differentially expressed between morning anticipating bees (tAM-cAM) and afternoon anticipating bees (tPM-cPM)

Category	Term	Count	%	PValue
Biol Process level 3	lipid metabolic process	42	5.75%	8.05E-04
Mol Function level 2	structural constituent of ribosome	35	4.79%	0.002001
Cell Component level 3	cytoplasm	192	26.27%	0.002085
Biol Process level 5	monosaccharide catabolic process	12	1.64%	0.002682
Biol Process level 5	hexose metabolic process	17	2.33%	0.003172
Cell Component level 4	cytosolic part	24	3.28%	0.003518
Mol Function level 4	calcium ion binding	26	3.56%	0.003556
Mol Function level 3	active transmembrane transporter activity	38	5.20%	0.003568
Biol Process level 4	alcohol catabolic process	12	1.64%	0.003809
Cell Component level 5	cytosolic part	24	3.28%	0.003985
Biol Process level 3	carbohydrate metabolic process	41	5.61%	0.004003
Cell Component level 4	cytoplasm	192	26.27%	0.004101
Cell Component level 3	ribosome	35	4.79%	0.004363
Biol Process level 4	carbohydrate catabolic process	14	1.92%	0.005684
Cell Component level 4	ribosome	35	4.79%	0.005692
Cell Component level 4	cytosol	37	5.06%	0.006287
Biol Process level 5	pyrimidine nucleotide biosynthetic process	6	0.82%	0.006442
Cell Component level 5	ribosome	35	4.79%	0.006626
Cell Component level 5	cytoplasm	192	26.27%	0.006702
Mol Function level 5	anion transmembrane transporter activity	13	1.78%	0.007166
Biol Process level 5	monosaccharide metabolic process	17	2.33%	0.007232
Cell Component level 5	cytosol	37	5.06%	0.007347
Biol Process level 4	monosaccharide metabolic process	17	2.33%	0.007603
Biol Process level 5	cellular carbohydrate catabolic process	13	1.78%	0.007611
Biol Process level 3	cellular biosynthetic process	87	11.90%	0.007615
Biol Process level 3	alcohol metabolic process	23	3.15%	0.009168
Biol Process level 4	cellular carbohydrate metabolic process	28	3.83%	0.009587
Cell Component level 3	ribosomal subunit	30	4.10%	0.009711
Biol Process level 4	cellular lipid metabolic process	32	4.38%	0.009743
Biol Process level 3	cellular lipid metabolic process	32	4.38%	0.010185
Biol Process level 2	biosynthetic process	99	13.54%	0.011755
Mol Function level 5	glutathione peroxidase activity	4	0.55%	0.011874
Cell Component level 4	ribosomal subunit	30	4.10%	0.012199
Biol Process level 2	response to chemical stimulus	27	3.69%	0.012857
Mol Function level 3	glutathione peroxidase activity	4	0.55%	0.012995
Biol Process level 5	pyrimidine base metabolic process	7	0.96%	0.013341
Mol Function level 4	secondary active transmembrane transporter activity	20	2.74%	0.013341
Biol Process level 2	cell adhesion	25	3.42%	0.013382
Cell Component level 5	ribosomal subunit	30	4.10%	0.013874
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	18	2.46%	0.014023
Biol Process level 1	biological adhesion	25	3.42%	0.014925
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	18	2.46%	0.015375
Cell Component level 4	cullin-RING ubiquitin ligase complex	5	0.68%	0.015954
Cell Component level 5	cullin-RING ubiquitin ligase complex	5	0.68%	0.016519
Cell Component level 5	SCF ubiquitin ligase complex	5	0.68%	0.016519
Mol Function level 2	isomerase activity	15	2.05%	0.016667
Biol Process level 3	chemosensory behavior	12	1.64%	0.017713
Mol Function level 2	transmembrane transporter activity	64	8.76%	0.018282
Biol Process level 4	extracellular transport	7	0.96%	0.019841
Biol Process level 3	extracellular transport	7	0.96%	0.020089

Table 11 continued.

Category	Term	Count	%	PValue
Cell Component level 3	cytoplasmic part	155	21.20%	0.021551
Mol Function level 3	oxidoreductase activity, acting on peroxide as acceptor	5	0.68%	0.02351
Mol Function level 2	peroxidase activity	5	0.68%	0.023818
Mol Function level 4	peroxidase activity	5	0.68%	0.024072
Mol Function level 3	identical protein binding	16	2.19%	0.026503
Biol Process level 4	reproductive behavior in a multicellular organism	7	0.96%	0.027675
Mol Function level 1	antioxidant activity	6	0.82%	0.027858
Biol Process level 3	reproductive behavior in a multicellular organism	7	0.96%	0.02801
	oxidoreductase activity, acting on the aldehyde or oxo group of			
Mol Function level 4	donors, disulfide as addor	4	0.55%	0.029324
Biol Process level 3	macromolecule biosynthetic process	61	8.34%	0.030268
Biol Process level 2	catabolic process	38	5.20%	0.032522
Biol Process level 2	behavior	30	4.10%	0.033223
Mol Function level 5	glucuronosyltransferase activity	5	0.68%	0.033534
Biol Process level 4	cellular macromolecule catabolic process	22	3.01%	0.036525
Cell Component level 4	cytoplasmic part	155	21.20%	0.036879
Biol Process level 5	translation	47	6.43%	0.04127
Biol Process level 4	translation	47	6.43%	0.044681
Biol Process level 3	macromolecule catabolic process	25	3.42%	0.045526
Biol Process level 2	translation	47	6.43%	0.046143
Biol Process level 3	cellular catabolic process	34	4.65%	0.046549
Mol Function level 5	symporter activity	11	1.50%	0.047684

Table 12. Significantly enriched GO categories for genes more highly expressed in morning anticipating bees (tAM-cAM) versus afternoon anticipating bees (tPM-cPM).

Category	Term	Count	%	PValue
Biol Process level 3	lipid metabolic process	28	7.98%	1.81E-05
Biol Process level 4	cellular lipid metabolic process	21	5.98%	6.38E-04
Biol Process level 3	cellular lipid metabolic process	21	5.98%	7.98E-04
Biol Process level 5	pyrimidine base metabolic process	6	1.71%	0.001615
Biol Process level 5	prosthetic group metabolic process	5	1.42%	0.004565
Biol Process level 3	prosthetic group metabolic process	5	1.42%	0.00549
Biol Process level 2	behavior	18	5.13%	0.011938
Biol Process level 5	lipid biosynthetic process	10	2.85%	0.015883
Mol Function level 3	identical protein binding	10	2.85%	0.01642
Biol Process level 2	response to chemical stimulus	15	4.27%	0.019367
Biol Process level 4	lipid biosynthetic process	10	2.85%	0.019588
Biol Process level 2	cell adhesion	14	3.99%	0.020006
Biol Process level 5	nucleobase metabolic process	7	1.99%	0.020939
Biol Process level 1	biological adhesion	14	3.99%	0.021106
Mol Function level 2	isomerase activity	9	2.56%	0.021242
Biol Process level 3	lipid biosynthetic process	10	2.85%	0.021582
Biol Process level 2	response to abiotic stimulus	12	3.42%	0.02255
Biol Process level 4	nucleobase metabolic process	7	1.99%	0.024441
Cell Component level 4	endoplasmic reticulum	15	4.27%	0.025273
Cell Component level 5	endoplasmic reticulum	15	4.27%	0.028211
Biol Process level 4	chromosome organization and biogenesis	13	3.70%	0.033076
Biol Process level 5	isoprenoid metabolic process	4	1.14%	0.033427
Biol Process level 4	isoprenoid metabolic process	4	1.14%	0.036563
Mol Function level 5	iron ion binding	9	2.56%	0.046023
Biol Process level 5	central nervous system development	10	2.85%	0.046937

Table 13. Significantly enriched GO categories for genes more highly expressed in afternoon anticipating bees (tPM-cPM) versus morning anticipating bees (tAM-cAM).

Category	Term	Count	%	PValue
Cell Component level 4	cytosolic part	21	5.53%	1.09E-05
Cell Component level 5	cytosolic part	21	5.53%	1.18E-05
Mol Function level 2	structural constituent of ribosome	27	7.11%	4.31E-05
Cell Component level 4	ribosome	27	7.11%	9.13E-05
Cell Component level 3	ribosome	27	7.11%	9.74E-05
Cell Component level 5	ribosome	27	7.11%	9.89E-05
Cell Component level 4	cytosol	28	7.37%	1.33E-04
Cell Component level 5	cytosol	28	7.37%	1.45E-04
Cell Component level 4	cytosolic ribosome (sensu Eukaryota)	16	4.21%	1.55E-04
Cell Component level 4	ribosomal subunit	24	6.32%	1.61E-04
Cell Component level 5	cytosolic ribosome (sensu Eukaryota)	16	4.21%	1.65E-04
Cell Component level 3	ribosomal subunit	24	6.32%	1.70E-04
Cell Component level 5	ribosomal subunit	24	6.32%	1.74E-04
Biol Process level 5	hexose metabolic process	14	3.68%	2.86E-04
Biol Process level 4	cellular carbohydrate metabolic process	22	5.79%	3.31E-04
Biol Process level 3	carbohydrate metabolic process	29	7.63%	3.95E-04
Biol Process level 3	cellular biosynthetic process	57	15.00%	5.21E-04
Biol Process level 5	monosaccharide catabolic process	10	2.63%	5.31E-04
Biol Process level 4	monosaccharide metabolic process	14	3.68%	5.32E-04
Biol Process level 3	alcohol metabolic process	18	4.74%	5.84E-04
Biol Process level 4	alcohol catabolic process	10	2.63%	6.13E-04
Biol Process level 5	monosaccharide metabolic process	14	3.68%	6.33E-04
Cell Component level 4	ribonucleoprotein complex	34	8.95%	9.65E-04
Cell Component level 2	ribonucleoprotein complex	34	8.95%	0.001027
Cell Component level 3	ribonucleoprotein complex	34	8.95%	0.001035
Cell Component level 5	ribonucleoprotein complex	34	8.95%	0.00105
Mol Function level 3	active transmembrane transporter activity	26	6.84%	0.001166
Cell Component level 4	cytoplasm	112	29.47%	0.001293
Cell Component level 5	cytoplasm	112	29.47%	0.001482
Biol Process level 4	carbohydrate catabolic process	11	2.89%	0.001484
Biol Process level 3	macromolecule biosynthetic process	42	11.05%	0.001561
Biol Process level 2	translation	34	8.95%	0.001649
Biol Process level 4	translation	34	8.95%	0.001969
Cell Component level 3	cytoplasm	112	29.47%	0.002152
Biol Process level 5	translation	34	8.95%	0.002646
Mol Function level 1	structural molecule activity	34	8.95%	0.002718
Cell Component level 4	cytoplasmic part	94	24.74%	0.003203
Biol Process level 2	biosynthetic process	61	16.05%	0.00345
Biol Process level 5	cellular carbohydrate catabolic process	10	2.63%	0.003544
Cell Component level 5	cytoplasmic part	94	24.74%	0.003654
Cell Component level 3	cytoplasmic part	94	24.74%	0.00432
Cell Component level 4	mitochondrial envelope	24	6.32%	0.004606
Cell Component level 4	large ribosomal subunit	15	3.95%	0.004612
Cell Component level 5	cytosolic large ribosomal subunit (sensu Eukaryota)	10	2.63%	0.004617
Cell Component level 3	mitochondrial envelope	24	6.32%	0.004751
Cell Component level 4	intracellular non-membrane-bound organelle	48	12.63%	0.004818
Cell Component level 5	large ribosomal subunit	15	3.95%	0.004833
Cell Component level 5	mitochondrial envelope	24	6.32%	0.004905
Cell Component level 2	non-membrane-bound organelle	48	12.63%	0.005218
Cell Component level 3	intracellular non-membrane-bound organelle	48	12.63%	0.005262
Cell Component level 5	intracellular non-membrane-bound organelle	48	12.63%	0.005287

Table 13 continued.

Category	Term	Count	%	PValue
Mol Function level 4	oxidoreductase activity, acting on the aldehyde or oxo group of donors, disulfide as aCell Componenteceptor	4	1.05%	0.005542
Mol Function level 2	transmembrane transporter activity	41	10.79%	0.005625
Mol Function level 5	ATPase activity, coupled to transmembrane movement of substances	13	3.42%	0.00582
Cell Component level 4	small ribosomal subunit	11	2.89%	0.006267
Cell Component level 3	small ribosomal subunit	11	2.89%	0.006317
Cell Component level 5	small ribosomal subunit	11	2.89%	0.006506
Mol Function level 4	ion transmembrane transporter activity	30	7.89%	0.008087
Mol Function level 4	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	13	3.42%	0.009239
Cell Component level 4	mitochondrial part	34	8.95%	0.00997
Cell Component level 3	mitochondrial part	34	8.95%	0.010418
Mol Function level 5	anion transmembrane transporter activity	9	2.37%	0.010643
Cell Component level 5	mitochondrial part	34	8.95%	0.010723
Biol Process level 2	cellular metabolic process	180	47.37%	0.010779
Biol Process level 1	metabolic process	196	51.58%	0.012676
Mol Function level 5	NADH dehydrogenase (quinone) activity	7	1.84%	0.013879
Cell Component level 4	mitochondrion	39	10.26%	0.016432
Biol Process level 4	alcohol biosynthetic process	4	1.05%	0.016737
Biol Process level 4	female germ-line cyst formation	4	1.05%	0.016737
Mol Function level 4	oxidoreductase activity, acting on NADH or NADPH, quinone or similar compound as aCell Componenteceptor	7	1.84%	0.016785
Biol Process level 5	monosaCell Componentharide biosynthetic process	4	1.05%	0.017606
Biol Process level 5	female germ-line cyst formation	4	1.05%	0.017606
Cell Component level 5	mitochondrion	39	10.26%	0.017722
Mol Function level 5	nucleoside diphosphate kinase activity	3	0.79%	0.019366
Biol Process level 3	macromolecule catabolic process	17	4.47%	0.019793
Mol Function level 4	secondary active transmembrane transporter activity	13	3.42%	0.020121
Mol Function level 5	ATPase activity, coupled to transmembrane movement of ions	10	2.63%	0.021606
Mol Function level 3	substrate-specific transmembrane transporter activity	34	8.95%	0.024129
Biol Process level 4	polyol metabolic process	4	1.05%	0.025108
Biol Process level 5	glycerol metabolic process	4	1.05%	0.026381
Cell Component level 1	envelope	25	6.58%	0.027257
Cell Component level 4	organelle envelope	25	6.58%	0.027471
Cell Component level 2	organelle envelope	25	6.58%	0.027902
Cell Component level 3	organelle envelope	25	6.58%	0.028029
Mol Function level 4	calcium ion binding	15	3.95%	0.02804
Cell Component level 5	organelle envelope	25	6.58%	0.029034
Mol Function level 5	glucuronosyltransferase activity	4	1.05%	0.032328
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transporter activity	13	3.42%	0.034472
Mol Function level 4	rRNA binding	4	1.05%	0.035905
Biol Process level 4	female gamete generation	24	6.32%	0.035915
Mol Function level 5	glutathione peroxidase activity	3	0.79%	0.036629
Mol Function level 3	oxidoreductase activity, acting on NADH or NADPH	8	2.11%	0.038143
Biol Process level 5	oogenesis	24	6.32%	0.038529
Mol Function level 3	glutathione peroxidase activity	3	0.79%	0.038599
Biol Process level 2	catabolic process	23	6.05%	0.039565
Mol Function level 2	vitamin binding	5	1.32%	0.040717
Mol Function level 4	magnesium ion binding	6	1.58%	0.042275
Cell Component level 4	mitochondrial inner membrane	18	4.74%	0.042333
Cell Component level 4	organelle inner membrane	18	4.74%	0.042333
Cell Component level 3	organelle inner membrane	18	4.74%	0.042758
Biol Process level 4	cellular macromolecule catabolic process	14	3.68%	0.043554
Cell Component level 5	mitochondrial inner membrane	18	4.74%	0.044197
Cell Component level 5	organelle inner membrane	18	4.74%	0.044197

Table 13 continued.

Category	Term	Count	%	PValue
Biol Process level 5	acetyl-CoA metabolic process	6	1.58%	0.044248
Mol Function level 4	NADH dehydrogenase activity	7	1.84%	0.045265
Cell Component level 4	cytosolic small ribosomal subunit (sensu Eukaryota)	6	1.58%	0.045402
Biol Process level 1	reproduction	32	8.42%	0.045469
Mol Function level 4	primary active transmembrane transporter activity	13	3.42%	0.045495
Cell Component level 5	cytosolic small ribosomal subunit (sensu Eukaryota)	6	1.58%	0.04633
Mol Function level 3	ligase activity, forming carbon-sulfur bonds	4	1.05%	0.046669

Table 14. Significantly enriched GO categories for the 352 differentially expressed genes associated with unique spatiotemporal memories and not with the time of day, training group, or activity state.

Category	Term	Count	%	PValue
Biol Process level 3	extracellular structure organization and biogenesis	7	3.12%	0.004418
Biol Process level 4	regulation of synaptic growth at neuromuscular junction	4	1.79%	0.008722
Biol Process level 3	regulation of synaptic growth at neuromuscular junction	4	1.79%	0.009285
Biol Process level 4	ovarian nurse cell to oocyte transport	5	2.23%	0.009366
Biol Process level 4	synapse organization and biogenesis	6	2.68%	0.01007
Biol Process level 3	ovarian nurse cell to oocyte transport	5	2.23%	0.010136
Biol Process level 5	synaptogenesis	5	2.23%	0.013184
Biol Process level 5	oogenesis	15	6.70%	0.014909
Biol Process level 3	synaptic growth at neuromuscular junction	4	1.79%	0.015135
Biol Process level 4	female gamete generation	15	6.70%	0.015883
Mol Function level 4	calcium ion binding	10	4.46%	0.019178
Mol Function level 4	thiol-disulfide exchange intermediate activity	3	1.34%	0.025059
Mol Function level 5	pyrophosphatase activity	18	8.04%	0.027078
Biol Process level 4	female germ-line cyst formation	3	1.34%	0.030614
Biol Process level 5	female germ-line cyst formation	3	1.34%	0.030851
Biol Process level 3	gamete generation	17	7.59%	0.031805
Biol Process level 2	sexual reproduction	17	7.59%	0.036431
Cell Component level 4	cullin-RING ubiquitin ligase complex	3	1.34%	0.038372
Mol Function level 5	acetylglucosaminyltransferase activity	4	1.79%	0.039375
Cell Component level 5	cullin-RING ubiquitin ligase complex	3	1.34%	0.040377
Cell Component level 5	SCF ubiquitin ligase complex	3	1.34%	0.040377
Biol Process level 5	neuromuscular junction development	4	1.79%	0.040774
Mol Function level 3	hydrolase activity, acting on acid anhydrides	18	8.04%	0.04549
Mol Function level 5	ATPase activity, coupled to transmembrane movement of substances	7	3.12%	0.045991
Biol Process level 4	regulation of apoptosis	6	2.68%	0.048232
Biol Process level 5	regulation of apoptosis	6	2.68%	0.048913

Table 15. Significantly enriched GO categories for genes found to be more highly expressed in morning anticipating bees (tAM-cAM), as compared to afternoon anticipating bees (tPM-cPM), after removal of genes significant for time of day, training group, and activity state.

Category	Term	Count	%	PValue
Biol Process level 5	oogenesis	13	10.83%	0.001338
Biol Process level 4	female gamete generation	13	10.83%	0.001368
Biol Process level 4	ovarian nurse cell to oocyte transport	5	4.17%	0.001468
Mol Function level 3	active transmembrane transporter activity	12	10.00%	0.001647
Biol Process level 3	ovarian nurse cell to oocyte transport	5	4.17%	0.001649
Mol Function level 4	calcium ion binding	9	7.50%	0.002118
Biol Process level 3	extracellular structure organization and biogenesis	6	5.00%	0.0024
Mol Function level 5	ATPase activity, coupled to transmembrane movement of substances	7	5.83%	0.004767
Biol Process level 3	gamete generation	14	11.67%	0.004954
Biol Process level 2	sexual reproduction	14	11.67%	0.00523
Biol Process level 1	reproduction	15	12.50%	0.005403
Mol Function level 4	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	7	5.83%	0.00567
Biol Process level 4	synapse organization and biogenesis	5	4.17%	0.008034
Mol Function level 5	pyrophosphatase activity	14	11.67%	0.008445
Biol Process level 4	female germ-line cyst formation	3	2.50%	0.01158
Biol Process level 5	female germ-line cyst formation	3	2.50%	0.011806
Mol Function level 4	hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	14	11.67%	0.012222
Mol Function level 3	hydrolase activity, acting on acid anhydrides	14	11.67%	0.014144
Mol Function level 5	P-P-bond-hydrolysis-driven transmembrane transporter activity	7	5.83%	0.014619
Mol Function level 2	transmembrane transporter activity	16	13.33%	0.015078
Mol Function level 4	primary active transmembrane transporter activity	7	5.83%	0.015953
Biol Process level 5	synaptogenesis	4	3.33%	0.018245
Biol Process level 4	anatomical structure formation	7	5.83%	0.020834
Biol Process level 2	anatomical structure formation	7	5.83%	0.023685
Biol Process level 3	anatomical structure formation	7	5.83%	0.023955
Biol Process level 5	ovarian follicle cell development	6	5.00%	0.025467
Biol Process level 3	ovarian follicle cell development	6	5.00%	0.027668
Biol Process level 4	regulation of synaptic growth at neuromuscular junction	3	2.50%	0.028461
Biol Process level 3	regulation of synaptic growth at neuromuscular junction	3	2.50%	0.030127
Mol Function level 5	ATPase activity, coupled to transmembrane movement of ions	5	4.17%	0.036075
Biol Process level 4	germ-line cyst formation	3	2.50%	0.039107
Biol Process level 5	germ-line cyst formation	3	2.50%	0.039834
Biol Process level 3	germ-line cyst formation	3	2.50%	0.041354
Biol Process level 3	synaptic growth at neuromuscular junction	3	2.50%	0.041354
Cell Component level 4	mitochondrial envelope	9	7.50%	0.043155
Cell Component level 2	membrane	34	28.33%	0.046283
Cell Component level 3	membrane	34	28.33%	0.046561
Biol Process level 4	regulation of transforming growth factor beta receptor signaling pathway	2	1.67%	0.048636
Biol Process level 4	ring canal formation, actin assembly	2	1.67%	0.048636
Biol Process level 5	ring canal formation, actin assembly	2	1.67%	0.049131
Biol Process level 5	negative regulation of transforming growth factor beta receptor signaling pathway	2	1.67%	0.049131
Biol Process level 5	regulation of transforming growth factor beta receptor signaling pathway	2	1.67%	0.049131
Cell Component level 5	mitochondrial envelope	9	7.50%	0.049503

Table 16. Significantly enriched GO categories for genes found to be more highly expressed in afternoon anticipating bees (tPM-cPM), as compared to morning anticipating bees (tAM-cAM), after removal of genes significant for time of day, training group, and activity state.

Category	Term	Count	%	PValue
Biol Process level 5	lipid biosynthetic process	6	5.77%	0.002159
Biol Process level 4	lipid biosynthetic process	6	5.77%	0.002231
Biol Process level 3	lipid biosynthetic process	6	5.77%	0.002368
Biol Process level 3	lipid metabolic process	9	8.65%	0.004092
Biol Process level 4	cellular lipid metabolic process	8	7.69%	0.004436
Biol Process level 3	cellular lipid metabolic process	8	7.69%	0.0048
Mol Function level 2	transferase activity	17	16.35%	0.019835
Mol Function level 3	transferase activity, transferring glycosyl groups	5	4.81%	0.025786
	positive regulation of retinal cell programmed cell death	2	1.92%	0.031674
Biol Process level 5		2	1.92%	0.031674
Cell Component level 4	Golgi stack	3	2.88%	0.031992
Mol Function level 5	acetylglucosaminyltransferase activity	3	2.88%	0.033085
Cell Component level 5	Golgi stack	3	2.88%	0.033661
Mol Function level 1	catalytic activity	40	38.46%	0.036336
Cell Component level 3	ubiquitin ligase complex	3	2.88%	0.046182
Cell Component level 4	ubiquitin ligase complex	3	2.88%	0.049664

REFERENCES

- Alaux, C., Sinha, S., Hasadsri, L., Hunt, G. J., Guzman-Novoa, E., DeGrandi-Hoffman, G., Uribe-Rubio, J. L., Southey, B. R., Rodriguez-Zas, S., Robinson, G. E. (2009a). Honey bee aggression supports a link between gene regulation and behavioral evolution. *Proc. Natl. Acad. Sci USA* 106, 15400-15405.
- Alaux, C., Le Conte, Y., Adams, H. A., Rodriguez-Zas, S., Grozinger, C. M., Sinha, S., and Robinson, G. E. (2009b). Regulation of brain gene expression in honey bees by brood pheromone. *Genes Brain Behav.* 8, 309-319.
- Allada, R. and Chung, B. Y. (2010). Circadian organization of behavior and physiology in *Drosophila*. *Annu Rev Physiol.* 72, 605-624.
- Ament, S. A., Corona, M., Pollock, H. S., and Robinson, G. E. (2008). Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. *Proc. Natl. Acad. Sci USA* 105, 4226-4231.
- Angeles-Castellanos, M., Mendoza, J., and Escobar, C. (2007). Restricted feeding schedules phase shift daily rhythms of c-Fos and protein Per1 immunoreactivity in corticolimbic regions in rats. *Neurosci.* 144, 344-355.
- Beling, I. (1929). Über das Zeitgedächtnis der Bienen. *Z. Vergl Physiol.* 9, 259-338.
- Ben-Shahar, Y., Leung, H. T., Pak, W. L., Sokolowski, M. B., and Robinson, G. E. (2003). cGMP-dependent changes in phototaxis: a possible role for the foraging gene in honey bee division of labor. *J. Exp. Biol.* 206, 2507-2515.
- Buttel-Reepen, H. B. v., (1900). *Sind die Bienen reflexmaschinen?: Experimentelle Beiträge zur Biologie der Honigbiene.* Biol Zbl. 20, 177-193.
- Cash, A. C., Whitfield, C. W., Ismail, N., and Robinson, G. E. (2005). Behavior and the limits of genomic plasticity: power and replicability in microarray analysis of honey bee brains. *Genes, Brain and Behavior* 4, 267-271.
- Dibner, C., Schibler, U., and Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu. Rev. Physiol.* 72, 517-549.
- Doorn, W. G. v., and Meeteren, U. v. (2003). Flower opening and closure: a review. *J. Exp. Botany.* 54, 1801-1812.
- Eban-Rothschild A. D. and Bloch, G. (2008). Differences in the sleep architecture of forager and young honey bees (*Apis mellifera*). *J. Exp. Biol.* 211, 2408-2416.

- Eckel-Mahan, K. and Sassone-Corsi, P. (2009). Metabolism control by the circadian clock and vice versa. *Nat. Struct. Mol. Biol.* 16, 462-467.
- Escobar, C., Cailotto, C., Angeles-Castellanos, M., Delgado, R. S., and Bujis, R. M. (2009). Peripheral oscillators: the driving force for food-anticipatory activity. *Eur. J. Neurosci.* 30, 1665-1675.
- Forel, A. (1910). *Das Sinnesleben der Insekten*. Munich: Reinhardt.
- Frisch, K. von (1967). *The Dance Language and Orientation of Bees*. Cambridge, MA: Harvard University Press.
- Gimenes, M., Benedito-Silva, AA., and Marques, M.D. (1993). Chronobiologic aspects of a coadaptive process: the interaction of *Ludwigia elegans* flowers and their more frequent bee visitors. *Chronobiol. Int.* 10, 20-30.
- Green, D. J. and Gillette, R. (1982). Circadian rhythm of firing rate recorded from single cells in the rat suprachiasmatic brain slice. *Brain Res.* 245, 198-200.
- Harbison, S. T., Mackay, T. F., and Anholt, R. R. (2009). Understanding the neurogenetics of sleep: progress from *Drosophila*. *Trends Genet.* 25, 262-269.
- Hendricks, J. C., Finn, S. M., Panckeri, K. A., Chavkin, J., Williams, J. A., Sehgal, A., and Pack, A. I. (2000). Rest in *Drosophila* is a sleep-like state. *Neuron* 26, 295-298.
- Honeybee Genome Sequencing Consortium (2006). Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* 443, 931-949.
- Huang, D. W., Sherman, B. T., and Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols* 4, 44-57.
- Hung, H. C., Kay, S. A., and Weber, F. (2009). HSP90, a capacitor of behavioral variation. *J. Biol. Rhythms* 24, 183-192.
- Kaiser, W. (1988). Busy bees need rest, too: Behavioural and electromyographical sleep signs in honeybees. *J. Comp. Physiol. A.* 163, 565-584.
- Kaiser, W. and Steiner-Kaiser, J. (1983). Neuronal correlates of sleep, wakefulness, and arousal in a diurnal insect. *Nature* 301, 707-709.
- Katzenberg, D., Young, T., Finn, L., Lin, L., King, D. P., Takahashi, J. S., and Mignot, E. (1998). A CLOCK polymorphism associated with human diurnal preference. *Sleep* 21, 569-576.

- Kleber, E. (1935). Hat das Zeitgedachtnis der Bienen biologische Bedeutung? *Z. Vergl. Physiol* 22, 221-262.
- Klein, B. A., Olzowy, K. M., Klein, A., Saunders, K. M., and Seeley, T. D. (2008). Caste-dependent sleep of worker honey bees. *J. Exp. Biol.* 211, 3028-3040.
- Kononenko, N. I. and Berezetskaya, N. M. (2010). Modeling the spontaneous activity in suprachiasmatic nucleus neurons: Role of cation single channels. *J. Theor. Biol.* (article in press)
- Linnaeus, C. (1751). *Philosophia Botanica*. New York, NY: Oxford University Press.
- Mackiewicz, M., Naidoo, N., Zimmerman, J. E., and Pack, A. I. (2008). Molecular mechanisms of sleep and wakefulness. *Ann. N. Y. Acad. Sci.* 1129, 335-349.
- Matile, P. (2006). Circadian rhythmicity of nectar secretion in *Hoya carnosa*. *Bot. Helv.* 116, 1-7.
- Meredith, A. L., Wiler, S. W., Miller, B. H., Takahashi, J. S., Fodor, A. A., Ruby, N. F., and Aldrich, R. W. (2006). BK calcium-activated potassium channels regulate circadian behavioral rhythms and pacemaker output. *Nat. Neurosci.* 9, 1041-1049.
- Miche, S. and Colwell, C. S. (2001). Cellular communication and coupling within the suprachiasmatic nucleus. *Chronobiol. Int.* 18, 579-600.
- Michener, C. D. (2000). *The Bees of the World*. Baltimore, MD: The Johns Hopkins University Press.
- Mistlberger, R. E. (2009). Food-anticipatory circadian rhythms: concepts and methods. *Eur. J. Neurosci.* 30, 1718-1729.
- Moore, D. and Rankin, M.A. (1983). Diurnal changes in the accuracy of the honeybee foraging rhythm. *Biol. Bull.* 164, 471-482.
- Moore, D., Siegfried, D., Wilson, R., and Rankin, M. A. (1989). The influence of time of day on the foraging behavior of the honeybee, *Apis mellifera*. *J. Biol. Rhythms* 4, 305-325.
- Moore, D. and Doherty, P. (2009). Acquisition of a time-memory in forager honey bees. *J. Comp. Phys. A* 195, 741-751.
- Oishi, K., Shiota, M., Sakamoto, K., Kasamatsu, M., and Ishida, N. (2004). Feeding is not a more potent Zeitgeber than the light-dark cycle in *Drosophila*. *NeuroReport* 15, 739-743.

- Park, D. and Griffith, L. C. (2006). Electrophysiological and anatomical characterization of PDF-positive clock neurons in the intact adult *Drosophila* brain. *J. Neurophysiol.* 95, 3955-3960.
- R Development Core Team (2006). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Ramsey, K. M., Yoshino, J., Brace, C. S., Abrassart, D., Kobayashi, Y., Marcheva, B., Hong, H. K., Chong, J. L., Buhr, E. D., Lee, C., Takahashi, J. S., Imai, S., and Bass, J. (2009). Circadian clock feedback cycle through NAMPT-mediated NAD⁺ biosynthesis. *Science* 324, 651-654.
- Renner, M. (1957). Neue Versuche uber den Zeitsinn der Honigbiene. *Z. Vergl. Physiol.* 40, 85-118.
- Robinson, G. E., Grozinger, C. M., and Whitfield, C. W. (2005). Sociogenomics: social life in molecular terms. *Nat. Rev. Genet.* 6, 257-70.
- Robinson, G. E., Fernald, R. D., and Clayton, D. F. (2008). Genes and social behavior. *Science* 322, 896-900.
- Ruben, E. B., Shemesh, Y., Cohen, M., Elgavish, S., Robertson, H. M., and Bloch, G. (2006). Molecular and phylogenetic analyses reveal mammalian-like clockwork in the honey bee (*Apis mellifera*) and shed new light on the molecular evolution of the circadian clock. *Genome Res.* 16, 1352-1365.
- Schulz, D. J. and Robinson, G. E. (1999). Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *J. Comp. Physiol. A* 185, 481-488.
- Shaw, P. J., Cirelli, C., Greenspan, R. J., and Tononi, G. (2000). Correlates of sleep and waking in *Drosophila melanogaster*. *Science* 287, 1834-1837.
- Shen, H., Watanabe, M., Tomasiewicz, H., Rutishauser, U., Magnuson, T., and Glass, J. D. (1997). Role of neural cell adhesion molecule and polysialic acid in mouse circadian clock function. *J. Neurosci.* 17, 5221-5229.
- Ueda, H. R., Matsumoto, A., Kawamura, M., Iino, M., Tanimura, T., and Hashimoto, S. (2002). Genome-wide transcriptional orchestration of circadian rhythms in *Drosophila*. *J. Biol. Chem.* 277, 14048-14952.
- Van Nest, B., Wagner, A., and Moore, D. Unpublished.
- Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics using S 4th ed.* New York, NY: Springer .

- Vollmers, C., Gill, S., DiTacchio, L., Pulivarthi, S. R., Le, H. D., and Panda, S. (2009). Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc. Natl. Acad. Sci USA* 106, 21453-21458.
- Wahl, O. (1932). Neue Untersuchungen über das Zeitgedächtnis der Bienen. *Z. Vergl. Physiol.* 16, 529-289.
- Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge, MA: Harvard University Press.
- Webb, I. C., Baltazar R. M., Lehman, M. N., and Coolen, L. M. (2009). Bidirectional interactions between the circadian and reward systems: is restricted food access a unique zeitgeber? *Eur. J. Neurosci.* 30, 1739-1748.
- Welsh, D.K., Logothetis, D. E., Meister, M., and Reppert, S. M. (1995). Individual neurons dissociated from the rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* 14, 697-706.
- Whitfield, C. W., Cziko, A. M., and Robinson, G. E. (2003). Gene expression profiles in the brain predict behavior in individual honey bees. *Science* 302, 296-299.
- Whitfield, C. W., Ben-Shahar, Y., Brillet, C., Leoncini, I., Crauser, D., LeConte, Y., Rodriguez-Zas, S., and Robinson, G. E. (2006). Genomic dissection of behavioral maturation in the honey bee. *Proc. Natl. Acad. Sci USA* 103, 16068-16075.
- Welzl, H. and Stork, O. (2003). Cell adhesion molecules: key players in memory consolidation? *News Physiol. Sci.* 18, 147-150.
- Wang, Y., Mutti, N. S., Ihle, K. E., Siegel, A., Dolezal, A. G., Kaftanoglu, O., and Amdam G. V. (2010). Down-regulation of honey bee IRS gene biases behavior towards food rich in protein. *PLoS Genetics* 6, e1000896.
- Wang, Y. C. and Huang, R. C. (2006). Effects of sodium pump activity on spontaneous firing in neurons of the rat suprachiasmatic nucleus. *J. Neurophysiol.* 96, 109-118.
- Xu, K., Zheng, X., and Sehgal, A. (2008). Regulation of feeding and metabolism by neuronal and peripheral clocks in *Drosophila*. *Cell Metab.* 8, 289-300.
- Zheng, X. and Sehgal, A. (2010). AKT and TOR signaling set the pace of the circadian pacemaker. *Current Biol.* 20, 1203-1208.