We tested the hypothesis that queen mandibular pheromone (QMP) causes changes in gene expression in the brain of the adult worker honey bee, and that these changes can be correlated to the downstream behavioral responses induced by QMP. In support of the first hypothesis, cage experiments revealed that QMP transiently regulated expression of several hundred genes and chronically regulated the expression of 19 genes. Several of these genes were also affected by QMP in experiments with bee colonies in the field, demonstrating robust gene regulation by pheromone. To evaluate the second hypothesis, we focused on one function of QMP: delaying the transition from working in the hive (e.g., brood care, or “nursing”) to foraging. We compared the list of QMP-regulated genes with the lists of genes differentially regulated in nurse and forager brains generated in a separate study. QMP consistently activated “nursing genes” and repressed “foraging genes,” suggesting that QMP may delay behavioral maturation by regulating genes in the brain that produce these behavioral states. We also report here on an ortholog of the Drosophila transcription factor kruppel homolog 1 that was strongly regulated by QMP, especially in the mushroom bodies of the bee brain. These results demonstrate chronic gene regulation by a primer pheromone and illustrate the potential of genomics to trace the actions of a pheromone from perception to action, and thereby provide insights into how pheromones regulate social life.

Materials and Methods

Genetic Manipulation. Worker bees were derived from queens that were instrumentally inseminated with semen from a single, different drone, according to established procedures (18). Because male bees are haploid, the coefficient of relatedness

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among offspring of such an instrumentally inseminated queen is 0.75. Bees were obtained from three “queen colonies,” each headed by one of these queens, R8, R11, or R16. Bees from “genotype” R8 were used for the microarray experiments, and bees from R11 and R16 were used to confirm some of the microarray results with real-time quantitative RT-PCR (qRT-PCR), as described below.

**Rearing.** Colonies were maintained at the University of Illinois Bee Research Facility according to standard commercial procedures. To provide bees of known age, honeycombs containing late-stage pupae were removed from source colonies and placed in an incubator to emerge (33°C, 95% relative humidity). For cage studies, bees were collected 16 h after eclosion and placed in small (10 × 10 × 7 cm) Plexiglas cages (35 bees per cage) for 8 h before pheromone exposure began. Bees were provided with water and food (45% honey, 45% pollen, 10% sugar water). Cages were kept in a humidity-controlled (85% relative humidity), dark incubator at 33°C. Pheromone exposure consisted of 0.1 queen equivalent (the typical amount found in one queen) of QMP (QMP+) or a solvent control (QMP−) introduced on a glass slide. This dose is sufficient for inhibiting ovary development in caged worker bees (10). The duration of pheromone exposure was 1, 2, 3, or 4 days. For each replicate, one entire QMP+ and one QMP− cage were flash-frozen in liquid nitrogen to prevent any additional changes in gene expression that may have been induced by the QMP+ cage (19). Heads were removed and stored at −80°C until dissection. Bees were sampled at the same time of day to minimize any variation caused by circadian rhythms.

For field studies, the source colony was split into three colonies, allocating roughly equal quantities of adult bees, brood, and pollen and nectar stores. One colony retained the original queen (QR), one was left queenless (QL), and the third was headed by one of these queens, R8, R11, or R16. Bees from R8 were used for the microarray experiments, and bees from R11 and R16 were used to confirm some of the microarray results with real-time quantitative RT-PCR (qRT-PCR), as described below.

**Pheromones.** QMP was obtained from PharmaTech (Delta, Canada). For cage experiments, 0.1 QMP was applied to glass slides in 10 μl of 1% H2O-isopropl alcohol and allowed to dry (9). QMP was applied to a fresh slide at the same time every day. For field experiments, commercially formulated QMP strips (PhraTech) were used and replaced daily (20).

**Confirmation of Pheromonal Activity.** To confirm QMP activity in cages, the effect of QMP on ovary development was assessed (10). Cages of bees were set up as described above and maintained for 10 days, then collected and stored at −20°C until dissection. The degree of ovary development was assessed according to Veltuis (21). Two cages from each of the three genotypes were analyzed. A total of 12 of 30 QMP+ bees developed ovaries (with developed eggs and follicle cells), whereas 0 of the 30 QMP− bees developed ovaries (P < 0.01, χ² test), demonstrating that the QMP was active and the bees were sensitive to it. To confirm QMP activity in the field, the effect of QMP on the number of queen cells built was determined. Queen cells are distinctive large cells on the honeycomb built by worker bees to rear new queens (8); QMP partially inhibits this behavior (11). The number of queen cells built in QL colonies was 31, 29, and 12 (R8, R16, and R11, respectively) compared with 1, 5, and 5 queen cells built in the QMP− colonies. In QR colonies, no queen cells were built in R8 and R16, whereas three were built in R11. QMP clearly suppressed queen cell-building behavior in the field.

**Brain Dissection.** Whole bee heads were partially lyophilized to facilitate brain dissection (22). Dissections were performed over dry ice so tissue never thawed. Because ocelli and the subesophageal ganglion frequently fractured during dissection, these were removed during all dissections, while the remainder of the brain was included. Mushroom bodies were dissected from freeze-dried brains as in ref. 22.

**Microarrays and Data Analysis.** The microarray contained ~9,000 cDNAs, representing ~7,600 different genes, 40% of which have been annotated primarily by using comparisons to *Drosophila* genes and the molecular function classification scheme of the Gene Ontology Consortium (16). This number is estimated to account for 50% of the genes in the honey bee genome, based on comparisons with the *Drosophila* genome.

For cage studies, direct competitive hybridization comparisons were made for matched samples of QMP+ and QMP− bees. Each sample consisted of 10 brains pooled (10 bees taken randomly from a cage of 35 bees after the entire cage was killed). Eight arrays were analyzed for each time point (1, 2, 3, or 4 days of pheromone exposure), a total of 32 microarrays. The eight replicates were comprised of four biological replicates (different cages) and four technical replicates. For field studies, each sample consisted of 10 brains pooled. Samples from the QR, QMP+, and QL colonies split from the R8 source colony were compared with each other in a loop design that used three microarrays, with four technical replicates, for a total of 12 arrays (23).

Dissected bee brains were homogenized in Trizol (Invitrogen Life Technologies), and RNA was extracted according to the manufacturer’s protocols. mRNA was then purified by using an Oligotex mRNA kit (Qiagen, Valencia, CA). mRNA synthesis and hybridization to microarrays followed protocols modified from ref. 24 and described in ref. 14. Arrays were scanned with Genepix software and normalized to the median intensity with regional and intensity-dependent Lowess normalization. Dyes used to label the QMP+ and QMP− samples were reversed in half of the replicates to control for dye-by-gene interactions. The following filtering protocols were followed, as in Whitfield *et al.* (14). cDNAs with average expression intensities across each set of eight arrays <350 or absent from ≥1 array were removed from the analysis. cDNAs expressed at equal levels in hypopharyngeal glands in the honey bee head (14) were removed because material from these glands can sometimes contaminate dissected brains. Only cDNAs expressed at all time points and also in the Whitfield *et al.* nurse vs. forager analysis were analyzed, to facilitate comparisons across the two studies. This left ~6,360 cDNAs, representing ~5,000 unique genes.

Bayesian analysis (25) was used to determine which cDNAs were significantly regulated by QMP. This method generates an estimated mean and 95% confidence interval (CI) for the relative level of expression of each cDNA for each experimental group of bees. cDNAs were classified as differentially expressed if their 95% CIs did not overlap between groups (one-tailed t test). This approach enables identification of small, but reproducible, differences in gene expression (26, 27). Notably, the expression of most significantly regulated genes changed by ~10%. The expression of only a few cDNAs found to be significantly regulated during the time course, only 1,158 showed differences of >10%, and only 111 showed differences of >20%.
**Results**

**QMP Influences Brain Gene Expression in the Laboratory.** There were 1,210 cDNAs with significantly higher expression and 1,397 cDNAs with significantly lower expression in the brains of QMP+ bees relative to QMP− bees. This is about eight times more than the number of false positives expected (25). Several hundred cDNAs were significantly regulated on each day of the 4-day time course, with maximum numbers on days 2 and 3 and the fewest on day 1 (Fig. 1). The magnitude differences were smallest on day 1 as well: expression of only 3 ESTs changed by 40% on day 1, whereas 21, 20, and 21 ESTs, respectively, were regulated by 40% on the other three days. Expression levels for most cDNAs changed during the time course, but a small subset was chronically regulated across days 2, 3, and 4 (13 up-regulated and 6 down-regulated). Eight of these chronically regulated genes are annotated, with high sequence similarity to known *Drosophila* or human genes (Table 1). They include the transcription factor *Kr-h1* (28), the G protein-coupled receptor *frizzled 2* (29), and a transmembrane protein involved in regulating glial cell thickness (*push/poe*) (30). See Table 5, which is published as supporting information on the PNAS web site, for a complete list of regulated genes.

**QMP-Mediated Gene Expression Correlates with Downstream Behavioral Effects.** If QMP regulation of gene expression in the brain is related to the QMP-mediated delay in the transition from hive work to foraging (15), then the following pattern is predicted: exposure to QMP activates genes in the brain associated with nursing and represses genes associated with foraging. The prediction was tested by comparing our data with results from a previous study (14). That study showed widespread differences in gene expression between nurses and foragers, with 1,360 showing significantly more mRNA abundance in nurse brains (“nurse list”) and 1,310 significantly more mRNA abundance in forager brains (“forager list”). Results of this compare agree with the prediction. A significantly larger proportion of QMP-up-regulated cDNAs was on the nurse list than were QMP-down-regulated cDNAs (Table 2). Likewise, a significantly larger proportion of QMP-down-regulated cDNAs were on the forager list than were QMP-up-regulated cDNAs. (Table 2). These trends were even more striking if only the chronically regulated cDNAs are considered (Table 2). See Table 5 for a complete listing of all QMP-regulated genes that are also on the nurse or foragers lists.

**QMP Influences Brain Gene Expression in Bee Colonies.** There were 697 cDNAs that showed significant differences in brain expression in bees sampled from QMP+ and QL colonies (Fig. 2). Overall, these expression differences were most similar to the results from cages sampled on day 3. For example, of the cDNAs significantly up-regulated in the field, 59 were also significantly up-regulated in the cages (vs. 20 that were down-regulated in the cages), and of the cDNAs down-regulated in the field, 74 were down-regulated in cages (vs. 7 that were up-regulated in the cages). Relative to cage bees, there were fewer cDNAs with significant differences in colony bees, and they showed expression differences of smaller magnitude. Among the genes significantly regulated in the brain by QMP in both cage and colony

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**Table 1. Annotated genes chronically regulated by QMP in the honey bee brain**

<table>
<thead>
<tr>
<th>Closest Drosophila (or human) match</th>
<th>Similarity score</th>
<th>Fold difference QMP+/QMP−</th>
<th>Possible function</th>
<th>Nurse/forager</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q9H2Y7 (human)</td>
<td>2E-08</td>
<td>1.11</td>
<td>WD-repeat protein</td>
<td>Nurse</td>
</tr>
<tr>
<td>CG41668</td>
<td>1E-13</td>
<td>1.12</td>
<td>PDZ domain</td>
<td>Nurse</td>
</tr>
<tr>
<td>cft</td>
<td>5E-23</td>
<td>1.16</td>
<td>Carboxylesterase</td>
<td></td>
</tr>
<tr>
<td>Traf1</td>
<td>1E-24</td>
<td>1.16</td>
<td>Tumor necrosis factor receptor-associated factor</td>
<td>Forager</td>
</tr>
<tr>
<td>CG7474</td>
<td>4E-85</td>
<td>1.17</td>
<td>Tubulinyl-tyrosine ligase</td>
<td></td>
</tr>
<tr>
<td>poe</td>
<td>9E-34</td>
<td>0.86</td>
<td>Calmodulin binding, synaptogenesis</td>
<td></td>
</tr>
<tr>
<td>frizzled 2</td>
<td>5E-96</td>
<td>0.70</td>
<td>G protein-coupled receptor</td>
<td>Forager</td>
</tr>
<tr>
<td>kr-h1</td>
<td>1E-100</td>
<td>0.59</td>
<td>Zn-finger transcription factor</td>
<td>Forager</td>
</tr>
</tbody>
</table>

Genes found to be chronically regulated by QMP with high sequence similarity to known *Drosophila* or human genes are listed below. Similarity score: BLAST (E value) (this corresponds to the EST sequence only, except for *Kr-h1*, for which full sequence was obtained, and *frizzled 2*, for which additional sequence was identified from the genome project, http://hgc.bcm.tmc.edu/blast?organism=Amellifera). Fold difference column: the maximum difference between values obtained for bees maintained in cages with or without QMP. Nurse/forager column indicate in which behavioral group the gene was found to be significantly upregulated in an independent microarray-based study (14).
Expression differences were generally lower in colony bees. A regression line is shown. In general, cDNAs down-regulated in colony bees of these cDNAs under colony and cage (day 3) conditions are plotted, and the proportion of QMP showed the same trends.

Comparison of QMP-regulated brain gene expression in bees from QR and QMP colonies relative to the QL colony; these genes may be specifically regulated by QMP and/or coregulated in the QR and QMP colonies relative to the QL colony, whereas 17 were significantly regulated by exposure to QMP (Table 6, which is published as supporting information on the PNAS web site), whereas 17 were significantly regulated by >10%. This proportion was relatively high compared with other functional categories of genes (Table 3).

Microarray analysis showed that brain expression of three transcription factors (Kr-h1, Utx, and klpfuss) was significantly affected by QMP over more than 1 day (Fig. 3A). In the colony experiments, only klpfuss was significantly regulated. The cage results were verified with qRT-PCR, using individual bee brains. (A) mRNA levels expressed in three transcription factors (Kr-h1, utx, and klpfuss) were significant in cage experiments. (B) mRNA levels determined by qRT-PCR for bees exposed to QMP for 3 days from the same genotype as in A. Number of individually analyzed brains is indicated on bars. Data are means ± SE (converted to the same arbitrary scale as the mean), normalized to the QMP sample. Statistical analysis was done by one-tailed t test. (C) Same as B, except bees were from a second genotype.

were: transcription factors HLH3B and klpfuss, gap junction proteins inx2 and inx3, Tor, a phosphatidylinositol 3-kinase, Nvr2, an Na+/K+ exchanging ATPase complex, RacGAP, a GTPase activator, and Rab6, a GTPase.

As was the case in the laboratory experiments, a significantly larger proportion of QMP-up-regulated cDNAs were on the nurse list than were QMP-down-regulated cDNAs. Likewise, a significantly larger proportion of QMP-up-regulated cDNAs were on the nurse list than were QMP-down-regulated cDNAs (P < 0.001, χ² tests). Genes chronically regulated by QMP showed the same trends.

Table 2. QMP activates genes in the bee brain associated with nursing and represses genes associated with foraging

<table>
<thead>
<tr>
<th>Location</th>
<th>QMP</th>
<th>% on nurse list</th>
<th>% on forager list</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage</td>
<td>Total QMP ↓</td>
<td>1,397</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Chronically ↓</td>
<td>1,210</td>
<td>33</td>
</tr>
<tr>
<td>Colony</td>
<td>QMP ↑</td>
<td>374</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>QMP ↑</td>
<td>323</td>
<td>42</td>
</tr>
<tr>
<td>Cage and colony</td>
<td>QMP ↑</td>
<td>59</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>QMP ↑</td>
<td>74</td>
<td>53</td>
</tr>
</tbody>
</table>

QMP-regulated cDNAs were compared to the lists of cDNAs significantly up-regulated in nurse bees or down-regulated in foragers. A significantly larger proportion of QMP-up-regulated cDNAs were on the nurse list than were QMP-down-regulated cDNAs. Likewise, a significantly larger proportion of QMP-up-regulated cDNAs were on the nurse list than were QMP-down-regulated cDNAs (P < 0.001, χ² tests). Genes chronically regulated by QMP showed the same trends.

Table 3. Effects of QMP on different functional categories of genes

<table>
<thead>
<tr>
<th>Functional category</th>
<th>No. annotated</th>
<th>% Regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein phosphatases</td>
<td>35</td>
<td>2.9</td>
</tr>
<tr>
<td>Synaptic vesicle transport</td>
<td>66</td>
<td>1.5</td>
</tr>
<tr>
<td>Ion channels</td>
<td>73</td>
<td>6.8</td>
</tr>
<tr>
<td>Protein kinases</td>
<td>87</td>
<td>8.0</td>
</tr>
<tr>
<td>Cytoskeletal organization and biogenesis</td>
<td>89</td>
<td>4.5</td>
</tr>
<tr>
<td>Receptors</td>
<td>106</td>
<td>5.7</td>
</tr>
<tr>
<td>Peptidases</td>
<td>108</td>
<td>5.6</td>
</tr>
<tr>
<td>RNA-binding proteins</td>
<td>127</td>
<td>3.9</td>
</tr>
<tr>
<td>Oxido-reductases</td>
<td>129</td>
<td>8.5</td>
</tr>
<tr>
<td>Transcription factors</td>
<td>129</td>
<td>13.2</td>
</tr>
</tbody>
</table>

For each functional category, the number of genes significantly regulated by QMP (>10%) for at least 1 day in cage experiments are shown. Categories of molecular function are from the Gene Ontology Consortium; annotation is from ref. 16.

QMP Effects on Transcription Factor. The gene with the biggest difference in mean expression levels in the cage experiments was a transcription factor, Kr-h1. Because transcription factors regulate the expression of other genes, they may serve as markers for regulation of pheromone-mediated “transcriptional programs” that control downstream behavioral effects. A total of 129 cDNAs annotated as transcription factors were significantly regulated by QMP, whereas 17 were significantly regulated by >10%. This proportion was relatively high compared with other functional categories of genes (Table 3).

Microarray analysis showed that brain expression of three transcription factors (Kr-h1, Utx, and klpfuss) was significantly affected by QMP over more than 1 day (Fig. 3A). In the colony experiments, only klpfuss was significantly regulated. The cage results were verified with qRT-PCR, using individual bee brains. (A) Expression levels (QMP−/QMP+) determined by microarray analysis on days (D) 1–4 for cage bees and colony bees. (B) Expression levels determined by qRT-PCR for bees exposed to QMP for 3 days from the same genotype as in A. Number of individually analyzed brains is indicated on bars. Data are means ± SE (converted to the same arbitrary scale as the mean), normalized to the QMP− sample. Statistical analysis was done by one-tailed t test. (C) Same as B, except bees were from a second genotype.
brain expression of Kr-h1 mRNA levels in mushroom bodies. Differences were significant in trial 1 ($P < 0.05$) and trial 3 ($P < 0.01$) but not in trial 2 ($P = 0.09$). Other notations are as in Fig. 3.

Two additional experiments were conducted to further establish Kr-h1 as a candidate gene to study long-term, behaviorally relevant changes in brain gene expression caused by pheromone exposure. First, we determined whether QMP effects on Kr-h1 expression in the brain were associated with behaviorally related differences in expression. Previous microarray studies indicated that Kr-h1 expression is higher in foragers relative to nurses (14). qRT-PCR analysis agreed, showing that brain Kr-h1 expression was increased in foragers relative to 1-day-old bees (Fig. 4A). Second, we determined whether Kr-h1 is expressed in the mushroom bodies, as a first step toward exploring the effects of pheromone on gene expression in brain regions involved in higher-order integration and processing. Kr-h1 was expressed in the mushroom bodies, with expression significantly higher in foragers relative to 1-day-old bees (Fig. 4B) and also significantly higher in QMP+ bees than QMP− bees (Fig. 4C). Kr-h1 expression levels were not significantly different in optic lobes of QMP− vs. QMP+ bees (data not shown), suggesting that expression of this gene is specifically regulated in the mushroom bodies rather than the whole brain. Cloning A. mellifera kr-h1 (GenBank accession no. AY338499, see Fig. 5, which is published as supporting information on the PNAS web site) revealed that it contains eight zinc finger domains from amino acids 62–289 and has significant sequence similarity to the Drosophila Kr-h1 protein and the partial Anopheles gambiae protein. There are two transcripts for Kr-h1 present in Drosophila, Kr-h1 RA and RB (28). The RA transcript is present in larvae and adults, whereas the RB transcript is present only in embryos and has an additional 55 aa on the N terminal. The cloned A. mellifera kr-h1 does not contain this N-terminal extension, and thus matches the RA transcript most closely. However, it is ~200 aa shorter than the RA, because of deletions throughout the gene.

**Discussion**

This study has demonstrated that QMP causes changes in expression levels of many genes in the brain of adult honey bees, and that these changes correlate with some of the downstream behavioral effects of the pheromone. QMP effects on gene expression were detected both in a controlled laboratory environment and in bee colonies in the field, which represent a more natural environment. QMP causes transient changes in expression of several hundred genes, but causes chronic changes in only a small subset of genes in the brain.

The effects of QMP on brain gene expression changed over time. One possible explanation for this is that the transcriptional response to QMP is dynamic, with one set of genes activating/repressing expression of another downstream set of genes in “transcriptional cascades” reminiscent of what occurs during Drosophila neural development (31). These genes may only need to act transiently to produce the necessary response to queen pheromone, such as neuronal remodeling of pheromone-responsive networks. For example, bees raised in queenless conditions for 4 days have smaller synaptic structures in the antennal lobe, suggesting that QMP may influence synaptic growth and remodeling during this time frame (12). Thus, the dynamic nature of the response to QMP might be related to the fact that the bee’s olfactory system continues developing for the first few days of adult life (32).

Alternatively, it simply might be difficult to detect small, but statistically significant, differences in gene expression across consecutive days, even with the relatively large number of replicates used in this study. In most cases, the observed significant changes in gene expression were relatively subtle (~20%). Perhaps the relatively small QMP-induced expression differences are caused by effects of QMP on a small set of neurons. Small, but statistically significant, differences in gene expression are detectable in microarrays studies such as ours with numerous replicates (14, 27) and can have biological significance (26, 27).

Some genes chronically regulated by QMP may be involved in stably altering neuronal activity and responsiveness. For example, one of the chronically down-regulated genes encodes a homolog of the Drosophila protein Pushover (also named Poe), which has been shown to play a role in regulating glial cell thickness (30) and neuronal excitability (33). Such alterations in central brain processing regions could lead to changes in responsiveness to various stimuli, thereby altering behavior (34).

There was considerable overlap in the genes regulated by QMP in the cages and in colonies, but gene expression changes in the colonies were smaller and involved fewer genes. This might be because the “QMP strip” used in the colony releases a lower amount of pheromone per bee than the glass slide in the cages; thus, the colony bees may have been exposed to an almost 2-fold lower dose of QMP (K. N. Slessor, personal communication). Another factor is that the colony environment is more varied with respect to pheromonal and other stimuli. In particular, the brood in the colonies releases a pheromone that produces many of the same effects as QMP (35–38), and this may have attenuated the response to QMP in the colony experiment. If so, then the set of genes that were found to be regulated in both the colony and the cage experiments may be robustly regulated by or...
only sensitive to QMP. Further cage experiments using brood pheromone can help address these questions. QMP and a live queen had similar effects on some, but not all, genes. This finding is consistent with observations showing that QMP is not as effective as a full queen extract in producing behavioral and physiological responses. For example, an additional four components were recently identified that improve the performance of synthetic queen pheromone in the attraction of workers to the queen, and even this nine-component blend is not quite as effective as the full extract (9). The genes that were specifically regulated only under queenright conditions may be responding to other unidentified components of queen pheromone and may prove to be reliable markers for future elucidation of a more complete pheromone blend.

QMP consistently activated genes correlated with nursing behavior and repressed genes correlated with foraging behavior, in both cage and colony experiments. This finding suggests that the effects of QMP on the timing of the transition from working in the hive to foraging (15) may be caused by QMP regulation of genes in the brain that produce these behavioral states. At least some QMP-regulated genes may be involved in regulating the transitions to nursing and foraging, rather than the maintenance of these states, because the bees we used were too young or simply unable to perform these behaviors in laboratory cages. The fact that many QMP-regulated genes were not found to be associated with nursing or foraging behavior may reflect the fact that QMP also is involved in the regulation of several other behavioral and physiological processes besides age-related division of labor, such as inhibiting ovary development and the rearing of new queens. Further microarray experiments may identify distinct transcriptional programs that relate to other pheromone-regulated processes.

The proportion of transcription factors regulated by QMP was relatively high compared with other functional groups of genes. This result suggests transcription factors may be important targets of pheromone activation. To aid in the future identification of specific transcriptional programs, we catalogued the transcription factors whose expression levels were significantly regulated by QMP. Only one transcription factor was found to be chronically regulated; most showed significant changes in expression on only 1 day. transiently regulated transcription factors may simply initiate a downstream program and be subsequently turned off in a matter of hours as is the case for CREB (cyclic AMP-response element binding protein), which initiates changes in neuronal plasticity (reviewed in refs. 39 and 40).

Kr-h1 was the most highly and robustly regulated gene identified in this study. Furthermore, Kr-h1 has the unique trait of being chronically regulated by a pheromone. Kruppel homologs are zinc finger transcription factors that play important roles in orchestrating development and cell differentiation (reviewed in (17), including neural development (31). Kr-h1 in particular was identified as an ec dysome-sensitive transcript during Drosophila morphogenesis (28), and in a gain-of-function screen for genes involved in motor axon guidance and synaptogenesis in Drosophila larvae (41). Differences in expression patterns and sequence divergences within the DNA-binding regions of Kruppel-like proteins across mammalian lineages suggest that members of this family have undergone duplication and acquired novel functions in different species during evolution (42).

The A. mellifera Kr-h1 protein is highly similar to Drosophila Kr-h1 and the Anopheles gambiae ortholog, suggesting that it functions similarly in all three insects. However, in the case of the honey bee, a species with a highly derived form of social organization, it has evolved to be regulated by a pheromone, perhaps in addition to intrinsic factors common to the dipteran species with solitary lifestyles. QMP regulation of Kr-h1 occurs in the mushroom bodies, the sites of integration of sensory information, so Kr-h1 may be involved in organizing stable changes in gene expression and neuron structure that are necessary to transduce the chemosensory QMP stimulus to downstream changes in behavior and physiology. Further experiments will be necessary to map Kr-h1 expression throughout the brain to further elucidate the function of Kr-h1 in response to pheromone. Given that Kr-h1 is strongly down-regulated by QMP, it is puzzling that Kr-h1 is repressed by QMP in young honey bees but up-regulated in older bees, because both groups were taken from colonies with a queen. Perhaps this is because foragers typically contact the queen less than do younger bees, or foragers are simply less responsive to QMP. Further experiments will be needed to explore this issue.

QMP affects the expression of many genes in the bee brain, and in particular changes expression of genes associated with behaviors that also are regulated by this pheromone. Some effects on gene expression are relatively transient, which may reflect short-term modifications in synaptic plasticity, and some are more chronic, which may reflect long-term changes in neuronal responsiveness and behavioral state. Some of the genes identified here may represent transcriptional programs regulating particular pheromone-mediated physiological and behavioral processes. Further genomewide analysis of the transcriptional effects of QMP and other pheromones should lead to new insights into how pheromones regulate social life.

We thank K. Priuet for expert assistance with the bees; A. Barron, C. Schook, and L. Wright for technical assistance; R. Velarde for samples of 1-day-old and forager bees; S. Rodriguez-Zas for help with statistical analysis; M. Band for fabricating microarrays of outstanding quality; C. Keeling, S. Hoover, K. N. Slessor, and M. L. Winston for helpful advice; and R. Maleszka and members of the Robinson laboratory for comments that improved the manuscript. This work was supported by a Beckman Institute postdoctoral fellowship (to C.M.G.), a National Science Foundation Bioinformatics postdoctoral fellowship (to C.W.W.), and grants from the Burroughs Welcome Trust, the National Institutes of Health, and the U.S. Department of Agriculture (to G.E.R.).