Hygienic behavior of the honey bee (*Apis mellifera*) is independent of sucrose responsiveness and foraging ontogeny

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Abstract

Hygienic behavior in honey bees is a behavioral mechanism of disease resistance. Bees bred for hygienic behavior exhibit an increased olfactory sensitivity to odors of diseased brood, which is most likely differentially enhanced in the hygienic line by the modulatory effects of octopamine (OA), a noradrenaline-like neuromodulator. Here, we addressed whether the hygienic behavioral state is linked to other behavioral activities known to be modulated by OA. We specifically asked if, during learning trials, bees from hygienic colonies discriminate better between odors of diseased and healthy brood because of differences in sucrose (reward) response thresholds. This determination had to be tested because sucrose response thresholds are susceptible to OA modulation and may have influenced the honey bee's association of the conditioned stimulus (odor) with the unconditioned stimulus (i.e., the sucrose reward). Because the onset of first foraging is also modulated by OA, we also examined whether bees from hygienic colonies differentially forage at an earlier age compared to bees from non-hygienic colonies. Our study revealed that 1-day- and 15- to 20-day-old bees from the hygienic line do not have lower sucrose response thresholds compared to bees from the non-hygienic lines. In addition, hygienic bees did not forage at an earlier age or forage preferentially for pollen as compared to non-hygienic bees. These results support the idea that OA does not function in honey bees simply to enhance the detection of all chemical cues non-selectively or control related behaviors regardless of their environmental milieu. Our results indicate that the behavioral profile of the hygienic bee is sculpted by multiple factors including genetic, neural, social and environmental systems.
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Introduction

Honey bees exhibit a wide range of behaviors that appear to be linked by their susceptibility to octopamine (OA) modulation, but the nature and scope of such associations are far from understood. Octopamine is a key neuroactive substance that modulates a diverse array of behaviors in arthropods and other invertebrate species; essentially, it is the chemical and functional equivalent of norepinephrine in vertebrate animals (Evans, 1985; Liebersat and Pflüger, 2004; Roeder, 2005, for reviews). In the honey bee (*Apis mellifera*), OA has been shown to contribute to the shaping of behaviors performed both inside and outside the nest; it influences nestmate recognition (Robinson et al., 1999), responsiveness to brood pheromone (Barron and Robinson, 2005; Barron et al., 2002; Schulz et al., 2002) and the transition from in-hive tasks to foraging (Schulz and Robinson, 1999, 2001; Schulz et al., 2003; Wagner-Hulme et al., 1999). In insects, the effects of this monoamine encompass alterations in olfactory and gustatory information processing, arousal and motor activity (Hammer and Menzel, 1995; Farooqui et al., 2003; Liebersat and Pflüger, 2004).

Another honey bee behavior that may be rooted in how OA functions within the nervous system is hygienic behavior (Spivak et al., 2003). When hygienic behavior is expressed in a colony, it is displayed by 15- to 20-day-old bees (middle-age bees) that are typically younger than most foragers (Arathi et al., 2000). Honey bees that have the genetic predisposition to perform hygienic behavior are characterized by their ability to detect, uncap and remove diseased and mite-parasitized brood from the nest, limiting disease transmission and reducing the

reproductive success of the parasitic mite *Varroa destructor* (Rothenbuhler, 1964; Spivak, 1996; Spivak and Reuter, 2001a, b). All bees are able to perform the motor tasks of uncapping and removing abnormal brood from the nest, but there is great variability among colonies in the rates of initiation and completion of these components of hygienic behavior. We have established previously in laboratory experiments that individual bees from colonies bred for hygienic behavior are better able to detect and discriminate between odors associated with healthy and diseased brood compared to non-hygienic bees (Masterman et al., 2000, 2001). We hypothesize that such increased olfactory sensitivity and responsiveness result in quick and efficient detection, uncapping and removal of diseased and parasitized brood by hygienic colonies in the field (Arathi and Spivak, 2001; Gramacho and Spivak, 2003). Although we do not have conclusive evidence that oral administration of OA to colonies of bees in the field increases the probability that individuals within the colony will perform hygienic behavior, we do have solid evidence that oral administration of OA to individual non-hygienic bees enhances the sensitivity of their chemoreceptors to odors of diseased brood, as measured by electroantennograms (Spivak et al., 2003). A similar administration of OA to hygienic honey bees does not increase olfactory responsiveness, which is already enhanced compared to non-hygienic bees (Spivak et al., 2003). Furthermore, the highly selective OA receptor antagonist, epinastine, blocks the innate olfactory enhancement in hygienic bees (Spivak et al., 2003). Lastly, honey bees performing hygienic behavior have been shown to possess a significantly greater level of OA immunoreactivity in the protocerebral neurons (cluster-3 cells) of the brain (Spivak et al., 2003). Collectively, these findings provide a link between OA and hygienic behavior, especially the ability to detect the odors of diseased brood to which subsequent hygienic behavioral routines are tied. Our working model is that hygienic behavior is expressed as a continuum. At one end, the rapid detection of olfactory cues at low concentrations by hygienic bees increases the probability of rapid expression of hygienic-related motor patterns. At the opposite end, the delayed detection of olfactory cues by non-hygienic bees may or may not trigger the motor patterns, depending on the genetic and environmental conditions of the colony. Although numerous factors within the colony and a given bee phenotype will determine whether hygienic behavior will be expressed, an enhancement of diseased-brood detection will increase the probability that the behavioral continuum will be shifted towards the uncapping and removal of diseased brood.

Assuming that OA plays a role, direct or indirect, in the differential modulation of olfactory sensitivity, the question still remains whether this component per se factors into the ability of hygienic bees to discriminate odors related to diseased brood better than non-hygienic bees in laboratory trials. The reason for this is as follows: odor discrimination trials are based on an associative conditioning paradigm that pairs an unconditioned stimulus, sucrose, with a conditioned stimulus, the odor of healthy or diseased pupae. The bee uses an unconditioned proboscis extension response (PER) to obtain the sucrose reward during conditioning. If, however, hygienic bees have a lower response threshold for the sucrose reward, then any interpretation

of the olfactory discrimination results may be confounded; for example, differences in odor discrimination may be based more on variables such as motivation, reinforcement or learning and less on differences in odor detection. Documenting the potential differences in sucrose responsiveness among the two honey bee lines is even more compelling in the light of other research, which indicates that OA administration can lower the threshold concentration at which honey bees respond with proboscis extension to sucrose when touched to the antennae (Mercer and Menzel, 1982; Pankiw and Page, 2003; Scheiner et al., 2002). Bees with lower sucrose response thresholds tend to perform better in both olfactory and tactile associative conditioning trials (Scheiner et al., 1999, 2001). In addition, the reinforcement pathway involved in PER and the sucrose reward is octopaminergic (Hammer, 1993; Farooqui et al., 2003).

Our aim was to determine whether hygienic behavior is inextricably linked to other OA-susceptible behaviors. Thus, we examined whether hygienic and non-hygienic honey bees differed in their sucrose response thresholds, a responsiveness that is modulated by OA (Pankiw and Page, 2003; Scheiner et al., 2002). We also examined whether the developmental onset of foraging differed among the two honey bee genotypes. Precocious foraging can be elicited by oral administration of OA (Schulz and Robinson, 2001), and the ontogeny of foraging behavior is clearly associated with rising levels of OA in the brain (Wagner-Hulme et al., 1999). Because the expression of hygienic behavior is also associated with OA and because hygienic bees are typically of pre-foraging age, one could predict that hygienic bees might exhibit precocious foraging behavior. Therefore, we tested the sucrose response thresholds of hygienic and non-hygienic bees in the laboratory and their foraging ontogeny in the field.

Materials and methods

Breeding

The breeding program for hygienic behavior began in 1993. Colonies of bees derived from *A. mellifera ligustica* were used as breeding stock from which lines of hygienic and non-hygienic colonies were selected. A standard field assay was used to select colonies for hygienic behavior by freeze-killing approximately 200 cells of wax-capped pupae and recording the time it took for the bees in the colony to detect, uncap and remove the dead brood (Spivak and Downey, 1998; Spivak and Reuter, 1998). If the dead brood was removed within 48 h, the colonies were considered hygienic, and those that took over 4 days to remove freeze-killed brood were considered non-hygienic. The hygienic and non-hygienic lines were maintained by raising daughter queens from the most hygienic and non-hygienic colonies. The daughter queens were then instrumentally inseminated with a mixture of semen from drones of other hygienic and non-hygienic colonies.

Experiment I: sucrose response thresholds

The experiments were conducted in the summer of 2003 at the University of Minnesota. Five hygienic and three non-hygienic colonies were chosen to be the source of bees (parental colonies). The parental colonies were maintained in standard Langstroth beekeeping equipment and the colony populations, and stores of nectar and pollen were equalized so that those variables would not influence the experiment.

Two age sets of bees were used in this experiment: 1-day-old bees and 15- to 20-day-old bees, the latter age range is when bees predominantly

perform hygienic behavior (Arathi et al., 2000). To obtain 1-day-old bees, frames of capped pupae from each parental colony were transferred into separate cages and placed into an incubator (34°C and 50% RH) until adults emerged. Recently emerged bees from each line were placed in different cages inside the incubator overnight where they were fed 30% (w/v) sucrose. It was important to control the concentration of sucrose that bees were offered since previous studies showed that an individual bee's perception of sucrose solution could be modulated by the bee's recent feeding experiences (Pankiw et al., 2001). When the bees were 24 h old, their sucrose response threshold was tested in the laboratory. This process was repeated until at least 30 bees from each of the parental hygienic and non-hygienic colonies were tested. A total of 240 hygienic and 242 non-hygienic bees were tested between 14 July and 8 September, 2003.

To obtain bees that were between 15 and 20 days, another portion of recently emerged bees (approximately 200 bees randomly selected from the same five parental hygienic and three parental non-hygienic colonies) were marked with paint on the thorax to identify them by age and genetic line (they were not marked according to colony of origin within genotype). These bees were introduced into a host hygienic or non-hygienic colony in the field, each serving as foster colonies for the bees to control for the environment of the young bees as they aged. Bees were marked every 3 days for a period of 1 month. The brood areas and pollen stores were equalized between the host colonies, and each had a laying queen. When marked bees in host colonies were 15–20 days, they were removed from the colony, and their sucrose response threshold was tested in the laboratory. In all, 111 hygienic and 112 non-hygienic bees were tested in this part of the experiment.

Sucrose response threshold assay

To test the sucrose response threshold of the bees, they were individually harnessed with tape into plastic tubes so that only the head was visible and were fed water until satiation. Two hours later, each bee was tested for its response to water. Any bee that extended its proboscis to water was allowed to drink until satiation, until it no longer extended its proboscis to the water stimulus. Allowing bees to drink water before the test controlled for effects of thirst on the sucrose sensitivity of bees (Pankiw et al., 2001). Immediately afterwards, the sucrose response threshold of each bee was tested. The following sucrose solutions were presented to the bees' antennae in ascending concentrations: 0.1%, 0.3%, 1%, 3%, 10%, 30% (wt/vol). Sucrose solutions were prepared using distilled water and Sigma brand sucrose (99.5% purity). The lowest concentration eliciting a positive response (proboscis extension) is the individual bee's response threshold to sucrose (following Pankiw and Page, 2000). The inter-bee time interval between concentrations was 2 to 3 min depending on how many bees were being tested at one time.

For data presentation, the sucrose concentrations were transformed to log10 values, which resulted in a linear response relationship and followed the convention of Page et al. (1998). The proportion of bees from each line that extended their proboscis at each sucrose concentration was compared using likelihood ratio Chi-squared tests.

Experiment II: foraging ontogeny and preference

The experiments were conducted in the summer of 2004. Three hygienic and three non-hygienic colonies were established by transferring approximately 2000 bees that were adhering to three combs of brood and one of nectar and pollen from large "parent" colonies in the field into small "daughter" colonies. Young queens from the hygienic and non-hygienic lines were introduced into the daughter colonies. Combs of sealed worker pupae within 1–2 days of emergence as adults, from each parental colony in each line, were placed in cages in an incubator. Newly emerged cohorts of 300 1-day-old hygienic and non-hygienic bees were marked with paint on the thorax to indicate their age and were placed into their respective daughter colonies every 4 days such that each daughter colony housed either hygienic or non-hygienic bees. Five age cohorts, corresponding to five colors, were added to each nucleus colony over 20 days. One day before foraging observations began, the colonies were equalized with respect to brood areas, pollen and nectar stores and number of bees. These control measures minimized variation among the colonies and ensured that differences among colonies in pollen stores would not affect the age

at which bees began to forage (e.g., Fewell and Winston, 1992; Pankiw and Page, 2001). When the oldest marked bees in the colonies were 27 days old and the youngest were 10 days old, foraging observations were performed twice daily for 4 days. The first observations began at 09:30, and second observations began at 15:30 h. The entrance of each colony was closed for 20 min at a time, and all returning marked foragers were collected into individual wire cages. Captured foragers were anesthetized with ice and recorded as: pollen foragers (by the presence of pollen on their corbicula), nectar foragers (by the sucrose concentration of crop contents, measured with a refractometer), both pollen and nectar foragers or empty foragers (by the lack of pollen or nectar). Foragers were sampled without replacement in their colonies. The day after foraging observations ended, all marked bees remaining in the colonies were collected and counted. From this census, the number of marked bees was calculated in each age cohort that was lost during the course of the experiment in each colony (due to mortality, drift or other unknown causes). The proportion of marked bees observed foraging each day was calculated from the adjusted total of originally marked bees minus the marked bees that were lost from each age cohort. The data were analyzed using likelihood ratio Chi-squared tests, comparing the proportion of each age cohort foraging between the hygienic and non-hygienic lines (pooling colonies within line) and comparing the proportion of foragers among colonies within each line.

Results

Experiment I: sucrose response thresholds

We tested the sucrose response threshold of 51, 78 and 113 1-day-old bees from the three non-hygienic colonies and 30, 38, 40, 43 and 89 1-day-old bees from the five hygienic colonies. There were no differences in the sucrose response thresholds of bees among colonies within each line ($P > 0.05$ for all comparisons), so data from colonies within line were pooled. A between genotype comparison revealed that the 1-day-old hygienic ($n = 240$) and non-hygienic bees ($n = 242$) did not differ in their responsiveness to sucrose at any concentration (Fig. 1A).

The 15- to 20-day-old hygienic ($n = 111$) and non-hygienic bees ($n = 112$) also did not differ in their responsiveness to sucrose at each concentration (Fig. 1B). Because the older bees were collected from their host hygienic or non-hygienic colonies and were not marked by colony of origin, it was not possible to compare differences among colonies within lines.

Experiment II: foraging ontogeny and preference

The day before foraging observations began, the three hygienic and three non-hygienic colonies had, respectively, 1078 ± 393 and 1199 ± 124 cm^2 of unsealed brood (eggs and larvae); 2741 ± 864 and 2331 ± 747 cm^2 of sealed brood (pupae); 173 ± 97 and 171 ± 33 cm^2 pollen stores; and 2219 ± 699 and 1957 ± 741 nectar/honey stores. There were no significant differences in these measures between the lines (t tests; $P > 0.05$ for all measures). Therefore, the colonies were relatively equal in brood areas and resource stores, especially in unsealed brood and pollen stores, which are known to influence the need and onset of foraging.

A total of 1500 marked bees were introduced into each of the six colonies. Over the 4 days of foraging observations, 878 marked bees were collected foraging from the hygienic colonies, and 914 marked bees were collected from the non-
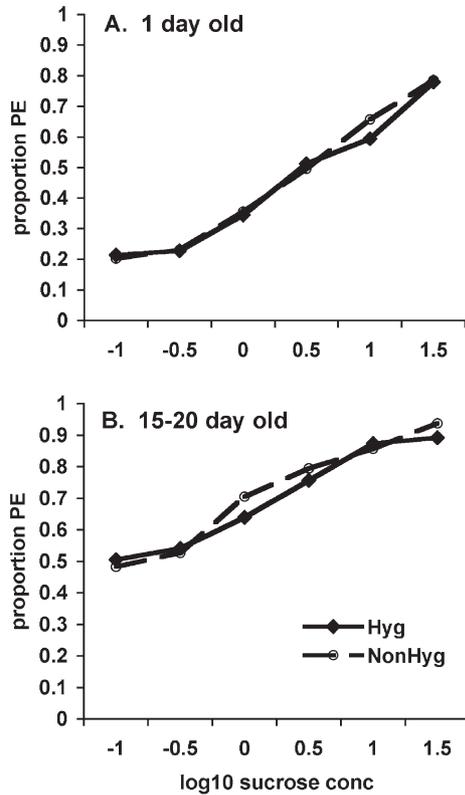
Fig. 1. The proportion of hygienic and non-hygienic bees responding with proboscis extension (PE) to antennal stimulation with increasing concentrations of sucrose. Percent concentrations were converted to a log10 scale: −0.1, −0.5, 0, 0.5, 1.0 and 1.5 correspond to 0.1%, 0.3%, 1%, 3%, 10% and 30% (w/v), respectively. Likelihood ratio Chi-squared tests revealed no significant differences in the proportion of bees responding to each sucrose concentration. (A) Results of 1-day-old bees from each line across multiple sucrose concentrations: 0.1%: $\chi^2 = 0.428$, $P = 0.26$; 0.3%: $\chi^2 = 0.139$, $P = 0.45$; 1%: $\chi^2 = 0.381$, $P = 0.36$; 3%: $\chi^2 = 0.215$, $P = 0.05$; 10%: $\chi^2 = 0.071$, $P = 0.85$; 30%: $\chi^2 = 0.228$, $P = 0.27$. (B) Results of 15- to 20-day-old bees from each line across multiple sucrose concentrations: 0.1%: $\chi^2 = 0.738$, $P = 0.26$; 0.3%: $\chi^2 = 0.837$, $P = 0.45$; 1%: $\chi^2 = 0.295$, $P = 0.36$; 3%: $\chi^2 = 0.497$, $P = 0.05$; 10%: $\chi^2 = 0.714$, $P = 0.85$; 30%: $\chi^2 = 0.220$, $P = 0.27$.

hygienic colonies. The day after foraging observations ended, 1626 hygienic and 1351 non-hygienic marked bees remained in the colonies. Therefore, 44.4% of the originally marked hygienic bees and 49.7% of the non-hygienic bees were lost or died over the course of the experiment. Table 1 shows the total number of marked bees per age cohort from the three hygienic and three non-hygienic colonies that were collected foraging over the 4 days of observations. There were no significant differences in the mean number of marked foragers per colony from the two lines, showing that all colonies had the same relative number of foragers in each age cohort.

There were no significant differences between the hygienic and non-hygienic lines (colonies pooled) in the proportion of successful foragers (bees returning with pollen and/or nectar) within the two youngest age cohorts of bees, 11- to 14-day-old and 15- to 18-day-old, which could be considered precocious foragers. Significant differences between the hygienic and non-hygienic lines were observed only within the 19- to 22-day-old and 23- to 36-day-old cohorts (Fig. 2; Table 2), when the non-

Table 1
Comparison of the number of marked bees in each of the five age cohorts collected while foraging over the 4 days of the experiment

Age cohort	Line	Total marked foragers collected (3 colonies combined)	$X \pm SD$ marked foragers collected per colony	Students t test [a]
11–14	Hyg	150	50.0 ± 21.9	$t = -0.170$
	Non-Hyg	158	52.7 ± 16.0	$P = 0.8732$
15–18	Hyg	129	43.0 ± 23.5	$t = 0.077$
	Non-Hyg	125	41.7 ± 18.6	$P = 0.9423$
19–22	Hyg	188	62.7 ± 26.8	$t = -1.068$
	Non-Hyg	239	79.7 ± 6.7	$P = 0.3458$
23–26	Hyg	216	72.0 ± 34.4	$t = -0.399$
	Non-Hyg	247	82.3 ± 28.8	$P = 0.7101$
27–30	Hyg	195	65.0 ± 18.3	$t = 1.310$
	Non-Hyg	145	48.3 ± 12.3	$P = 0.2603$

[a] Student's t tests show lack of significant differences between the numbers of foragers from the hygienic and non-hygienic colonies for each age cohort.

hygienic colonies had more successful foragers than hygienic colonies. The proportion of bees collecting pollen was significantly different between the lines only within the 27–30-day cohort, when hygienic bees collected more pollen (Fig. 3; Table 2). Significantly more non-hygienic bees returned with both pollen and nectar within the 19–22-day and 27–30-day cohorts; and significantly more hygienic bees returned empty within the 15–18-day and 27–30-day cohorts (Table 2). There were no significant differences between the lines in the proportion of nectar foragers within any age cohort.

A comparison of colonies within each line revealed some significant differences, although there was no colony that consistently had more foragers or foraged preferentially for pollen. Table 3 shows the data for the two most important foraging categories for this study, proportion of successful foragers and pollen-only foragers. Colony 2 within the hygienic line had a significantly higher proportion of marked successful foragers within the 11- to 14-day-old and 15- to 18-day-old cohorts, compared to the other two hygienic colonies. Colony 3 within the hygienic line had a significantly higher proportion of
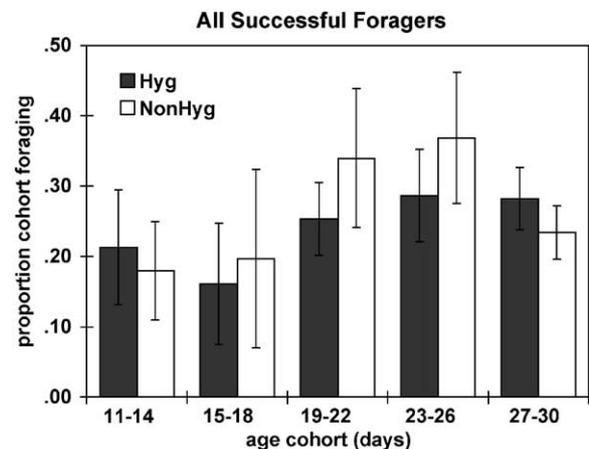

Fig. 2. The mean ± SD proportions of marked, successful foragers in each of the five age cohorts in the 3 hygienic and 3 non-hygienic colonies. Successful foragers refer to bees that returned with pollen and/or nectar, excluding those that returned empty (had no pollen on hind legs and no nectar in crop).

Table 2

Results of likelihood ratio Chi-squared tests between the proportion of foragers that returned empty or with pollen or nectar[a] from the hygienic and non-hygienic lines (colonies pooled) within each of the 5 age cohorts

	Age cohort of foraging bees				
	11–14 days	15–18 days	19–22 days	23–26 days	27–30 days
Successful foragers[a]	ns	ns	P = 0.009 NH[b]	P = 0.006 NH	ns
Pollen-only foragers	ns	ns	ns	ns	P = 0.031 H
Nectar-only foragers	ns	ns	ns	ns	ns
Pollen + nectar foragers	ns	ns	P = 0.003 NH	ns	P = 0.010 NH
Empty foragers	ns	P = 0.028 H	ns	ns	P = 0.004 H

[a] Successful foragers refer to bees that returned with pollen and/or nectar, excluding those that returned empty (had no pollen on hind legs and no nectar in crop). Pollen-only, nectar-only and pollen + nectar foragers are subdivisions of the successful forager category.

[b] P values followed by H or NH indicate the line, hygienic or non-hygienic, that had a significantly higher proportion of foragers in that age cohort.

pollen foragers in the 3 oldest cohorts. There were no consistent differences within the non-hygienic line.

Discussion

Our results demonstrate that bees from the hygienic line do not have lower sucrose response thresholds and do not forage at an earlier age as compared to bees from the non-hygienic line. The potential effects of OA on brood odor discrimination and the expression of hygienic behavior (Spivak et al., 2003) do not involve an automatic increase in sucrose responsiveness and foraging ontogeny, even though both of these other two activities are altered by OA. With regards to our previous PER conditioning studies which documented differences in olfactory sensitivities among the two lines (Masterman et al., 2000, 2001), the present results strengthen our conclusion that the detection of
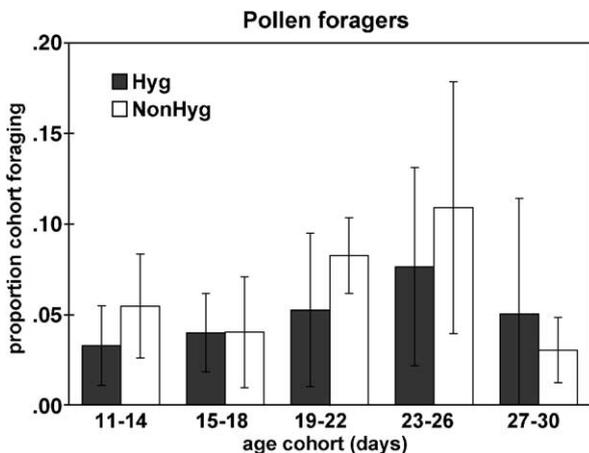

Fig. 3. The mean ± SD proportions of marked foragers returning with pollen only (no nectar in crop) in each of the five age cohorts in the 3 hygienic and 3 non-hygienic colonies.

Table 3

Percentages of successful and pollen-only foragers collected from each of the three colonies within the hygienic and within the non-hygienic line, from each marked age cohort[a]

		Age cohort (days)				
		11–14 days	15–18 days	19–22 days	23–26 days	27–30 days
Successful foragers[b]	Hyg 1	14.3	11.3	19.6	21.1*	27.1
	Hyg 2	30.2*	26.0*	29.2	32.3	24.5
	Hyg 3	19.4	11.1	27.3	32.6	33.0
	NonHyg 1	26.1*	17.0*	29.3	42.0	20.7
	NonHyg 2	14.3	33.5[#]	45.3*	26.1*	21.7
	NonHyg 3	13.6	8.6[@]	27.3	42.4	27.8
Pollen-only foragers	Hyg 1	1.5	1.5	3.0	3.0	2.3
	Hyg 2	5.7	5.2	2.6	6.2	0.5
	Hyg 3	2.6	5.3	10.1*	13.7*	12.3*
	NonHyg 1	3.2	1.6*	5.8	5.3	1.6
	NonHyg 2	8.7	7.4[#]	9.3	8.7	2.5
	NonHyg 3	4.5	3.0*[#]	9.6	18.7*	5.0

[a] All statistical comparisons are made within lines, not between them. A value in a column followed by an asterisk indicates that, within a line, a given colony had a significantly different percentage of foragers (P < 0.05, based on likelihood ratio Chi-squared tests). Values followed by differing symbols indicate that they are all significantly different from each other.

[b] Successful foragers refer to bees that returned with pollen and/or nectar, excluding those that returned empty (had no pollen on hind legs and no nectar in crop). Pollen only foragers is a subdivision of the successful forager category.

brood odors, and not the reinforcing properties of the sucrose reward, is what distinguish the two lines. Specifically, the ability of hygienic bees to respond appropriately to a brood odor as the conditioned stimulus was clearly not based on a heightened sensitivity to sucrose as the unconditioned stimulus (e.g., Mercer and Menzel, 1982; Scheiner et al., 2002).

Consistent with other studies (Pankiw and Page, 2000), we demonstrated that sucrose responsiveness increased over time as bees aged; for example, middle-age bees had a greater sucrose responsiveness (that is, they extended their proboscises to lower sucrose concentrations) compared to 1-day-old bees. This elevated responsiveness to sucrose has been associated with foraging and other behaviors, although hygienic behavior is clearly not one of them. Pankiw (2003) has described a collection of behavioral characters that can be predicted by a bee's sucrose response threshold. Bees that have a lower sucrose response threshold tend to forage at an earlier age, collect pollen and water and tend to collect nectar having a lower sugar concentration (Pankiw and Page, 1999, 2000). This set of characters may be considered a behavioral syndrome, whereby a suite of correlated behaviors is expressed across multiple situations (Sih et al., 2004). Although OA is known for its ability to orchestrate and coordinate multiple behavioral activities and subroutines (Hoyle, 1985), OA need not be the 'glue' that unifies the expression of all interrelated behaviors, even if collectively they are susceptible to OA modulation. Some behavioral events may reveal a convergence of OA modulation at any given time or not. Such associations may be age- and context-dependent, and the mode of OA action, site of

release, receptor distribution and sensitivity pattern will all contribute to the formation of subtle differences in task-specific behaviors that are not easily predicted. For example, honey bees bred to hoard pollen, which have inherently low sucrose response thresholds, do not have elevated OA levels in the brain as measured by HPLC (Schulz et al., 2004).

In a recent study by Barron and Robinson (2005), the selective effects of OA were observed across an array of tasks affecting the division of labor in honey bee colonies. Oral administration of OA was not found to cause a general increase in olfactory responsiveness to all odors: bees responded to brood pheromone and increased their foraging response, but they did not change their retinue response to the queen, which is mediated by the queen mandibular pheromone. In addition, OA caused a change in flight-related activity that was selective: foraging activity was increased, but not the rate of undertaking, which involves flying out of the hive during corpse disposal. Corpse removal, discarding of dead adult bees that have fallen to the bottom of the nest, is distinct from hygienic behavior, which involves the detection and removal of live but disease-infested or parasitized brood from wax cells.

We do not yet know if hygienic bees have a greater olfactory sensitivity to other odors, such as floral odors, or hive odors, such as brood pheromone. OA increases a bee's responsiveness to brood pheromone, which can stimulate bees to forage precociously for pollen (Barron et al., 2002). However, we observed that there were no differences between the hygienic and non-hygienic lines in their preference to collect pollen, and the hygienic line did not forage for pollen at an earlier age. Care was taken to establish that the colonies had equal amounts of larvae and pollen stores so that the need for pollen would be similar among the colonies. Each colony had the same relative number of foragers, and no colony consistently had more successful foragers or foraged preferentially for pollen. These results indicate that individual colonies most likely were regulating the number of foragers according to relatively fine differences in their ability to locate resources or other inherent causes and were not influenced by the perceived need for resources due to colony environment (quantity of brood, pheromone and stored pollen).

In summary, we found that the hygienic and non-hygienic lines did not vary in their sucrose responsiveness, onset of foraging behavior or tendency to collect pollen. Thus, our working model is further supported that hygienic bees have an innately enhanced ability to detect the odors of diseased brood independently of sucrose sensitivity and foraging state. We are doubtful, however, that differences in the levels of OA will be found to account for the increased olfactory enhancement of hygienic bees, especially as preliminary results using HPLC purification methods indicated no differences between the two lines (G. Robinson, K. Mesce and M. Spivak, personal observations). Rather, we favor the hypothesis that the two lines differ in the distribution and responsiveness of their OA receptors, which is most consistent with our previous electrophysiological and anatomical studies (Spivak et al., 2003). Clearly, OA does not function in the honey bee as a simple chemical code to instruct the enhanced detection of

chemical cues non-selectively or promote related behaviors regardless of context (Barron and Robinson, 2005). The hygienic phenotype of an individual bee or an entire genetic line is sculpted by a unique and dynamic blend of wide-ranging interactions, including the influence of OA (Spivak et al., 2003). Undoubtedly, the honey bee is an ideal model system to understand how the genetic architecture (Rueppell et al., 2004) of an animal interacts with its neural and modulatory systems to create a defined set of behavioral routines. How these behavioral programs are further refined and regulated by social and environmental influences is a worthy challenge for the future.

Acknowledgments

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