J Comp Physiol A (1987) 160:239–245

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology


# Olfactory sensitivity and discrimination of mixtures in the honeybee *Apis mellifera*

Wayne M. Getz and Katherine B. Smith
Division of Biological Control, University of California, Berkeley, California 94720, USA

Accepted October 8, 1986

**Summary.** The work reported here is motivated by questions relating to the perception of olfactory cues in the discrimination of nestmates and kin in the honeybee *Apis mellifera*. Two sets of experiments are discussed. The first deals with the perception of individual compounds in mixtures made up from various pairs of volatile (citral, geraniol, linalool, and limonene) and nonvolatile (un- and dodecanoic acids) compounds. The second deals with the ability of worker honeybees to discriminate between mixtures made up from the same two compounds (un- and dodecanoic acids; tri- and pentacosane) combined in different proportions. All experiments employ differential conditioning of the proboscis extension reflex as an assay of the ability of workers to discriminate between two odors. Results show that workers can relate mixtures to their component parts, and that workers can discriminate between mixtures of two very similar compounds as long as the proportions are relatively dissimilar.

## Introduction

The physiology of olfactory receptors in insects has been extensively studied (for a review see Kaissling 1986) and the neural pathways associated with olfaction have been mapped out in some detail, especially in the honeybee *Apis mellifera* (see Mobbs 1985). Despite this, little is known about the mechanisms of odor perception; and most organismal level studies involving olfaction have focused on learning (for example: Menzel et al. 1974; Bitterman et al. 1983) and not on perception per se.

Early experiments on odor learning in honeybees involved free flying insects (see von Frisch 1967) and, more recently, classical conditioning of the proboscis extension reflex (Vareschi 1971; Bitterman et al. 1983). From this and other work (Kriston 1973; Menzel et al. 1974; also see Menzel 1985) it is known that floral-like odors are usually learned within one or two trials while geraniol (a component of the Nassanov gland secretion which is used as a marker by workers) is learned even more rapidly. Further, as reviewed by Menzel (1985), it is possible to condition honeybees to associate feeding with such known worker repellents as propanol, and associative learning is generally more rapid with odors than visual stimuli. The latter is not surprising since odors play a central role in both the ecology and sociobiology of the honeybee. For example, odors are used to discriminate between flowers thereby enhancing foraging efficiency (see Seeley 1985 Chapt. 9). More interestingly, recent results indicate that worker honeybees preferentially rear full sisters over half sisters (Noonan 1985; Page and Erickson 1984; Visscher 1985; but see Breed et al. 1984). These results implicate kin discrimination based on genetically determined olfactory cues.

Olfaction mediates nestmate discrimination in several species of social insects (reviewed in Gadagkar 1985) including carpenter ants (*Camponotus* spp.), (Carlin and Hölldobler 1986), sweat bees (*Lasioglossum zephyrum*) (Smith and Wenzel, ms.), and paper wasps (*Polistes* spp.) (Gamboa et al. 1986). It is also known that honeybee workers each have a distinct olfactory signature (Breed 1983, 1985; Getz and Smith 1983, 1986). In fact, this olfactory signature has both volatile (Getz et al. 1986) and non-volatile components (for example, in the cuticular wax – see Carlson and Bolten 1984).

The process of kin discrimination has two aspects: the origin of the labels (also referred to in

the literature as cues and discriminators); and the perception of the labels. Differential training of the proboscis extension reflex can be used to explore questions relating to both the origin and perception of kin discrimination odor cues. Work relating to the origin of such cues is reported elsewhere (Getz et al. 1986).

Here we report the results of experiments designed to address two questions on the perception of olfactory stimuli by worker honeybees. The first relates to the perception of the individual components within an odor comprised of two components. It has a bearing on assumptions that have been made in modeling the kin recognition process (see: Breed and Bennett, in press; Crozier and Dix 1979; Getz 1981, 1982; Lacy and Sherman 1983). For example, several mechanisms for discrimination have been suggested including recognition of a labeling genotype (assuming a one-to-one correspondence between phenotype labels and alleles) and the rejection of unknown labels (Getz 1982). The latter assumes that individual components can be perceived in the overall phenotype odor, while the former does not necessarily rely on discrimination at the odor component level.

The second question relates to the sensitivity of the honeybee olfactory system in discriminating between odors and compounds that are chemically very similar. It has a bearing on how dissimilar two odor phenotypes must be before they are perceived as being different. The terminology *phenotype matching* is used to describe the process in which the labeling phenotype of an 'observee' is compared in an observer's memory to a referent template (for a discussion see: Gadagkar 1985; Holmes and Sherman 1983). How this matching might take place is an intriguing question and, in the context of olfactory cues, is discussed in detail elsewhere (Getz and Chapman 1986).

## Materials and methods

Worker honeybees used as test individuals in all the proboscis extension training trials were obtained from an observation hive located in our laboratory. The queen had been reared and instrumentally inseminated the previous season with semen from two drones using stocks maintained by local bee breeders. Late in the afternoon on the day before an experiment, 40 to 50 workers (primarily foragers) were removed from the entrance to the hive or the outermost comb. As in earlier studies (Getz et al. 1986), these workers were cooled until motionless and then harnessed in small brass tubes with their mouthparts, antennae and legs free to move. They were then fed a 1.5 *M* sucrose solution until satiated and left overnight in the dark, at room temperature (around 18 °C). The following morning, one half to one hour prior to training, each bee was fed several droplets of sucrose solution to assuage its hunger.

Differential training followed the basic methods described in Getz et al. (1986). Individual bees were classically conditioned using a 1.5 *M* sucrose solution as a positive unconditioned stimulus ($US^+$) to extend their proboscis when stimulated with one odor (positive conditioned stimulus, $CS^+$). Note that the $US^+$ was applied immediately after a 3 s application of the $CS^+$ (for more details see: Bitterman et al. 1983; Getz et al. 1986). At the same time they were also trained using a 1.0 *M* solution of sodium chloride as a negative unconditioned stimulus ($US^-$) not to extend their proboscis when stimulated with a second odor ($CS^-$). Note that in this case the $US^-$ was only applied if the proboscis was extended during a 3 s application of the $CS^-$. Bees were trained to discriminate between either two volatile odors applied to the antennae on a stream of air or two 'waxy' solids that had been allowed to deposit on 50 mm long, 1 mm outer diameter, hollow, glass rods which were held to the antennae. The volatile odors were obtained from 0.25 $cm^2$ pieces of filter paper soaked in one of the following compounds and placed in 50 ml glass flasks through which compressed air was blown at the rate of 240 ml/min: citral (approximately 95% purity with 63% *cis* and 32% *trans* isomers), geraniol (95% to 97% purity), limonene (purity not given), and linalool (purity not given). Odors consisting of a mixture of two volatile compounds were obtained by combining streams of air each blown through a different flask at the rate of 120 ml/min. The waxes were obtained by dipping the rods in either chloroform solutions containing various concentrations of the two fatty acids, undecanoic (approximately 99% purity) and dodecanoic (greater than 99% purity) acids, or hexane solutions containing various proportions of the two *n*-alkanes, tricosane (greater than 99% purity) and pentacosane (98% purity or greater), and letting the solvent evaporate. Rods thus prepared were stored in a refrigerator and used within 1 to 4 days of preparation. In one set of experiments, 20 g of fatty acid were placed in 50 ml flasks to obtain (as described above) volatiles given off by these solids.

In each experiment, approximately 30 bees were differentially conditioned to two odors using an 8 trial training sequence; that is, every 5 to 10 min each bee was placed in turn on a platform below an air exhaust system and either presented with one odor ($CS^+$) and the associated $US^+$ or presented with the other odor ($CS^-$) and when necessary the associated $US^-$. The odors were presented in the following sequence:

$$\text{Training sequence} = \begin{cases} \text{trial:} & 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 \\ \text{CS:} & + & - & - & + & - & + & + & - \end{cases} \quad (1)$$

The following two sets of experiments were performed.

*Mixture perception experiments.* In these experiments, all odors were presented as airborne volatiles. Immediately following the 8-trial training sequence, a 9th trial was conducted in which 1/3 of the test bees were presented with the $CS^+$, 1/3 with the $CS^-$, and 1/3 with a novel but related odor denoted by NS. The response data for the three groups could then be compared and inferences made on how workers perceived the NS in relation to the $CS^+$ and $CS^-$. For the pairs of compounds listed below, but labeled *A* and *B* here for generality, three different cases were examined:

Case 1. $CS^+ = A$, $CS^- = B$, and $NS = A + B$.

Case 2. $CS^+ = A + B$, $CS^- = A$, and $NS = B$.

Case 3. $CS^+ = A$, $CS^- = A + B$, and $NS = B$.

The response of individuals to this 9th trial was recorded. For purposes of comparison bees were classified as *learners* or *nonlearners* according to whether they responded correctly or not

during trials 7 and 8 in sequence (1) above; that is, learners extended their proboscis during trial 7 but not during trial 8, while non-learners responded during both trials or during neither trial. Less than 2% of individuals got both wrong, and the data from them were discarded for purposes of analysis. The following 8 pairings of compounds were tested: $A$ = limonene, $B$ = linalool and vice versa; $A$ = geraniol, $B$ = limonene and vice versa; $A$ = geraniol, $B$ = citral and vice versa; and, $A$ = undecanoic acid, $B$ = dodecanoic acid and vice versa.

*Mixture sensitivity experiments.* In these experiments all odors were presented as mixtures of waxes that had crystallized on glass rods, as described above. The training sequence (1) was immediately followed by an identical *evaluation* sequence of 8 trials, that is:

Evaluation sequence

$$= \begin{cases} \text{trial:} & 9 & 10 & 11 & 12 & 13 & 14 & 15 & 16 \\ \text{CS:} & + & - & - & + & - & + & + & - \end{cases} \quad (2)$$

If an individual bee extended its proboscis during presentation of a $CS^-$ (trials 10, 11, 13 and 16) or did not extend its proboscis during presentation of a $CS^+$ (trials 9, 12, 14 and 15) then a 1 (error) was recorded for each of these occasions, otherwise a 0 was recorded. Thus each bee made from 0 to 8 errors and an error frequency histogram was constructed for the approximately 35 bees tested (range was 27–46) in each of the comparisons of odor mixtures listed below. The average error for the group for each comparison can then be used as a measure of how well the group performed at discriminating between the two mixtures. The results obtained for any two comparisons can be compared statistically using an $n \times 2$ chi-squared contingency table analysis. Although 9 error categories are possible, the tails of the histograms were lumped to ensure that expected cell frequencies were at least 2 (Sachs 1982, p. 464).

For each pair of compounds $A$ and $B$, the following comparisons were performed, where the notation $CS = x{:}y$ signifies that the CS is made up of $x$% of compound $A$ and $y$% of compound $B$. If the $CS^+$ and $CS^-$ are regarded as vectors (points connected to the origin) in $x$-$y$ space, then the angle $\theta$ between these odors provides a measure of their separation (for further details see Getz and Chapman 1986). Note that the angle $\theta$ can be calculated using the formula

$$\cos\theta = (x_+ x_- + y_+ y_-)/\sqrt{(x_+^2 + y_+^2)(x_-^2 + y_-^2)},$$

where $x_+$ corresponds to the proportion of compound $A$ in the $CS^+$, etc.

Comparison 1. $CS^+ = 100{:}0$, $CS^- = 100{:}0$, $\theta = 0°$

Comparison 2. $CS^+ = 50{:}50$, $CS^- = 50{:}50$, $\theta = 0°$

Comparison 3: $CS^+ = 100{:}0$, $CS^- = 90{:}10$, $\theta = 6°$

Comparison 4. $CS^+ = 0{:}100$, $CS^- = 10{:}90$, $\theta = 6°$

Comparison 5. $CS^+ = 50{:}50$, $CS^- = 90{:}10$, $\theta = 39°$

Comparison 6. $CS^+ = 50{:}50$, $CS^- = 10{:}90$, $\theta = 39°$

Comparison 7. $CS^+ = 50{:}50$, $CS^- = 100{:}0$, $\theta = 45°$

Comparison 8. $CS^+ = 50{:}50$, $CS^- = 0{:}100$, $\theta = 45°$

Comparison 9. $CS^+ = 90{:}10$, $CS^- = 10{:}90$, $\theta = 77°$

Comparison 10. $CS^+ = 10{:}90$, $CS^- = 90{:}10$, $\theta = 77°$

Comparison 11. $CS^+ = 100{:}0$, $CS^- = 10{:}90$, $\theta = 84°$

Comparison 12. $CS^+ = 0{:}100$, $CS^- = 90{:}10$, $\theta = 84°$

Comparison 13. $CS^+ = 100{:}0$, $CS^- = 0{:}100$, $\theta = 90°$

Comparison 14. $CS^+ = 0{:}100$, $CS^- = 100{:}0$, $\theta = 90°$

These Comparisons were carried out for mixtures made up from the following pairs of compounds: $A$ = undecanoic, $B$ = dodecanoic acid; and $A$ = tricosane, $B$ = pentacosane.

## Results

### *Mixture perception experiments*

For each pair of compounds the results can be tabulated in a $3 \times 3$ table, where the three columns respectively indicate the response in the ninth trial of the subgroup (1/3 of total sample) presented with compounds $A$, $B$ and $A+B$, and the three rows respectively correspond to the three Cases listed in the Methods. Based on the hypothesis that workers can not only associate a mixture with its individual components, but separate out these components in the context of differential training of the proboscis extension reflex, Table 1 A indi-

**Table 1.** Percentage response[a] of individuals to three odors after being differentially trained to two of these odors[b]

| A. Hypothetical | | | | B. Overall[c] | | | |
|---|---|---|---|---|---|---|---|
| | $A$ | $B$ | $A+B$ | | $A$ | $B$ | $A+B$ |
| 1 | 100 $CS^+$ | 0 $CS^-$ | ? NS | 1 | 100 (126) | 23 (129) | 86 (132) |
| 2 | 0 $CS^-$ | 100 NS | 100 $CS^+$ | 2 | 63 (197) | 89 (197) | 97 (195) |
| 3 | 100 $CS^+$ | 0 NS | 0 $CS^-$ | 3 | 94 (102) | 41 (204) | 53 (20) |
| C. Learners[d, e] | | | | D. Non-learners[d, f] | | | |
| | $A$ | $B$ | $A+B$ | | $A$ | $B$ | $A+B$ |
| 1 | 100 (80) | 6 (83) | 81 (85) | 1 | 100 (46) | 54 (46) | 94 (47) |
| 2 | 33 (42) | 87 (47) | 97 (39) | 2 | 72 (156) | 90 (155) | 97 (150) |
| 3 | 90 (48) | 22 (51) | 30 (47) | 3 | 95 (154) | 47 (153) | 60 (154) |

[a] Sample sizes are in parenthesis. All tests of significance are chi-squared contingency table, except where otherwise specified

[b] Differential training involves a $CS^+$ and $CS^-$ (see text for details). The NS is a novel stimulus

[c] In each row, each entry is significantly different from the other two entries at least at $P < 0.005$, except for entries 2 and 3 in row 3 where $P > 0.1$

[d] Learners are defined to be those individuals that responded correctly to trials 7 and 8 during training. The remaining individuals are non-learners

[e] In each row, each entry is significantly different from the other two entries at $P < 0.001$, except for entries 2 and 3 in row 2 ($P = 0.09$ Fisher's exact test) and entries 2 and 3 in row 3 ($P > 0.5$)

[f] In each row, each entry is significantly different from the other two entries at least at $P < 0.05$, except for entries 1 and 3 in row 1 where $P = 0.12$ (Fisher's exact test)

**Table 2.** Combined results on learning in mixture perception experiments

A. By Case[a]

| Case | Percent learners[b, c] | Sample size |
|---|---|---|
| 1. | 64 | 387 |
| 2. | 22 | 590 |
| 3. | 24 | 607 |

B. By pairs of compounds[d]

| Pairs | Percent learners[b, e] | Sample size |
|---|---|---|
| Limonene and linalool | 48 | 445 |
| Limonene and geraniol | 37 | 488 |
| Citral and geraniol | 31 | 429 |
| Un- and dodecanioc acids | 22 | 222 |

[a] Case 1 corresponds to row 1 in Table 1 B

[b] Learners are defined to be those individuals that responded correctly to trials 7 and 8 during training. The remaining individuals are non-learners

[c] The first column entry is significantly different from the other two column entries at $P<0.001$ (chi-squared contingency tables)

[d] Results from symmetrical pairs are combined, e.g. $A$ = linalool, $B$ = limonene and $A$ = limonene, $B$ = linalool

[e] This is the average percentage across Cases 1–3. Because sample sizes vary between Cases and between pairs, however, it is only valid to statistically compare learning rates on a per Case basis. In Case 1, for example, learning for the first pair of compounds was significantly greater than for the other three pairs at $P<0.01$; while in Case 3, learning for the last pair of compounds was significantly less than for the other 3 pairs at $P<0.001$ (chi-squared contingency tables)

cates a 'perfect' result. Note that we do not necessarily need to assume that the components are individually perceived per se, only that a mixture is associated as being similar to its components in some sense. Also, it is not a *priori* known how individuals will resolve the conflict of whether to respond to $A+B$ when trained to respond to $A$ but not to respond to $B$. Hence the entry in row 1, column 3 is filled with a question mark.

The results combined across all 8 pairings of compounds are given in Table 1 B, but are split into *learners* and *non-learners* in Tables 1 C and 1 D (recall that learners are individuals responding correctly during trials 7 and 8). The actual percent of learners is summarized by Case categories in Table 2 A and by compound pairing categories in Table 2 B. In both sets of tables, differences between entries certain are significant; details are given in the footnotes to these tables.

For Case 1, 7 of the 8 pairings followed the same pattern as the row 1 entries in Table 1 B. For the pair $CS^+$ = limonene and $CS^-$ = linalool, however, the responses were $A=100\%$, $B=11\%$, $A+B=50\%$; the second response being closer to the theoretical value of 0% (NS, $0.1<P<0.05$ chi-squared contingency table analysis with $n=129$), and the third response being significantly lower than the corresponding entry of 86% ($P<0.001$, chi-squared contingency table analysis with $n=132$).

For Case 2, the results were more variable across pairs than for Case 1, but again the results closest to the hypothetical Table 1 A involved limonene and linalool; namely, $CS^+$ = limonene + linalool, $CS^-$ = limonene and NS = linalool. The corresponding row 2 entries were $A=18\%$, $B=100\%$, $A+B=100\%$. The first response is significantly less than the corresponding entry of 63% in Table 1 B at $P<0.001$ (chi-squared contingency table analysis with $n=197$). In contrast to this, linalool in a limonene background, that is $CS^+$ = limonene + linalool, $CS^-$ = linalool, and NS = limonene gave poor results with $A=80\%$, $B=76\%$, $A+B=96\%$. The $A$ and $B$ responses for these two sets are significantly different at $P<0.001$ (chi-squared and Fisher's exact tests with $n=53$ and $n=52$ respectively).

For Case 3, the results were again more variable across pairs than for Case 1. Also, as in Case 2, limonene in a linalool background, that is $CS^+$ = limonene and $CS^-$ = limonene + linalool, gave results closest to Table 1 A, namely $A=97\%$, $B=10\%$, $A+B=37\%$ (both are significantly less than the corresponding entries of 41% and 53% in Table 1 B, respectively at the levels $P<0.05$ and $P<0.001$ using a chi-squared contingency table analysis).

From Table 2 A, we see that the task of training individuals to discriminate between two compounds is more easily accomplished than training them to discriminate between a mixture and one of the components in that mixture (the significance of these results are given in footnote c). The entries in Table 2 B summarize how easily individuals can be differentially trained with respect to the different pairs of compounds tested, when the results are combined across all three Cases (the significances of these results are given in footnote e).

### *Mixture sensitivity experiments*

For each of the two pairs of mixtures, the results for the set of Comparisons 1–14 can be summarized by plotting the average error for each Comparison in terms of the angle measuring the distance between the two mixtures used in the differential training procedure. This is illustrated in

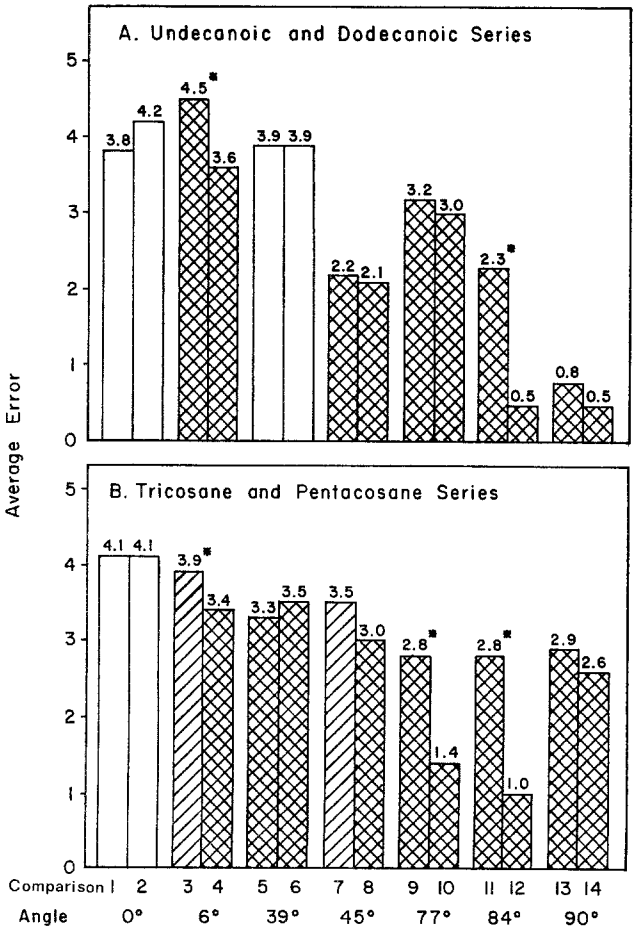


**Fig. 1.** The average error for each of the 14 Comparisons is plotted in **A** for the un- and dodecanoic series, and in **B** for the tri- and pentacosane series of experiments. Asterisks indicate that results for the two Comparisons representing the same angle are significantly different at $P<0.05$ (see text for statistical details). The data for Comparisons 1 and 2 can be combined in each series to provide a no-learning control. The data for each of Comparisons 3–14 were compared with this control. Single and double hatching respectively denote that differences are significant at $P<0.05$ and $P<0.001$ (see text for further details)

Fig. 1A for un- and dodecanoic acids, and in Fig. 1B for tri- and pentacosane. Since there is no reason to assume that the error rate can be characterized as a linear function of the angle between odors (a monotonic non-linear relationship such as cos $\theta$ may be more plausible, as discussed in Getz and Chapman 1986), the non-parametric Spearmen rank correlation test with ties (see Sachs p. 401) was used to test the significance of the increase in discrimination (that is, reduction in average error) with increase in angle. Correlation coefficients for the two sets of data were respectively $r=0.87$ and $r=0.89$. Both are significant at $P<0.001$. Note that in Fig. 1, asterisks indicate whether the results for Comparison with the same angle differed significantly ($P<0.05$, $n\times 2$ chi-squared analysis described in the Methods). For each series of 14 Comparisons, a set of data to be used as a control for *no-learning* was obtained by combining the results of Comparisons 1 and 2. Hatching indicates whether the data for the individual Comparisons 3–14 differed significantly from the combined data of Comparisons 1 and 2. Note that some Comparisons differed significantly from the no-learning control even though the average errors were very similar. These differences were due to the shapes of the histograms (for example, flat versus peaked) rather than the mean error values. For example the significance of the differences between Comparison 3 and combined Comparisons 1 and 2 in Table 1B is related to the fact that respectively 76% and 56% of individuals are in the 4-error category of the two histograms.

## Discussion

Conclusions can be drawn from the results with varying degrees of confidence. First and most obvious is the fact that worker bees could be trained to discriminate between all compounds (but excluding similar mixtures) that were paired in both sets of experiments. This was not surprising for the volatile compounds, citral, geraniol, limonene, and linalool. These compounds are constituents of pheromones and/or plant odors, and have previously been used by Bitterman et al. (1983) in differential conditioning experiments to investigate learning in honeybees. Work by Vareschi (1971), however, indicates that worker honeybees conditioned to respond to undecanoic acid will, to some extent, respond as though trained to dodecanoic acid, and vice versa. Further, Vareschi's work indicates that these two fatty acids stimulate the same population of sensory cells in the antennae. Thus *a priori* it was not clear that worker honeybees could be differentially conditioned to discriminate between these two substances. In fact, the difference between the first two results (same odor – Comparisons 1 and 2) and the last two results (Comparisons 13 and 14) in Fig. 1A (un- versus dodecanoic acids) indicates a stronger level of discrimination than we have previously obtained in any differential conditioning experiments including training workers to discriminate between lavender and unmodified air from a compressed air cylinder (Getz et al. 1986). The lavender, however, was delivered on a stream of air so that the high level of discrimination between the two fatty acids could, in part, be attributed to the antennae mak-

ing contact with the fatty acid deposits on the glass rod. From Fig. 1 B we see that workers also learn to discriminate between tricosane and pentacosane, although not as strongly as with the fatty acids.

Fatty acids and odd-numbered $n-$alkanes are either found in or known to be constituents of cuticular waxes in the honeybee, *A. mellifera* (see Carlson and Bolten 1984) and, therefore, may potentially provide cues for kin discrimination within the hive. The difference between individuals, however, may be in the relative proportion rather than the presence or absence of these compounds in the cuticular wax. If the results of Comparisons 1 and 2 in Fig. 1 A and B are taken as baseline data when no odor discrimination is possible (odors 1 and 2 in these runs are the same compound or the same mixture of compounds), then discrimination is evident in many of the remaining 12 Comparisons (hatched bars in Fig. 1). Although there is a trend toward increased levels of discrimination as odors separate (as measured by the angle $\theta$) in the odor component space, the results are variable. For example, the strongest level of discrimination was not between the two pure $n-$alkanes (Comparisons 13 and 14, Fig. 1 B) but involved the comparison of a 90:10 to a 0:100 mixture of tri-:pentacosane (Comparison 12) and to a 10:90 mixture of the same (Comparison 10). We feel these results are not just attributable to experimental variation, but to the fact that different mixtures may have different textures. Because all the molecules are not the same size in mixtures, the composites they form as they are deposited could lead to texture differences. We noticed in our preparation of mixtures that some mixtures took much longer to deposit on the glass rods than others. Thus one of the problems with the glass rod technique used in these experiment are that sensory modalities of touch are confounded with those of olfaction.

The results of the mixture perception experiments indicate that, although worker bees have some ability to associate different mixtures made up from the same compounds with each other, the success of this task depends on the particular compounds involved. For example, they seem to be able to most easily separate out limonene from linalool. On the other hand, although the mixture sensitivity experiments indicate quite clearly that workers can discriminate between different mixtures of un- and dodecanoic acids, they have difficulty in separating out these components for association purposes. From these results, however, one cannot infer that workers are actually able to identify the components of mixtures. Rather, one can only infer that if a particular odor (say NS $= B$) is more like one odor (say $CS^+ = A + B$) than another (say $CS^- = A$), then an association is made between the first two odors (that is between NS and $CS^+$).

Comparing Tables 1 C and 1 D, it is clear that the group of test bees responding correctly to trials 7 and 8 (learners) performed much better than the remaining group of test bees (non-learners). The proportion of learners in an experiment depended both on the task being performed (Table 2 A) and on the pair of compounds used (Table 2 B). In both learners and especially non-learners, we see from the entries in column 3, row 1 of Tables 1 C and D that, when confronted with an ambiguous situation, most workers respond. Thus the difference between the hypothetical 0% in entries (2, 1), (3, 2) and (3, 3) in Table 1 A, and the corresponding entries of 33%, 22% and 30% in Table 1 C could in part be due to this tendency to respond when confused. This becomes more evident when we consider that the non-response percentage levels (that is, the percentage response subtracted from 100%) corresponding to entries (2, 2), (2, 3) and (3, 1) in Table 1 C are 13%, 3% and 10%, which are much closer to the hypothetical 0% than the first 3 entries mentioned.

Although extension of the proboscis is an out-of-context response for kin discrimination based on odor cues, and care must be taken in interpreting results, it is clear that differential training of this reflex can provide valuable insights relating to the question of kin discrimination in honeybees using chemical cues and also to mechanisms of olfaction.

*Acknowledgements.* We would like to thank E. Bernays, R. Chapman and R. Flath for technical advice on the preparation of odor mixtures, provision of various chemicals and comments on our work. WMG is indebted to D. Brückner for encouragement to pursue this research. This work was supported by NSF Grant BNS-8518037 to WMG.

## References

Bitterman ME, Menzel R, Fietz A, Schäfer S (1983) Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). J Comp Psychol 97:107–119

Breed M (1983) Nestmate recognition in honeybees. Anim Behav 31:86–91

Breed M, Bennett B (in press) Kin recognition in highly eusocial insects. In: Fletcher D, Michener CD (eds) Kin recognition in animals, Wiley, New York

Breed M, Butler L, Stiller TM (1986) Kin discrimination by worker honeybees in genetically mixed groups. Proc Natl Acad Sci USA 82:3058–3061

Breed M, Velthuis HHW, Robinson GE (1985) Do worker honeybees discriminate among unrelated and related larval phenotypes. Ann Entomol Soc Am 77:737–739

Carlin NF, Hölldobler B (1986) The kin recognition system of carpenter ants (*Camponotus* spp.). I: hierarchical cues in small colonies. Behav Ecol Sociobiol 19:123–134

Carlson D, Bolten AB (1984) Identification of Africanized and European honeybees, using extracted hydrocarbons. Bull Entomol Soc Am 30(2):32–35

Crozier RH, Dix MW (1979) Analysis of two genetic models for the innate components of colony odour in social Hymenoptera. Behav Ecol Sociobiol 4:217–224

Frisch K von (1967) The dance language and orientation of bees. Harvard University Press, Cambridge, Massachusetts

Gadagkar R (1985) Kin recognition in social insects and other animals – A review of recent findings and a consideration of their relevance for the theory of kin selection. Proc Indian Acad Sci (Anim Sci) 94:587–621

Gamboa GJ, Reeves HK, Pfennig DW (1986) The evolution and ontogeny of nestmate recognition in social wasps. Annu Rev Entomol 31:431–454

Getz WM (1981) Genetically based kin recognition systems. J Theor Biol 92:209–226

Getz WM (1982) An analysis of learned kin recognition in Hymenoptera. J Theor Biol 99:585–597

Getz WM, Brückner D, Smith KB (1986) Conditioning honeybees to discriminate between heritable odors from full and half-sisters. J Comp Physiol A 159:251–256

Getz WM, Chapman RF (1986) An odor discrimination model with application to kin recognition in social insects. Int J Neurosci (in press)

Getz WM, Smith KB (1983) Genetic kin recognition: honeybees discriminate between full and half sisters. Nature (Lond) 302:147–148

Getz WM, Smith KB (1986). Honeybee kin recognition: learning self and nestmate phenotypes. Anim Behav (in press)

Holmes WG, Sherman PW (1983) Kin recognition in animals. Am Sci 71:46–55

Kaissling K (1986) Chemo-electrical transduction in insect olfactory receptors. Annu Rev Neurosci 9:121–145

Kriston I (1973) Zum Zusammenhang zwischen Signalbewertung und Lernprozeß: Die Bewertung von Duft- und Farbsignalen an der Futterquelle durch *Apis mellifica*. J Comp Physiol 84:77–84

Lacy RC, Sherman PW (1983) Kin recognition by phenotype matching. Am Nat 121:489–512

Menzel R (1985) Learning in honeybees in an ecological and behavioral context. Fortschr Zool 31:55–74

Menzel R, Erber J, Masuhr T (1974) Learning and memory in the honeybee. In: Barton Browne L (ed) Experimental analysis of insect behaviour. Springer, Berlin Heidelberg New York

Mobbs PG (1985) Brain structure. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology, biochemistry and pharmacology, vol 5, nervous system: Structure and function. Pergamon Press, Oxford

Noonan KC (1985) Kin recognition in the honeybee (*Apis mellifera*): in-colony studies of workers/brood recognition. PhD dissertation, University of Wisconsin, Madison

Page RE, Erickson EH (1984) Selective rearing of queens by worker honeybees: kin or nestmate recognition. Ann Entomol Soc Am 77:578–580

Sachs L (1982) Applied statistics: a handbook of techniques. Springer, Berlin Heidelberg New York

Seeley TD (1985) Honeybee ecology: a study of adaptation to life. Princeton University Press, Princeton, New Jersey

Vareschi E (1971) Duftunterscheidung bei der Honigbiene – Einzelzell-Ableitungen und Verhaltensreaktionen. Z Vergl Physiol 75:143–173

Visscher PK (1985) Genetic structure and kinship discrimination in honeybee (*Apis mellifera* L.) colonies. PhD dissertation, Cornell University, Ithaca, New York