

## **The Contributions of Kinship and Conditioning to Nest Recognition and Colony Member Recognition in a Primitively Eusocial Bee, *Lasioglossum zephyrum* (Hymenoptera: Halictidae)**

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Received February 2, 1977

**Summary.** 1. In the primitively social halictine bee, *Lasioglossum zephyrum*, colony unity is maintained through an interplay of both nest recognition and nest mate recognition, using odor cues.

2. Nests have odors which are attractive to members of their colonies and also to bees from other colonies. Bees are, however, usually able to distinguish between their own nest and a foreign nest if given a choice.

3. Bees from colonies which are relatively homogeneous genetically and in which the bees share a common larval environment recognize their own nest with less difficulty than bees from genetically heterogeneous colonies in which a common early environment is lacking among member bees.

4. A significant component of nest recognition behavior is based on genetic homogeneity, and/or larval conditioning, perhaps a form of imprinting to chemical cues.

5. Recognition of nestmates by guards, essential for intraspecific nest defense, seems not to be based on the aphrodisiac secreted by females.

6. Guards apparently learn individual odors of residents or a combination of the odors of several residents, providing a mechanism for distinguishing between nest mates and intruders attempting to enter the nest.

7. Though adult learning is important in nest mate recognition, an overriding contribution from genetic similarity or early conditioning also occurs.

### **Introduction**

Distinctive colony odors occur in numerous species of social insects, often serving as mechanisms for preserving colony unity. This phenomenon is well-

documented for ants and termites (Wilson, 1971) as well as for some bees, particularly *Apis* and *Bombus* (Michener, 1974). In *Apis* these odors are primarily the products of provisions brought into the nest and serve as cues for bees of one colony to distinguish nest mates from bees belonging to other colonies. Bees perceived by their odor to be entering the wrong nest are attacked by colony members (Kalmus and Ribbands, 1952). A similar mechanism operates in *Bombus* (Free, 1958). In addition, colony odors may promote avoidance of foreign nests, as suggested in allodapine bees by Michener (1971).

Functional evidence for nest odors has been reported in halictine bees. Batra (1966) noted that disoriented *L. zephyrum* foragers which enter foreign nests leave immediately, even in the absence of attack by residents. However, *L. versatum* foragers which enter foreign nests may even deposit pollen in those nests (Michener, 1966); it is interesting to note that this species is usually found in small dense aggregations of nests. Workers of *L. imitatum* can be transferred into foreign nests but only young bees (preforagers) will remain (Michener and Wille, 1961).

*Lasioglossum zephyrum* excavates burrows in exposed soil of river banks. Extensive banks may contain hundreds or thousands of individual nests. If a forager is to maintain her inclusive fitness, she must be able to identify and return to the nest which contains her mother and sisters (Hamilton, 1972). Error on her part would be minimized if this recognition were based on a genetic mechanism, such as recognition of odor produced by closely related bees. Alternatively, or simultaneously, some form of larval conditioning could occur. Both these processes operate in host recognition, using chemical cues in phytophagous parasitic insects (Bush, 1974).

Within the nest, each larva develops in a wax-lined cell and feeds on a pollen ball, both of which are constructed by adult members of the colony. In addition, these cells are occasionally visited by adults (Batra, 1966). The possibility therefore exists for preimperial conditioning of bees to odors within the colony. Since bees were collected from the wild as pupae, our experiments do not distinguish between effects of genetic mechanisms and larval conditioning. However, since *L. zephyrum* will form nonkin colonies under laboratory conditions, they provide an opportunity to evaluate the role of adult experience in nest recognition to determine if a significant contribution is made by some other factor which could only be either genetic, or due to larval conditioning, or a combination thereof.

Maintenance of colony unity involves both colony odor to attract returning foragers to their home nests and intraspecific nest defense to prohibit the entry of foreign bees. Intraspecific nest defense and agonistic action patterns of guard bees in *L. zephyrum* were studied by Bell et al. (1974), Bell and Hawkins (1974), and Barrows et al. (1975). The patterns involve aggressive behavior by guards stationed at nest entrances who admit nest mates but vigorously reject bees from other colonies if those bees are two or more days old. This occurs in artificial colonies of unrelated bees which are collected as pupae, from diverse nests placed in nests constructed of identical soil, and provisioned with identical pollen and honey water.

Bell et al. (1974) demonstrated that recognition of resident vs. nonresident bees may well be based on odors secreted by them. Barrows (1975) found that

females secrete odors which attract males and may also function as the aphrodisiac. The odors appear to vary among individuals or small groups, allowing males to distinguish among females. It seems possible that such odor cues have multiple functions, attraction and sexual stimulation of males plus recognition by guards of resident vs. nonresident females. Alternatively if the aphrodisiac is not the recognition odor this suggests a certain degree of complexity in the bee's chemical communication system.

As with the returning forager, the inclusive fitness of a guard depends on her ability to recognize proper odor cues and allow only colony members to enter the nest, which in nature would be closely related females. That adult learning is involved can be concluded from observation of guarding behavior seen in nonkin laboratory colonies. One would expect however, that for a primitively eusocial species, some genetic or imprinted mechanisms operate as well.

Five questions arising from these previous studies of *L. zephyrum*, or suggested by the theory of kin selection, are pursued in this paper: (1) Is there a nest odor which enables bees to discriminate their nest from others? (2) Do genetic homogeneity and/or larval conditioning play a role in nest discrimination? (3) Is the odor that triggers recognition of nest mates by guards identical to some part of the female sex pheromone? (4) What is the role of genetic homogeneity and/or larval conditioning in identification of colony members? (5) Is constant exposure to resident bees or nest odors necessary for guards of nonkin colonies to discriminate between resident and nonresident bees?

## Methods

Females of *Lasioglossum (Dialictus) zephyrum* were collected as pupae by excavating nests along the Kansas River, Douglas County, Kansas, and were reared in bee rooms at the University of Kansas. Pupae or newly emerged adult females were placed in glass observation nests, three to eight per nest ( $\bar{x}=6$ ), as described by Michener and Brothers (1971). Some of these colonies were formed with bees collected from the same nest; these bees are all presumably the progeny of a single female and such nests are termed "kin" nests in the text. Other colonies were formed with individuals from diverse nests; these contained offspring of different females not necessarily closely related and are termed "nonkin" nests. All nests were constructed of soil from a single site which was mixed and sifted before use. They were provisioned with identical honey and *Typha* pollen (Kamm, 1974) to minimize differences among colonies caused by soil or food. The experimental manipulations are as described by Bell et al. (1974) with necessary additional procedures described in the text.

## Results

### *A. Colony Odor*

*1. Is There a Distinctive Nest Odor?* Choice experiments between nests were conducted by removing all bees from a pair of nests. The nests were then connected with a glass T-tube which had a 5-mm inside diameter so that each nest entrance joined one end of the crossbar of the T-tube. Bees were then placed in the stem of the T-tube; when they reached the intersection of the T, they could enter either nest by making a 90° left or right turn. The stem of the T-tube was 4 cm in length, as were the side branches. These experiments

**Table 1.** Choice experiments between home nest and foreign nest or home nest and an empty tube

Colony type	Experimental condition	<i>n</i>	% choosing home nest	<i>p</i>
Nonkin	Home nest vs. empty tube	87	73	0.001
	Home nest vs. foreign nest	121	62	0.005
Kin	Home nest vs. empty tube	50	66	0.025
	Home nest vs. foreign nest	50	68	0.025

Probabilities are calculated by testing experimental results against random (50:50) selection by chi-square

were conducted in a darkened room. A GE ruby red photographic bulb (590–680 nm) was used so that the experimenter could record activities of the bees; they probably have little visual perception at this wavelength. Similar experiments were performed in which bees were offered the choice of entering a nest or an empty, clean piece of plastic tubing. In each experiment the T-tube was replaced after each trial to eliminate the possibility of trail formation, and the positions of the nests were reversed to compensate for any directional bias.

The T-tube experiments (Table 1) unambiguously indicate that in the absence of any cues other than odor, *L. zephyrum* is able to discriminate between its own nest and an empty tube, as well as between its own nest and a foreign nest. In all four experimental situations there are significantly more bees turning toward and entering their home nests than turning away from them ( $p < 0.001$ , chi-square). The percentage of these correct choices appears low, but may reflect a low concentration of the odor stimulus due to the 4-cm distance between the point of choice and the nest entrances.

*2. Do Genetic Homogeneity and/or Larval Conditioning Play a Role in Nest Discrimination?* The T-tube tests involved relative discrimination in which bees were forced to choose between two stimuli. Since absolute recognition in the absence of alternative stimuli is in general more difficult, such a task was used for a more sensitive assay of the performance of kin vs. nonkin bees in recognizing their own nests. Bees were removed from their nests and each was individually reintroduced into its own nest or a foreign nest by way of a plastic tube 20 cm long with a 4-mm inside diameter. They were gently prodded so that they walked toward the nest entrance without evidence of agitation, i.e., at a normal pace. As each bee approached a nest it stopped at a distance of 0.5 to 1 cm from the nest entrance and antennated the air. It then moved into the nest, hesitated, or retreated back into the tube. When a bee reached a distance of 1 cm from the nest entrance a stop watch was begun and the bee was observed for a total of 60 s. If the bee entered the nest within 5 s, it was scored as a quick entry, if it entered after 5 s or entered and then left before 60 s had elapsed, it was scored as a hesitant entry, and if it did not enter it was scored as a nonentry.

**Table 2.** Reactions of kin and nonkin bees upon approaching their own nest or a foreign nest

Colony type	Nest into which introduced	<i>n</i>	% quick entries	% hesitant entries	% non-entries
Nonkin	Home nest	31	23	42	35
	Foreign nest	32	47	40	3
Kin	Home nest	31	71	23	6
	Foreign nest	32	47	25	28

Kin bees enter their own nests more frequently and rapidly than they enter foreign nests ( $p < 0.001$ , chi-square). Nonkin bees do not perform as well (Table 2). If the behavior of all bees entering a home nest is considered, kin bees perform better than nonkin bees in that more of them enter nests normally and fewer hesitate or refuse to enter ( $p < 0.025$ ). However, there is no significant difference in the behavior of kin and nonkin bees in entering a foreign nest. It appears from these results that nonkin bees discriminate against their own nests. A chi-square test of the difference between their entry into foreign nests and entry into home nests is not significant ( $p > 0.1$ ), however. This suggests that, in this experiment, nonkin bees react to their own nest in the same way that they react to a foreign nest. Note that 46.9% of the bees tested entered foreign nests without hesitation and 32.8% entered with hesitation, suggesting that foreign nests do not appear to repel bees introduced into them, but are attractive.

### B. Intraspecific Nest Defense

3. *Is the Aphrodisiac a Releaser for Guard Recognition?* Females were isolated in small plastic vials immediately following adult emergence; they were later tested in two bioassays. Each was tested for sex pheromone secretion using the assay of Barrows (1975) and then introduced as a nonresident bee into an established colony, as described by Bell et al. (1974). Of the 30 young females tested, aged 1 to 3 days after emergence, all attracted males, whereas 35% were rejected and 65% accepted by guards when introduced into established nests. Older bees, despite continued production of sex pheromone, were consistently rejected by guards.

This suggests that females secrete above-threshold levels of sex pheromone within one day after adult emergence, and that the presence of this pheromone has no direct bearing on their acceptance into established colonies. These results imply that the sex pheromone does not function as an odor cue for discriminating between residents and nonresidents, and suggest the existence of complexity in the odor communication system of *L. zephyrum*.

4. *What is the Role of Genetic Homogeneity and/or Larval Conditioning in Identification of Colony Members?* The role of genetic homogeneity in producing odor similarity among these bees was investigated by Barrows et al. (1975).

At that time, certain evidence suggested that bees of the same kin group were more similar in odor than bees from different kin groups. The following study was conducted to provide more data on this issue.

Eleven kin nests were established, as described in Methods, so as to provide several pairs, and one triplet, of kin nests in which the presumed offspring from a single female were established in separate nests. These colonies of kin bees were allowed at least 10 days to develop a social structure, then adult bees older than 9 days after emergence were introduced into the nests as described by Bell et al. (1974). Of 116 nonresident, kin bees so introduced, 63% were accepted and 37% rejected. Introductions of nonresident, unrelated bees provided a control; of these 93% were rejected ( $n=30$ , see also Bell et al., 1974). Odors which provide cues for identification of nest mates by guard bees are apparently to a great extent influenced by either genetic homogeneity or common larval conditioning. Since, however, unrelated resident bees are commonly admitted by guards, some learning during the adult phase of the life cycle must also occur. If such learning occurs, then constant contact among individuals may be necessary.

*5. Is Constant Exposure to Resident Bees or Nest Odors Required for Guards to Discriminate between Resident and Nonresident Bees in Nonkin Colonies?* Barrows et al. (1975) reported preliminary evidence that guards which were held in isolation progressively lose the ability to distinguish resident from nonresidents. Further studies presented here corroborate this observation, but suggest an additional interpretation as well.

Colonies ( $n=39$ ) of four to six unrelated bees were established in artificial nests. After normal social interactions and a division of labor became apparent, the individuals were marked and the principal guard identified. All bees were then isolated for periods ranging from 1 to 12 days, with each placed in a small, clear plastic vial, provisioned with *Typha* pollen and honey water. The guard was then replaced in its nest, allowed 24 h to resume normal sentry behavior and then subjected to introduction of nest mates and nonnest mates.

It appears that the initially clear discrimination between nest mates and nonnest mates by the guard decreases with time. As the period of isolation increased, the percentage of nest mates accepted decreased from 90% after one day of isolation to 10% after 12 days (Fig. 1). This suggests a progressive loss of memory by the guard, perhaps extinction of a learned ability to recognize nest mates, until finally all bees are considered outsiders. The percentage of nonnest mates allowed entry into nests is unexpectedly variable during days 4 to 10 (Fig. 1), suggesting that the ability of the guard to discriminate between resident and nonresident bees is decreased. After 11 or 12 days of isolation, the guard bees tested admitted only 1 out of 21 resident and nonresident bees introduced to them. This, and a noticeable absence of typical sentry behavior, suggests that isolation for periods of over 10 days produces a breakdown in the behavior of the guard or of introduced bees.

Such effects of isolation have been noted in the course of other work as well. Often bees introduced into their own nests after several days of isolation behaved abnormally. Guards only intermittently performed sentry duties and

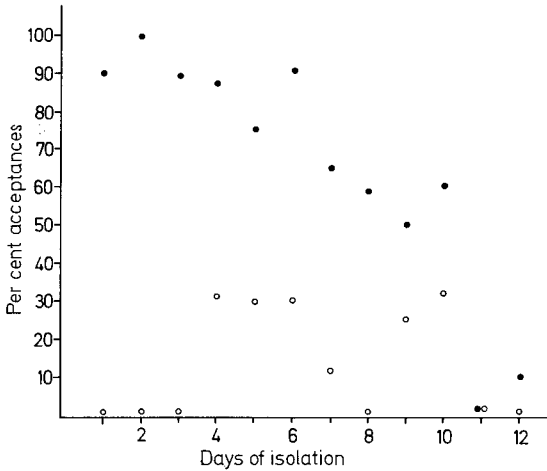


Fig. 1. Percentage acceptances of nest mates (closed circles) and nonnest mates (open circles) by guards that have been isolated from their nest mates for varying periods of time. The correlation coefficient ( $r$ ) of percent acceptance of nest mates with days of isolation is 0.88

former nest mates began to excavate separate tunnels, apparently losing their social cohesion. In extreme cases, guards did not take up their usual stations at the nest entrance and worker bees established their own tunnels in which they remained isolated. This indicates the importance of continuous social interaction for maintenance of a division of labor within a colony as described by Michener and Brothers (1971).

## Discussion and Conclusions

This paper argues against a simple explanation for nest recognition in *L. zephyrum*. In experiments conducted under conditions which eliminate the possibility of visual or tactile cues, bees are still able to determine to which branch of a T-tube their nest is connected. The only probable bases for this discrimination are odors present in the nest. The odors of a given nest attract bees which reside in it, but also attract bees which are residents of other colonies. However, if bees are given a choice between their own nest and another nest, they usually choose to enter their own.

In a more sensitive test, we found that bees approach and enter their home nest less hesitantly if the colony is composed of bees from the same kin group to which the approaching bee also belongs. Since care was taken to minimize exogenous odor sources, the odors may be metabolic products of the bees themselves. If the bees produce distinctive odors providing a means of nest recognition, these odors must certainly have genetic bases. Recognition of such odors may involve a genetic, preprogrammed response to the odors of related bees, or be the product of larval conditioning, or both. Such an explanation accounts for the significantly better discriminatory power of bees from kin

nests. These results are in agreement with expectations generated by the theory of kin selection.

Similarly, no simple explanation for intraspecific nest defense or recognition of nest mates is evident for *L. zephyrum*. The sex pheromone described by Barrows (1975) is apparently not the odor which functions as a cue for guards in nest-mate recognition. Additional investigation is necessary to identify and characterize the odors which are involved. Parallel experiments with kin and nonkin bees suggest that odors which form a basis for nest-mate recognition in nature are in part dependent on genetic similarity or common larval conditioning among bees in a colony although, as shown earlier (Barrows et al., 1975; Bell et al., 1974), such recognition by guards is possible in the laboratory, in colonies of unrelated bees. This suggests that a component of the guard's response is due to adult experience, a suggestion which is confirmed by the guard bee's loss of ability to recognize nest mates following isolation from them for various periods of time. As with nest recognition, there are significant contributions from genetic, or ontogenetic processes and adult learning to mechanisms of nest defense.

It appears that nest recognition and intraspecific nest defense, using odor cues, function in nature to maintain colony unity in genetically homogeneous colonies of bees. This system may prevent joining of older bees which might disrupt the social hierarchy or attempt to steal provisions. On the other hand, it is advantageous to the returning forager to identify its own nest. A system of recognition based on relatedness and reinforced by on-going experience may maintain the genetic homogeneity in the colony, thus reducing the forces for disruptive conflict among individuals. The relationship among genetic similarity, larval conditioning, and adult learning seen in both nest recognition and nest mate recognition suggests that there is a relationship between the odors that function in these two contexts. As yet there is no evidence to support this contention.

*Acknowledgments.* This work was supported by NSF grants GB 38502 and BNS-75-07654, W.J. Bell and C.D. Michener, co-principal investigators. We thank C.D. Michener and R. Jander for reviewing the manuscript and E. Barrows, J. Hackney, and J. Trager for assistance in conducting the experiments. Contribution number 1646 from the Department of Entomology, The University of Kansas.

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