J. comp. Physiol. 93, 195—202 (1974)

Recognition of Resident and Non-resident Individuals in Intraspecific Nest Defense of a Primitively Eusocial Halictine Bee

William J. Bell

Department of Entomology and Department of Physiology and Cell Biology,
University of Kansas, Lawrence, Kansas, USA

Received April 17, 1974

Summary. In laboratory colonies of *Lasioglossum zephyrum*, derived from unrelated pupae and in nests with identical soil and food sources, guards recognize non-resident conspecifics on the basis of odor discrimination. Odors which are important in this recognition mechanism seem to be individual bee odors. The system of recognition may be one of the following: 1. The guard recognizes the summation of odors emitted by all resident bees, and a non-resident intruder emitting an odor which is not part of this repertoire elicits aggressive responses by the guard, or 2. the guard becomes habituated to the odors of each resident bee, and a non-resident intruder emitting an odor to which the guard is not habituated, elicits aggressive responses.

Introduction

Guards defending nests of *Lasioglossum zephyrum* commonly distinguish colony members, which they admit, from conspecific non-resident bees which are rejected (Batra, 1964). In artificial colonies the bees were not usually close relatives, all received identical food throughout life, and soil in the nests was mixed and shifted from the same site. Hence, odor similarity due to genetic relatedness and odors adsorbed from different food sources and nest substrates, which may be important bases for recognition of non-resident bees in nature, were excluded. Nonetheless, the guards distinguished non-resident bees from nestmates. The purpose of this paper is to elucidate the stimuli which release aggressive behavior by nest-defending guards under these conditions.

Nests of *L. zephyrum* were prepared and maintained as described by Bell *et al.* (1974). Other aspects of methodology are explained for each section of the paper.

Table 1. Aggressive behavior of guards elicited by introducing living or dead non-resident or resident bees or probes into the nest entrance

Experiment	N	% agressive display	
		Proximity (1–10 mm)	Contact
1. Non-resident bee	228	50	92
2. Dead non-resident bee	32	41	97
3. Resident bee	50	0	0
4. Dead resident bee	20	0	10
5. Clean probe	60	18	83
6. Probe stung by guard and then introduced into the same nest	28	22	100
7. Probe stung by guard and then introduced into a different nest	32	12	94

Results

A. Tactile and Auditory Stimuli

Clean glass micro-pipettes were inserted into nest entrance tubes at about the same speed at which a returning forager normally enters the tube. Guards showed aggressiveness before being contacted by the pipette in 18% of introductions and in 83% after contact was made ($N = 60$). Thus tactile stimuli seem to be one important factor in releasing guard aggressiveness. The possibility was considered that perhaps resident and non-resident bees exhibit specific tactile stimuli which are perceived by the guard and used in the mechanism of recognition. Non-resident and resident bees were therefore killed by freezing and then introduced into their own nests or other nests (Table 1). In 32 cases of dead non-resident bee introductions, 41% elicited aggressiveness when contacting the guard. Dead resident bees failed to stimulate guard aggressiveness before contact and only 10% stimulated aggressiveness when contact was made ($N = 50$). It would appear that specific tactile stimuli exhibited by entering bees are not decisive factors in determining acceptance or rejection. The same experiment excludes auditory stimuli as a necessary factor in recognition since dead bees could not produce auditory signals.

B. Visual Stimuli

To study the possible role of visual stimuli, nests were placed in a darkroom illuminated only by far red light (590–680 mμ), not visible to bees (Goldsmith, 1961). Non-resident bees were introduced into nests where rejection had previously occurred. Of 35 introductions, 86% were

Table 2. Effects of maintaining bees away from their nests on success of reintroduction into their own nests

Time away from nest (hrs)	*N*	% strong rejection	% acceptance after some aggression	% prompt acceptance
1	23	17	17	66
2–4	15	14	46	40
5–8	16	13	25	62
24	20	35	30	35
48	30	40	25	35
72	16	50	13	37

rejected without visual cues. Thus although visual stimuli may be important when this modality can be employed, they appear not to be a major factor in recognition of non-resident bees.

C. Chemical Stimuli

When non-resident bees were introduced into recipient nests, 50% elicited an aggressive display by the guard prior to making contact (Table 1), thus suggesting that the guard may distinguish non-resident from resident bees through olfaction prior to receiving tactile stimuli. Upon contact, 92% of non-resident bees released guard aggressiveness, although no aggressiveness followed contact with resident bees removed from their nests and immediately re-introduced; thus contact chemoreception as well as olfaction may be important in recognition.

Chemical stimuli were studied along several lines to determine if either the odor of a nest (generated from feces, secretions lining the cells or other sources) adsorbed by bees in a nest or odors of the bees themselves were important in recognition of entering bees.

1. Acceptance of Resident Bees Maintained Away from Their Nests. Since nest odors, as defined above, but not individual bee odors, would be expected to diminish in concentration the longer a bee is away from its nest, experiments were performed using bees removed from their nests and maintained in clean tubes from 1 to 72 hours. The bees were re-introduced into their own nests and scored for rejection, acceptance, or acceptance following initial aggressiveness by guards (mainly intention movements; see Bell and Hawkins, 1974). The results (Table 2) show that the longer a bee is absent from its nest, the greater the probability that it will encounter guard aggressiveness when attempting to re-enter its own nest.

2. Guard Marking. Since the guard has an opportunity to contact each bee entering or leaving the nest, it was of interest to determine if the guard marked bees of its colony with an odor. Probes (filter paper strips) were placed in entrances of nests in which a definite guard had been identified. The guard was observed to attack the probe, stinging and biting; a liquid could often be seen on the paper. The probe was removed after 4 hours and an attempt was made to insert the probe past the guard and into the nest (Table 1). Guards showed aggressiveness in 22% of these attempts prior to being contacted, a value which is significantly greater than that observed when resident bees were introduced ($P < 0.001$), less than that observed when non-resident bees were introduced ($P < 0.001$), but exactly the same as the value obtained when a clean probe was used. Thus if an odor was adsorbed from the guard onto the probe, it was not a sufficient stimulus to inhibit the release of an aggressive display by the guard. Indeed, when actually touched by the contaminated probe, all guards exhibited aggressiveness.

To see if the probes used above might elicit more aggressiveness than clean probes when inserted into other nests, the same procedure was repeated except the probe was inserted into different nests. Fewer guards displayed aggressiveness than when the probe was inserted into the nest it was impregnated in, but the difference was not significant. Thus, if odors were adsorbed onto the probe, there is no evidence that they affected the number of aggressive responses by guards.

3. Adsorption of Nest Odors by Non-resident Bees. To see if a non-resident bee can adsorb the odor of another nest and therefore elicit less aggressiveness by the guard when introduced into the same nest, two experiments were performed. First, non-resident bees were placed in the entrance tubes of nests in which the resident bees were restricted to lower areas of the nests. After 4 hours resident bees were released and the non-resident bee was introduced. All non-resident bees were strongly rejected ($N = 20$). Second, a non-resident bee was placed in a tube 10 cm long containing feces and soil pushed out into the vial by bees of another nest. After 24 hours, the non-resident bee was introduced into the nest from which the feces and soil were extracted. The results were the same as above, although when the bees were returned to their own nests, the guard showed initial aggressiveness before admitting its nestmate in 50% of the introductions. Thus non-resident bees did not adsorb sufficient nest odor in these experiments to allow them to be considered resident bees. Since the bees encountered difficulty in entering their own nests, they may have lost their own nest odor during the period of absence from their nests or adsorbed sufficient foreign nest odor to release aggressiveness in the guards of their own nests.

To study nest odors further, air was blown from one nest into another for 24 hours. Then bees from the nest supplying the air were introduced into the nest receiving the air. All introduced bees ($N = 20$) were vigorously rejected. Second, all bees except the guard were removed from a nest and placed in clean tubes for about 2 minutes. While these bees were being re-introduced to their own nest, air was blown into the nest entrance tube from that of another nest. All resident bees were accepted immediately; the guards exhibited no aggressiveness ($N = 20$). Third, a foreign odor, peppermint, was blown into 6 nests for 24 hours and then reciprocal transfers were attempted. In all cases the non-resident bees were vigorously rejected by the guards ($N = 20$).

Next, bees of one nest were completely transferred to another nest and vice versa. Six such reciprocal transfers were made (12 nests, each with 4 bees). On days 1 to 6 after the exchange, the bees were introduced back into their original nests which were now populated by other individuals. Introductions after 1 or 2 days resulted in significant acceptances of non-resident bees (72 and 60% acceptance, respectively). Introduced bees were so aggressive in moving into the nest which they had previously occupied that the guard in its newly acquired nest seemed not to be as successful as when defending its previously occupied nest. After 3 days, however, acceptances became less common (20%), especially if the transplanted bees had begun making cells. By day 6 only 18% of non-resident bees were accepted.

In a final attempt to explore a possible role of nest odors in recognition, portions of the soil from different nests were transplanted from one nest to another. Following the transplants, reciprocal bee introductions were attempted. Even after 10 days, no acceptances were recorded ($N = 60$), indicating that nest odors in the soil transferred from one nest to another did not increase the acceptance of non-resident bees from nests donating the soil.

D. Motivational State

Since guards often must reject one bee and then accept the next bee which attempts to enter the nest, it appears that the level of aggressive motivation of the guard would have to be "plastic" enough to permit such a system to work effectively. Batra (1966) noted that a guard stopped fighting an intruder to permit a native forager to enter the nest, and then continued its battle with the intruder.

To explore the motivational state of guard bees engaged in aggressive behavior, non-resident bees were introduced and after contacting the guard, they were removed and a resident bee was quickly introduced. In 86% of resident bee introductions ($N = 16$), the guard showed aggressiveness, and in 43% the guard blocked the entrance to the resident bee when contacted. When the experiment was repeated using a clean probe to cause guard aggressiveness, followed by introduction of a resident bee ($N = 27$), aggressiveness by the guard was exhibited in 73% of the cases and the resident bee was rejected in 14%. These results may be interpreted as follows. Contact (= tactile stimulus) with either a bee or a neutral object releases guard aggressiveness, although contacting an actual non-resident bee enhances the effect since the bee provides both tactile and chemical stimuli. The level of aggressive motivation is raised by these stimuli and the decay from this level to the basal level is rather slow in that the guard is still aggressively motivated a few seconds later when a resident bee is introduced. Although no quantitative data are available on the length of time required for decay, guards accept resident bees if at least one minute has elapsed since the introduction of a non-resident bee.

Discussion

Odor recognition of non-resident bees by guard bees is involved in intraspecific nest defense. The diversity of odors required to permit this recognition system could be explained if bees have access to different pollen or nectar and if the nest burrows encounter soil diversity, as is likely in the field. Since laboratory colonies were maintained with identical pollen sources, honeywater and soil, such diversified odor-generating systems are eliminated. Other sources of odors were considered, however. Batra (1964) showed that bees engaged in cell-making discharge saliva and secretions of the Dufour's gland in producing cell lining material. Feces may contain a variety of odors which might promote nest odors differing from colony to colony. The results of this study indicate, however, that nest odors are less important sources of information used for recognition of non-resident vs resident bees than individual bee odors.

A guard may recognize nestmates according to their individual odors, which form a "group odor" or a summation of all individual bee odors in a colony, but during the absence of a nestmate the guard may "forget" the odor of the bee which was removed. Upon re-introduction of the nestmate, the guard exhibits aggressiveness owing to lack of recognition. Guards may also become habituated to the individual odors of bees in the colony. When bees were removed from their nests for long periods of time, perhaps habituation to the odor of the absent bees decayed, and the guards perceived the odors of the re-introduced resident bees and responded aggressively.

A few older non-resident bees were readily accepted into mature colonies. If individual odors were genetically determined by any limited number of genes, one would expect a certain number of odor duplicates, and guards would not be able to distinguish between resident or non-resident bees which emit these similar or identical odors.

Males of *L. zephyrum* can distinguish between females, probably as a result of diversity in individual sex pheromones. In this case olfaction seems clear. One might speculate that the odors important in recognition of non-resident bees could be the sex pheromones, especially since resident males are often rejected by the guard and since non-resident newly emerged females, which do not secrete sex pheromone, are accepted more readily than older females.

Multiple stimuli may be involved in nest defense. In a primitively social species, such a mechanism would seem likely, since one might expect such a species to have a diversity of control mechanisms. Evolution might eventually lead to a single mechanism which releases guard aggressiveness, but whether this has occurred, even in any of the highly eusocial bees, is not known. It seems likely that the recognition of nest or individual odors for nest defense by *L. zephyrum* makes use of abilities that are also important to the solitary ancestors of this species. Solitary bees may recognize their own nests in part by nest odors based on their own individual odors; recognition of odors of other females would have the same importance as in *L. zephyrum.* Use of these abilities for societal purposes would require little evolution from the solitary ancestor.

The author is grateful to Trish Jones and Profs. R. Jander and C. D. Michener for critically reading the manuscript. This research was supported by grants from the National Science Foundation (GB-38502 and GB-8588X).

References

Batra, S. W. T.: Behavior of the social bee, *Lasioglossum zephyrum*, within the nest. Insects Sociaux **11**, 159–186 (1964)

Batra, S. W. T.: Life cycle and behavior of the primitively social bee, *Lasioglossum zephyrum.* Univ. Kansas Sci. Bull. **46**, 359–423 (1966)

Bell, W. J., Breed, M. D., Richards, K. W., Michener, C. D.: Social, stimulatory and motivational factors involved in intraspecific nest defense of a primitively eusocial Halictine bee. J. comp. Physiol. in press (1974)

Bell, W. J., Hawkins, W.A.: Patterns of intraspecific agonistic interactions involved in nest defense of a primitively eusocial Halictine bee. J. comp. Physiol., in press (1974)

Goldsmith, T. H.: The color vision of insects. In: Light and life, W. D. McElroy and B. Glass, eds., p. 771–794. Baltimore: The Johns Hopkins Press 1961

Dr. W. J. Bell
Department of Entomology
University of Kansas
Lawrence, Kansas 66045, USA