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Social, Stimulatory and Motivational Factors Involved in Intraspecific Nest Defense of a Primitively Eusocial Halictine Bee

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Summary. The major releasing stimulus in intraspecific nest defense of *Lasioglossum zephyrum* is the odor emitted by a non-resident bee. Non-resident bees older than two days emit the releasing odor and elicit aggressiveness by guard bees, whereas younger non-resident bees are accepted more often. Defense motivation is a function of nest age and/or ontogeny. As nests become older and cells are constructed and provisioned, there is a gradual increase in guard aggressiveness, although no one attribute of nest ontogeny (such as cell construction) seems to be a definitive point at which nest defense is initiated, nor is there any specific day after the emergence of the first bee when nest defense begins. The guard plays the major role in rejecting intruders, although other members of the colony may do so if a non-resident bee passes the guard and enters the nest.

Introduction

Nest defense, including the rejection or ejection of conspecific non-resident bees, occurs in highly and primitively social, as well as solitary bee species. The purpose of this three-part study on *Lasioglossum zephyrum* is to eludicate the factors which influence nest defense, to determine the mechanism of recognition of non-resident vs resident bees (Bell, 1974), and to study the patterns of intraspecific agonistic interactions involved in nest defense (Bell and Hawkins, 1974).

This first paper deals with motivational, stimulatory and social factors which influence nest defense: ontogeny of the social organization of a colony into which a non-resident bee is introduced, age and characteristics of the colony from which the experimental non-resident bee is taken, the age of the non-resident bee, and the role of the guard in nest defense.

Methods and Materials

Colonies of *Lasioglossum zephyrum* were constructed artificially of females emerging in captivity from pupae collected in the field from many nests. Thus colony members were not likely to be related bees. Nests were constructed in sifted

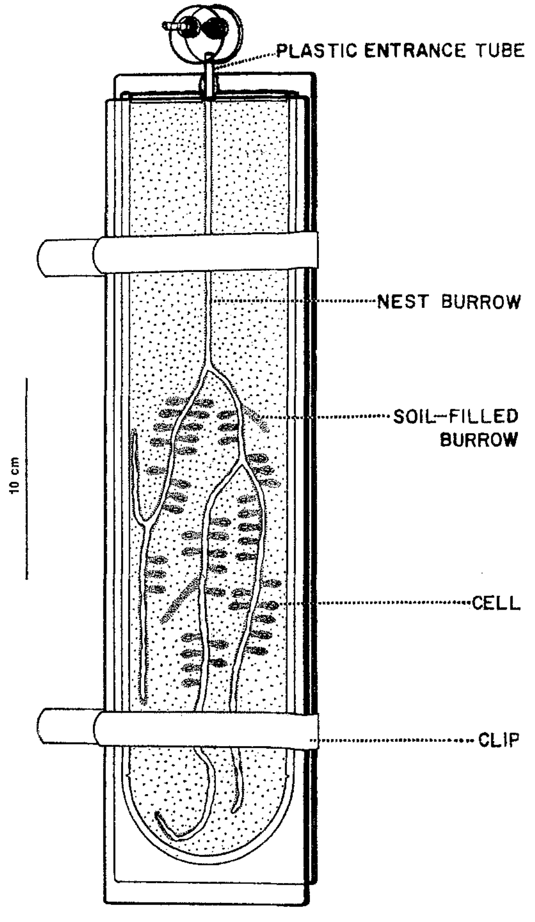

Fig. 1. Oblique frontal view of observation nest. At the top is the closed plastic vial in which bees feed but from which they cannot escape

soil (collected from the same location) between glass plates as described by Michener and Brothers (1971). All nests were capped as described by Kamm (1974) with a vial of 17.4 cm^3 provisioned with *Apis* honey-water and cat-tail (*Typha*) pollen (Fig. 1). Soil employed and provisions offered were identical in all nests. Some bees, shortly after emergence, were placed in isolation vials provisioned and maintained as described by Bell (1973); these bees remained in isolation and contacted other bees only when they were introduced as non-resident bees into recipient nests.

Bees were maintained in rooms with a changing summer photoperiod similar to that of Lawrence, Kansas. Temperature varied during the light phase similar to that in nature from 17° C at the onset of light to a high of 28° C approximatley 6 hrs later.

Bees within the nest were permitted entrance to the vial through a transparent 3 cm plastic tube. Guard bees generally stationed themselves at the end of the plastic tube where it opened into the vial. In most cases the guard constructed an inner ring of soil at the end of the tube so that only the guard's head with antennae protruding into the vial could be observed at the end of the tube.

Bees were marked for identification with small dots of colored "Dope" painted on the thorax.

Non-resident bees (those from other nests) or resident bees (those from the same nest) were introduced to recipient nests by holding a 3 cm tube containing the bee against the nest entrance with the vial removed. Individual non-resident bees were tested more than once, but their introductions were rotated. In cases where one bee was tested repeatedly however, there appeared to be no change in its acceptability by recipient nests.

The term rejection in the text is defined as the departure of a bee after it had been introduced into a nest and had interacted with the guard. Acceptance is defined as the entry into a nest of an introduced bee after interaction with the guard or in the absence of a guard.

Results

A. Chronology of Events in Nest Ontogeny

Within 1 to 7 days after the first bee emerged in a nest, tunnel digging was initiated (Fig. 2). In most nests cell construction began within 1 day after tunnel initiation and cell provisioning was common on the next day. Egg-laying occurred as early as the day of cell provisioning. The developmental state of a colony is characterized arbitrarily in this paper in two ways: 1. age, i.e. the number of days since the first bee emerged, and 2. ontogenetic characteristics if the colony: a) digging, burrows formed, but no cells, b) presence of empty, lined cells, c) provisioned, closed cells, and d) larval or pupal offspring. Substantial variation was observed with regard to the day on which digging, cell construction and provisioning first occurred, suggesting that the age of a nest may not correlate closely with the timetable of events in the ontogeny of a colony.

On the day when the first bee emerges, colonies do not have guards. Bees generally remain in the nest, exhibit little activity, and permit non-resident bees to enter. Thus in experiments described below, nests were employed only 24 hours or more after the first bee emerged; after 24 hours the nest is designated as a 1 day nest, after 48 hours a 2 day nest, etc.

B. Social Factors Involved in Nest Defense

Since non-resident bees are rejected at the nest entrance mainly by a specific female, the guard bee, and not by the queen or other females (Brothers and Michener, 1974), its role in nest defense was explored by introducing non-resident bees into either guarded ($N = 1175$) or unguarded ($N = 79$) nests. Significantly fewer numbers of acceptances were observed when a guard was present ($P < 0.001$), and significantly

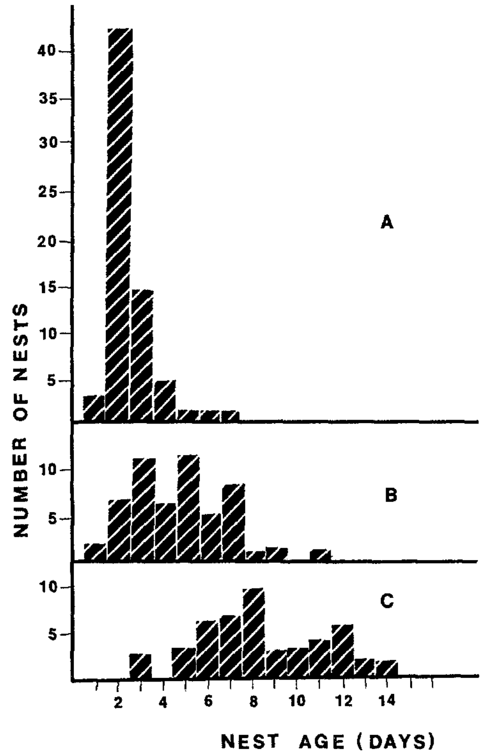

Fig. 2. Frequency distribution of the first occurrence of digging (*A*), cell construction (*B*), and cell provisioning (*C*) after the day on which the first bee emerged

greater numbers of acceptances were observed when the guard was absent ($P < 0.001$).

To further investigate the role of the guard, a non-resident bee was introduced into a nest after the resident bees had been removed. Resident bees were then replaced and observations made to determine if the non-resident bee was expelled or permitted to remain and what role the guard played in this situation. Table 1 shows that the most common result was aggressive expulsion of the intruder by resident bees. Aggressive behavior was shared between the guard and other bees in the nest. Expulsion was not always observed immediately, however, and in some cases the non-resident bee remained, but was expelled during the succeeding 24 hours. In some cases the non-resident departed on its own accord (20%), remained in the nest permanently (17%), or resident bees were aggressively rejected by the non-resident bee (7%) or they departed without aggressive actions toward the intruder (5%).

Table 1. Results of placing non-resident bees into evacuated nests and then replacing resident bees

Results	% ($N=40$)
1. Residents leave—non-resident aggressive	7
Residents leave—non-resident not aggressive	5
2. Non-resident leaves—residents aggressive	49
Non-resident leaves—residents not aggressive	20
3. All leave—residents and non-resident aggressive	0
All leave—residents and non-resident not aggressive	2
4. All stay—residents and non-resident initially aggressive	2
All stay—residents and non-resident not aggressive	15

C. Motivational and Stimulatory Factors Involved in Nest Defense

A total of 1175 introductions of non-resident bees were performed when guards were present. This procedure precluded the use of day 0 nests in which no guarding activity occurred. Tests were made using either non-resident bees residing in nests or isolated non-resident bees maintained in small vials. For each introduction the following information was recorded for both the non-resident bee and the recipient nest: age of the bee, age of the nest, presence of cells, provisioned cells or larvae, and number of bees in the nest. An acceptance or a rejection was scored and control data were obtained by introducing bees back into their own nests.

1. Age of the Non-resident Bee. Older bees are rejected more commonly than younger bees, independent of other variables (Figs. 3 and 4). There is no significant difference between frequency of acceptance of day 0 (newly emerged) or day 1 bees, whereas bees 2 days or older were rejected more often than younger bees ($P<0.001$; Mann-Whitney-U test; analysis of variance using Friedman's method of ranking).

2. Characteristics of Recipient Nests. Fig. 3 depicts the enormous variation in per cent acceptance of non-resident bees in relation to the characteristics of the recipient nests. As noted above, non-resident bees 2 or more days old are generally rejected by colonies exhibiting all types of characteristics tested. Bees aged 0 to 2 days are perhaps accepted more often in nests without cells than in nests with provisioned cells or larvae ($0.25< P<0.05$), sugges tingthat more mature colonies reject non-resident bees to a greater extent than newly established colonies.

3. Age of Recipient Nests. Comparing bees aged 0 to 2 days, introduced into nests of various ages, it appears that there is no significant

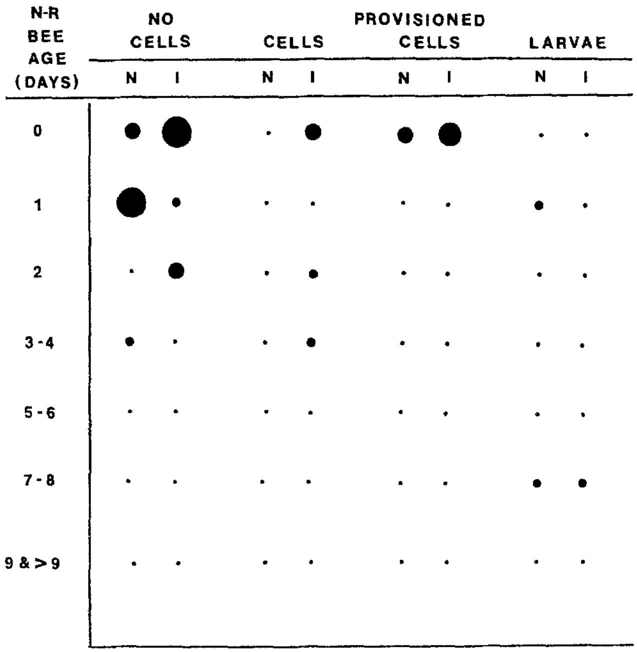

Fig. 3. Relationship between characteristics of the recipient nest and age of the non-resident bee. *N*, non-resident bees maintained in nests, *I*, non-resident bees maintained in isolation vials. Points represent per cent acceptance of non-resident bees: smallest to largest points, 0–15%, 16–30%, 31–45%, 46–60%, 61–75% acceptance. Each point represents 9 to 39 observed encounters with a median of 22 and a mean of 21.17

difference among nests except for day 1 nests vs nests over 7 days old ($P < 0.05$). There seems to be no definite point in the age of a nest after which non-resident bees are rejected (Fig. 4) and before which they are not.

4. Population Size of Recipient Nests. The number of bees in a nest seems not to be a factor in deciding whether or not a non-resident bee is accepted. No significant differences were observed in nest defense between colonies of different sizes, although nests with 1 bee accepted newly emerged bees in 83% of introductions, suggesting that a single pioneer bee is not a highly motivated guard and may actually be more motivated toward recruiting "joiners". If recognition of residents by guards is the mechanism for discriminating between residents and non-residents, the ability to make such a determination might diminish as the population of the nest increases. Guards of colonies as large as 12 individuals,

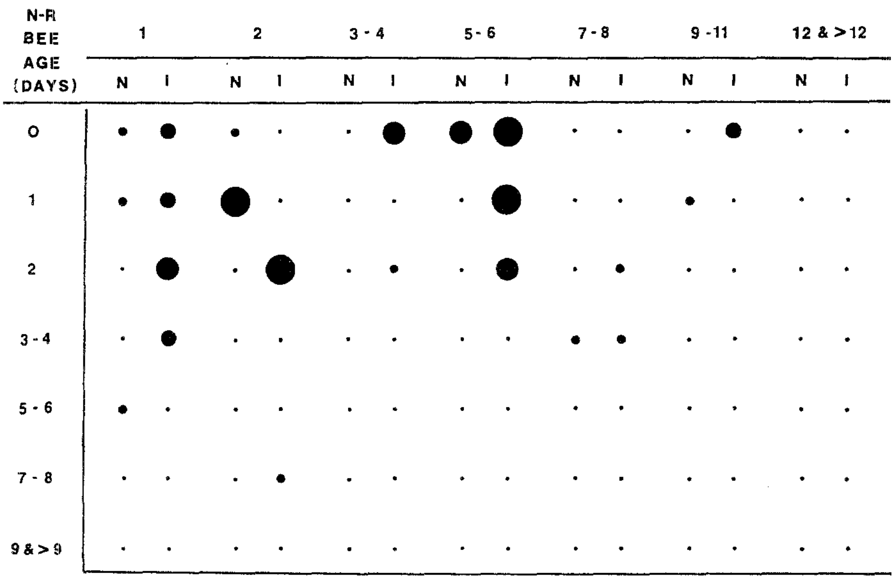

Fig. 4. Relationship between recipient nest age and age of non-resident bee. *Nest age* (= days after the first bee emerges in recipient nest), *bee age* (= days after non-resident bee emerges). Abbreviations same as in Fig. 2. Smallest to largest points represent 0–20%, 21–40%, 41–60%, 61–80%, 81–100%. Each point represents from 5 to 47 encounters with a median of 9 and a mean of 22.93

however, are as efficient in identifying and rejecting non-resident bees as guards of smaller colonies.

5. Acceptance of Nest Bees vs Bees Maintained in Isolation. In some combinations isolated bees are accepted more readily than non-resident bees taken from nests. On the whole, however, there is no significant difference, suggesting that if odors are important releasers of guard aggressiveness, these are individual odors and are not produced as a result of cell construction or provisioning, since isolated bees had no opportunity to work with soil.

6. Other Possible Factors. Characteristics of the nest, population size, and nest age of the non-resident bee (except where it correlates with the individual age of the non-resident bee) are not major factors in determining acceptance of non-resident bees. Once a bee is older than 2 days, it is usually rejected, regardless of its background.

7. Control Tests. In control tests where bees were introduced back into their own nests ($N = 154$), 98% were accepted, although in several instances they were accepted only after careful scrutiny by the guard. Frequency of acceptance among controls was thus significantly greater than in the combined results of non-resident bee introductions (14% acceptance) ($P < 0.001$).

Discussion

Stimulatory Factors in Nest Defense

A major stimulus factor in determining whether a conspecific non-resident bee will be accepted by the guard of another nest in *L. zephyrum* is the age of the non-resident. Newly emerged or one day old bees are often accepted into nests to which they do not belong, whereas older bees are commonly rejected. Perhaps a change occurs in releasing capability by bees which is functionally related to their age. The secretion of odors which identify the bee as a non-resident seems the most likely explanation, since Bell (1974) excluded other possible stimuli (i.e. auditory, behavioral, visual, tactile). Female sex pheromones of this species may differ among individuals, and this variation in sex pheromone may help guards recognize individuals. Newly emerged females are not attractive to males, and perhaps initiation of sex pheromone production correlates with the age at which young bees begin to be rejected by guards.

Motivational Factors in Nest Defense

Older colonies reject foreign bees more than younger colonies, although there is no definite point at which nest defense becomes more apparent. Similarly, colonies more advanced in their ontogeny reject non-resident bees more commonly than colonies which have not yet initiated cell construction and provisioning. Probably, establishment of the social hierarchy with guard, queen and foragers is requisite for promoting defense mechanisms. Since there is much variation among colonies in the timing of cell construction and other activities after the first bee emerges (Fig. 2), variation in the timing of establishing a guard and defensive potential (as suggested in Figs. 3 and 4) would be expected.

Lone bees do not guard their nests effectively. Their motivation toward defense is so low that introduced non-residents often join, forming a colony. These observations are consistent with observations that in most Halictine bees the over-wintering queens, which establish their own nest in spring, also do not guard, although once their progeny emerge and become active in the nest, guarding and nest defense become more common (reviews: Michener, 1969; Lin and Michener, 1972).

In developing a social hierarchy in colonies of *L. zephyrum*, a primary line of nest defense involves aggressive behavior exhibited by the guard at the nest entrance. Secondary defense includes activities of other members of the colony once a non-resident has entered the nest, and in these cases the rejection of a non-resident bee may not occur until several hours after the intruder is introduced. Non-resident bees were aggressively rejected in only 49% of introductions made when the nest bees were absent as compared to 86% when they were present and the non-resident bee had to pass the guard in order to enter the nest. This suggests the efficiency of the primary line of defense as compared to the secondary defense system.

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