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Interactions in Colonies of Primitively Social Bees

III. Ethometry of Division of Labor in *Lasioglossum zephyrum* (Hymenoptera: Halictidae)*

Denis J. Brothers and Charles D. Michener

Department of Entomology, The University of Kansas, Lawrence

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Summary. Thirty-seven laboratory colonies (two to six females in each) of a primitively social halictid bee were observed for a total of 13160 min to obtain data on intranidal differentiation in behavior. Nine behavioral patterns or categories were repeated often enough for numerical analysis of the behavior, i.e., ethometrics.

Although the castes are externally indistinguishable and intergrade with one another in ovarian development, the bee with the largest ovaries in each colony shows certain behavioral specializations that justify her designation as queen. She is the most active bee. Pre-eminent among characteristic queen behavioral categories is backing—the queen in every colony backed away from other bees more frequently than any other bee in the colony. Queens are usually maximal nudgers of other bees in the colony, while they are in the low ranks for pollen collecting and guarding. Both backing and nudging are directly related to ovarian size. Other behavioral categories have more complex relationships; e.g., following and guarding are usually minimal for queens and progressively more common for workers with smallest to largest ovaries. Thus the ovarially most queenlike workers are behaviorally the least queenlike for these attributes. The two workers in a colony of three bees are behaviorally different and their counterparts are easily recognizable in larger colonies. The maximal guard, which has ovaries larger than the average for workers, is usually the least active bee, showing minimal backing and minimal working on cells. The maximal pollen collector commonly shows minimal nudging and high passing. She usually has the smallest or nearly the smallest ovaries in the colony. "Other" workers (in colonies of more than three bees) are not specialized as either guards or pollen collectors although they do some of both; they tend to be rather inactive.

A principal components analysis of individuals in colonies of three bees, based on all behavioral characteristics except pollen collecting, clustered the bees into two groups, queens and workers, with guards and foragers at opposite extremes of the worker cluster. A discriminant function (canonical) analysis verified the distinctness of these groups, placing the workers into two clusters; when the "other" workers in colonies of four or more bees were also considered, they mostly fell between, but partially mixed with, foragers and guards.

In 11 out of 12 colonies, removal of queens resulted in another bee (commonly one of the "other" workers) showing queenlike behavior, sometimes noted in less than four hours. Such replacement queens had enlarged ovaries when later dissected; thus caste determination in the adult stage is verified.

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* Contribution number 1507 from the Department of Entomology, The University of Kansas, Lawrence, Kansas, 66045, U.S.A. Parts I and II of this series are indicated in the References as Michener, Brothers, and Kamm, 1971a and 1971b.

It appears as if the bees discriminate among individuals on the basis of ovarian size—for example, the queen concentrates her nudging on the bee with next largest ovaries, commonly the guard. The mechanism by which the queen inhibits ovarian development of her nest mates remains unknown but may be purely mechanical—disturbance by her great activity and frequent nudging of other bees, and drawing bees down, away from the entrance, by her frequent backing.

Oophagy was observed three times when the identity of the layer and egg eater were known. In each case the egg had been laid by a worker and was eaten by a queen.

Introduction

This paper reports the first development of ethometric techniques in the study of interactions among the adult females that constitute the colonies of a primitively eusocial insect. *Lasioglossum* (*Dialictus*) *zephyrum* (family Halictidae) was selected for study because its social behavior is poorly developed; it seemed likely that some attributes of early stages in social evolution would be recognizable in such a form. Colonies of females live in burrows in earth banks and at the season of maximum population (August) consist of an average of about 14 females, including potential queens that will overwinter in addition to the working bees of the colony. Sociality is facultative, for in spring many nests (and at all seasons, some nests) contain only one adult female. The life history and some aspects of the intranidal behavior have been described by Batra (1964, 1966).

Previous work on this bee has emphasized the following points: (1) Variation in female productivity is continuous so that it is difficult to define castes; arbitrarily the bee with the largest ovaries has been designated the queen, others are called workers even though some lay eggs. (2) Caste determination occurs in the adult stage. (3) There is no evidence of direct transfer of substances (like queen substance of *Apis*) among individuals since there is no food interchange or mutual grooming. (4) Momentary contacts are of common occurrence but rarely have an overtly aggressive aspect.

Michener, Brothers, and Kamm (1971a) speculated on the possible functions of repeated contacts, which appeared to be mutually stimulating, thus promoting digging and cell construction. It has since occurred to us that the contacts might somehow determine which females most commonly leave the nest and therefore are most likely to mate and feed. The present study was undertaken in order to shed more light on such possibilities.

A. Methods

Colonies of bees were maintained in soil between sheets of glass as described by Michener and Brothers (1971). Some colonies consisted of an overwintered queen and her progeny, like those described by Michener, Brothers, and Kamm (1971b), while others consisted of bees of more or less equal ages reared from pupae taken in the field near Lawrence, Kansas, as described by the same authors (1971a). In most cases the bees could fly to flowers of various kinds provided as food sources in rearing rooms (see Batra, 1964; Stockhammer, 1966), but some nests were closed with small plastic boxes in which honey and *Typha* pollen were provided, as described by Kamm (1974). The open nests were nearly vertical with entrances up; the closed nests were kept horizontal. No differences in the results were noted that could be attributed to nest type, closure, or orientation. For simplicity of expression, the nest entrance is spoken of as upward. A total of 23 nests was studied, 8 of them at two different times with partially different bees and 2 at four different times, making in effect 37 different or partially different colonies, each treated as a separate colony below. Of these, 12 contained two bees; 16, three bees; 5, four bees; 3, five bees; and 1, six bees.

In each nest the bees were marked for individual recognition with quick drying "Dope" paint. To inactivate the bees before removing them for marking, nests were placed in a refrigerator (about 6° C or even briefly below 0° C). The nests were then opened, bees marked or removed, and the nests returned to the rearing rooms. If the bees were to be allowed to fly in the bee rooms, the entrance burrows were closed with plugs of loose soil to reduce losses immediately after marking.

After the bees of a colony were making and provisioning cells, they were watched continuously for varying periods—a few minutes to a few hours but usually 15 min or more per session. All recognizable interactions among the bees, as well as certain other activities, were recorded. Because of the frequency of activities in colonies of four or more bees, records for such colonies were made verbally with a tape recorder. The impossibility of seeing and recording all interactions in larger colonies such as are common in the field in midsummer explains our decision to work with colonies of not over six bees. Such small colonies are also common in the field.

Observations were made by removing the opaque nest cover and watching the bees through the glass with as little other disturbance or vibration of the nest as possible. We do not believe that the light admitted by removal of the cover influenced activity of the bees, for there is no evidence of any activities consistently beginning or ceasing soon after removal of the cover. Neither is there any evidence of change in frequencies of activities after several hours of watching, as might be expected if there were gradual behavioral accommodation to light in the nest.

Data on any one colony were obtained during a period of 25 days or less (mean 8.4 days) and data for the various separate periods of observation of any one colony were combined. Data were discarded for any observation period if there were not at least as many records of behavior as the number of bees in the colony. The observation times for data that were used varied for different colonies from 15 to 1236 min (mean 356 min), and totalled 13160 min. For colonies with three or more bees the minimum observation time was 65 min and the mean was 438 min. Data from all appropriate colonies were pooled. The assumptions were made that once the pattern of activity in a colony had been established, there was no major disturbance in this pattern and that the relative frequency of each activity contributed by each bee remained relatively constant. This assumption is in general supported in Section C below.

The bees of 12 colonies were preserved in Dietrich's (Kahle's) solution immediately after the last observations. Examinations of the ovaries were made later, and the bees of each colony ranked with respect to ovarian development. A bee's rank was determined by counting the total number of oocytes visible. This ranking agreed with a subjective ranking on the basis of ovarian appearance.

The variation in the ovaries was like that illustrated by Michener, Brothers, and Kamm (1971a); no females when dissected had very slender ovaries like newly emerged females and like many workers of some related species such as *L. imitatum* (Michener and Wille, 1961).

For seven other colonies, instead of preserving all individuals at once, the queen as recognized behaviorally (see Section F) was removed, while the remainder of the bees were left in the nest, forming a new colony for purposes of our behavioral studies. When a replacement queen was recognized by behavior, she too was removed. When only two bees survived, after one had been recognized as the queen if possible, both were preserved. From the seven nests, queens were removed on 12 occasions. Examination of ovaries was as for colonies whose entire populations were simultaneously preserved.

B. Behavioral Categories

From an almost overwhelming volume of records of movements and actions of each bee, we extracted data on all interactions and other selected activities. The categories of behavior analyzed are listed below, 1 to 3 being interactions, the others being individual actions. A total of ten categories is involved, the two halves of each interaction being counted separately.

1. Nudging and Being Nudged. A bee moving along the burrow hesitates as she approaches another bee ahead of her and then moves forward in a short, sharp movement that brings her face into contact with the other bee. The mandibles are not open—this is not, or at least not usually, a biting movement. The moving bee is the *nudger* (1a), the stationary one, the *nudged* (1b). In most cases both bees face upward, the nudged bee often being near the burrow entrance.

2. Backing and Following. If, usually after nudging (or attempted nudging), the nudger backs along the burrow for a distance of several centimeters, she is now termed the *backer* (2a). The backer is usually followed by the nudged bee, now the *follower* (2b). This interaction commonly occurs after a bee has nudged another near the burrow entrance, but may occur when a bee moves down the burrow and encounters another bee facing it, the second bee then backing rather than allowing the first to pass. In some cases a nudger initiates backing behavior but the nudged bee does not follow; in such situations the backing distance is usually less than one centimeter and the nudger then moves forward and nudges the other bee again. Such instances of abortive backing (without following) were not tallied in this category.

3. Passing and Being Passed. Except at the nest entrance, bees usually pass only when facing one another. If two bees are both moving forward, toward one another, and pass, it is impossible to designate one the passer, the other the passed. Commonly, however, one is stationary or nearly so, the other moving, in which case the distinction between *passer* (3a) and *passed* (3b) is clear. Often the two bees are facing in the same direction, usually upward. One may be a guard or a bee behind the guard. The lower bee touches or nudges the upper; the upper turns around. At this point, instead of backing and following down the burrow as in 2 above, the bees pass. Near the nest entrance, at least in the artificial nests, passing sometimes occurs when a bee backs past the bee which touched it, so that both bees are continually facing upward.

For scoring of passing, each interaction was allocated two hatch marks. If the passer was distinguishable, both marks were allocated to her; if the passer could not be distinguished from the passed bee, each was given one mark. The final tabulation was adjusted by dividing each figure by two.

4. Guarding. One bee (the guard) is usually at the nest entrance blocking the hole with her head. Details of guarding behavior are described by Batra (1964, 1966) and for related species by other authors (e.g., Michener and Wille, 1961). While for other behavioral categories we recorded the number of times a given activity was observed, guarding is often carried on for long periods by a given bee and could not be scored in the same way. Guarding behavior was therefore expressed as the number of minutes of guarding by each bee, with any uncertain period or guarding period of less than one minute being counted as a minute.

5. Pollen Collecting. Records were made of which bees left and returned to the nest during observation periods and which ones carried pollen back to the nest on the scopa.

6. Working on Burrows. Note was made of instances of bees working on or extending the burrow system, biting at the walls, or tamping loose soil into them.

7. Working on Cells. Records were also made of bees making and lining cells. Since it is difficult to distinguish between short side burrows and cells in the first stages of construction, there may be some errors in the allocation of records to categories 6 and 7.

The reliability of data for the different behavioral categories varies. Our emphasis was on activities that are most common above the level of the cells in the nests. Greatest accuracy was therefore probably attained for behavioral categories 1a, 1b, 2a, 2b, 4, and 5. Interactions in categories 3a and 3b were extracted from the data by keeping a running tally of the relative positions of colony members, even if each passing was not directly noted; these data are thus also probably representative. Since categories 6 and 7 are activities which, for greatest accuracy, should have been scored in terms of the time involved in each and because they usually occur in positions not under maximal observation, these data are probably less representative. Diurnal cycles may influence repeatability of our results. Most of our observations were made in afternoons but some were in mornings. Pollen collecting may tend to occur more often in the mornings as in the case of the related *L.* (*D.*) *imitatum* (Michener and Wille, 1961), but other behavior did not show noticeable temporal biases.

C. Representative Data

To give the reader a feel for the data, information on the various behavioral categories for two colonies (one of six bees and the other of three bees) is presented in Tables 1 and 2. It is obvious that some types of behavior are commoner than others and that for each, some bees are more active than others.

Observations did not begin until the bees were several days old; therefore the temporally distinctive behavior (frequent guarding and excavating) of young adults reported by Batra (1964) and for related species by other authors (e.g., Michener and Wille, 1961) does not enter into our data. To investigate variations in relative activity from day to day, the data for a colony containing six bees were tabulated for eight days. There was considerable variation and significant heterogeneity over days (*a posteriori* test by STP for homogeneity; $p < 0.01$). However, temporal subgroups were not distributed over the data in a sequential fashion such as would be expected if there were progressive changes in the activity of particular bees. By inspection, also, no temporal trends in the variation could be discerned.

Since no trends were distinguishable, it seems that once the general pattern of activity in a nest has been established, day to day variations in this pattern are normal; any generalized patterns based on the proportions of each type of behavior contributed by each member of the colony can only be discerned with any certainty when the data over a number of days are considered together. It thus seems unlikely that major perturbations were introduced into our analyses by the use of data derived from different periods of observation, except perhaps for the three instances in which data collected on only two days were used.

D. Behavioral Trends within Colonies

Each of the ten behavioral categories was examined separately. For each activity, bees of each colony were ranked from 1 (exhibiting that activity least) to 2, 3, 4, 5, or 6 (showing it most, depending on the number of bees in the colony). Trends within each category of behavior were investigated by plotting the proportion of the total of that activity in the colony contributed by each bee, against the rank of that bee. Each plot was constructed in such a way as to reduce the variation between colonies of different sizes to a minimum. This often involved using the relative proportion of the activity (i.e., the absolute proportion multiplied by the number of bees in the colony) and plotting the total range of the ranks over the same interval for each colony size. Thus

Table 1. Frequencies of behavioral interactions (sections 1–3) and other types of behavior (section 4) observed in a colony of six bees during 717 minutes over 8 days. The letters represent different individual bees

1	Nudger *Y*	*O*	*G*	*W*	*B*	*U*	Total nudged
Nudged							
Y		80	60	38	27	73	278
O	21		17	7	16	20	81
G	81	39		32	21	41	214
W	88	18	37		24	59	226
B	58	31	19	28		31	167
U	126	33	59	38	12		268
Total nudger	374	201	192	143	100	224	1 234

2	Backer *Y*	*O*	*G*	*W*	*B*	*U*	Total follower
Follower							
Y		29	0	1	0	0	30
O	4		1	2	0	1	8
G	3	28		1	0	1	33
W	2	16	2		2	0	22
B	1	25	1	1		1	29
U	1	4	0	0	2		7
Total backer	11	102	4	5	4	3	129

3	Passer *Y*	*O*	*G*	*W*	*B*	*U*	Total passed
Passed							
Y		9.0	41.0	23.5	34.0	38.0	145.5
O	25.5		30.0	13.0	24.0	18.5	111.0
G	50.5	19.5		27.5	16.5	28.5	142.5
W	43.0	9.0	26.5		30.5	12.5	121.5
B	27.5	16.0	20.0	12.5		13.5	89.5
U	57.0	18.5	30.0	20.0	15.5		141.0
Total passer	203.5	72.0	147.5	96.5	120.5	111.0	751.0

4	*Y*	*O*	*G*	*W*	*B*	*U*	Total
guard (minutes)	130	0	53	152	88	245	668
pollen collector	0	0	7	1	0	0	8
worker on burrow	30	18	20	45	33	16	162
worker on cell	1	3	6	6	7	2	25
egg layer	0	2	0	0	0	0	2

Table 2. Frequencies of behavioral interactions (sections 1–3) and other types of behavior (section 4) observed in a colony of three bees during 296 minutes over 15 days. The letters represent different individual bees

1	Nudged *W*	 *U*	 *O*	Total nudged	2	Backer *W*	 *U*	 *O*	Total follower
Nudged					Follower				
W		4	28	32	*W*		2	2	4
U	27		17	44	*U*	15		3	18
O	34	39		73	*O*	44	14		58
Total nudger	61	43	45	149	Total backer	59	16	5	80
3	Passer *W*	 *U*	 *O*	Total passed	4	*W*	*U*	*O*	Total
Passed									
W		4.5	14.0	18.5	guard (minutes)	19	71	25	115
U	4.5		11.0	15.5					
0	12.0	25.0		37.0	pollen collector	0	4	3	7
Total passer	16.5	29.5	25.0	71.0	worker on burrow	6	6	6	18
					worker on cell	8	5	1	14
					egg layer	3	2	0	5

in Fig. 1 the bee most active in nudging (rank 3) in each colony of 3 bees contributed 41.0 to 89.3% of the total nudging in her colony, with a mean for 16 colonies of 63.0%, shown as the relative proportions 1.23 to 2.68 and 1.89 respectively. A limitation in the data for colonies of two bees must be borne in mind, viz., trends for each half of an interaction are identical, a situation not necessarily true for colonies with three or more members.

Nearly horizontal lines connecting means indicate little difference between the most active and least active individuals for a given behavioral category. Sloping straight lines show some difference between the extremes but with a uniform continuum of intermediates, indicating an absence of distinctive caste differentiation. The slope of the line indicates the degree of difference between most and least active individuals and in plots using absolute proportions also reflects the number of individuals per colony. A concave curve indicates greater distinctness of certain individuals (usually those in the highest rank) as compared with the others in the continuum. (Specialization of bees in the highest rank for a particular activity was ascertained by testing the frequency of colonies showing a concave curve over the last three ranks against an expected frequency of equal numbers of concave and non-concave curves, for all colonies of three or more bees, using a chi-square test.) In addition, types of behavior for which one or a few members of a colony are strongly specialized would be expected to occur in similar absolute proportions, for those specialized bees, regardless of colony size. In other words, varying numbers of other bees in the colony are unlikely to greatly influence behavior of such individuals. Lower levels of specialization, however, may be more influenced by colony size, resulting in similar relative proportions.

The following paragraphs are numbered to correspond to the behavioral categories described above.

1a. Nudging. As shown in Fig. 1, there is some overlap between the proportions of the total nudging by bees in the highest and second highest ranks. Nonetheless, the means for the highest rank are markedly higher than for other ranks and for colonies of three ($n = 16$) and four ($n = 5$) bees, the curve based on means is markedly concave. The total frequency of colonies showing concave curves over the three highest ranks was significantly greater than that with non-concave curves ($p < 0.05$; $n = 25$). There appears, at least in the smaller colonies, to be a specialized class of bees, one bee per colony, that nudge more than the other bees in the same colonies.

1b. Being Nudged. As shown in Fig. 2, variation among colony members in relative proportions of being nudged was considerable, especially in the smallest colonies; however, there is no distinctively specialized class of nudged bees, the lines connecting means being nearly straight. There was no significant deviation from equality in the frequencies of concave and non-concave curves for individual colonies ($p > 0.05$; $n = 25$).

2a. Backing. Fig. 3 shows that there is a marked gap for every colony between the absolute proportion of backing shown by bees in rank 1 and that shown by other bees. Thus there is a markedly specialized class of bees, one bee per colony, that back more frequently than the others. The frequency of colonies with concave curves over the three highest ranks was highly significantly greater than that with non-concave curves ($p \ll 0.01$; $n = 25$).

2b. Following. The same comments as for being nudged (1 b) apply here.

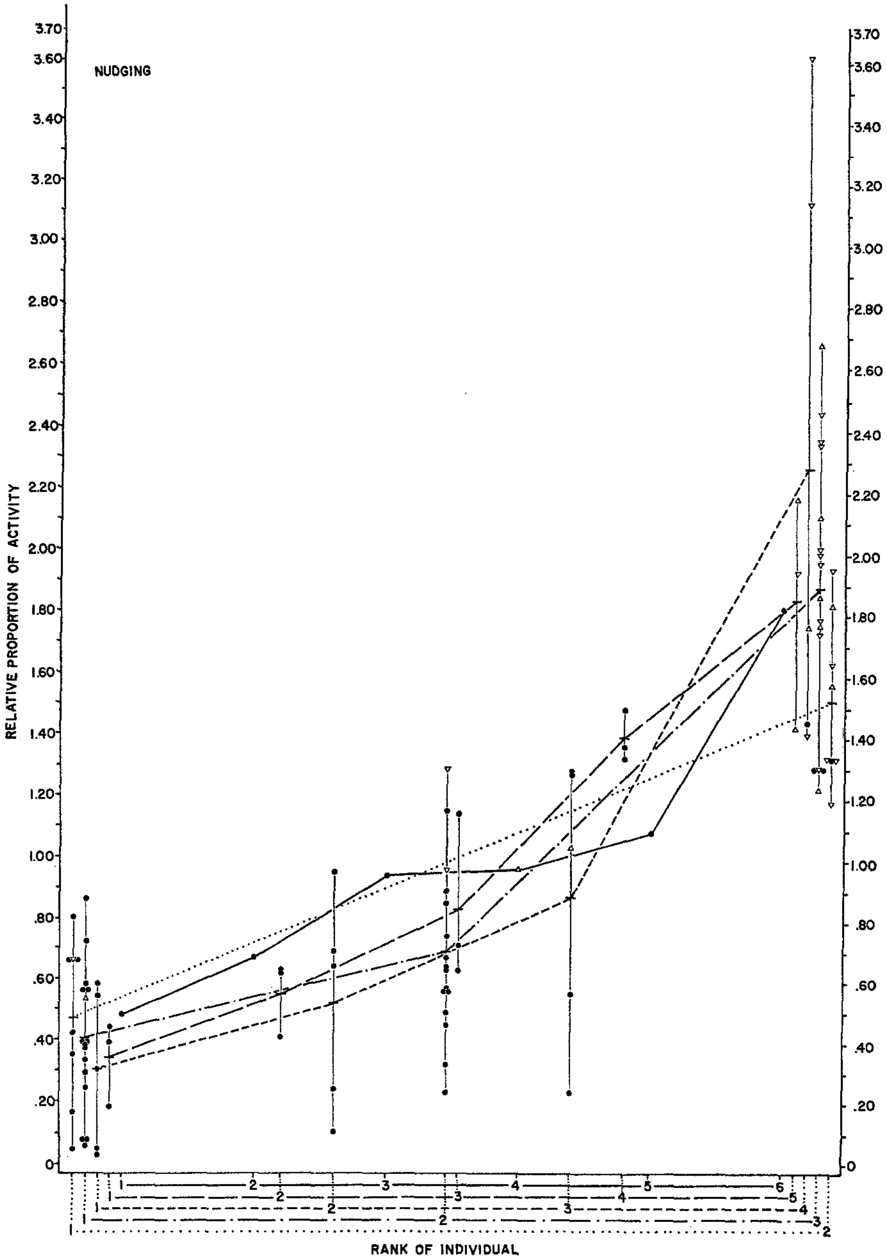
NUDGING
RELATIVE PROPORTION OF ACTIVITY
0
.20
.40
.60
.80
1.00
1.20
1.40
1.60
1.80
2.00
2.20
2.40
2.60
2.80
3.00
3.20
3.40
3.60
3.70
1 2 3 4 5 6
1 2 3 4 5
1 2 3 4
1 2 3
1 2
RANK OF INDIVIDUAL

Fig. 1

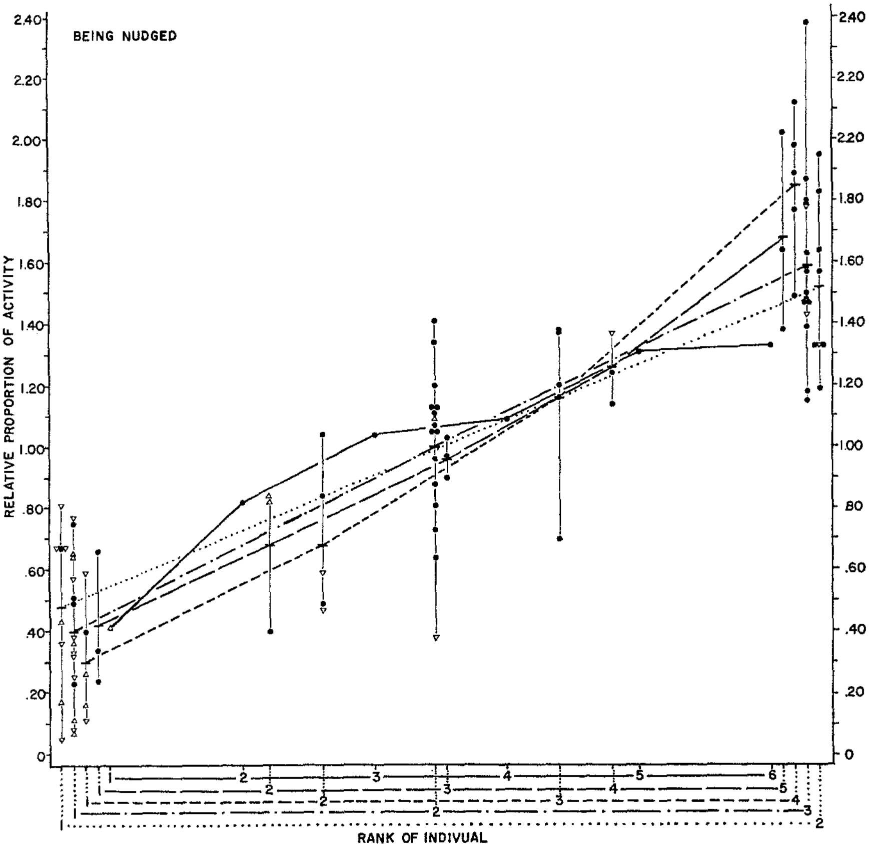

Fig. 2. Trends in being nudged (1b) for colonies of different sizes. (For explanation see Fig. 1)

Fig. 1. Trends in nudging (category 1a) for colonies of different sizes. Ranks shown on the abscissa are segregated according to colony size and linked by distinctive lines. Equivalent lines connect means (short horizontal bars) for each colony size. Rank 1 = minimal activity; ranks 2, 3, 4, 5, and 6 = maximal activity, depending on colony size. △ queen recognized by dissection; ▽ queen recognized from behavior only [maximal backer (2a); when backing data insufficient (in 3 colonies of 2 bees), then minimal guard (4)]; ● worker. (If a queen and one or more workers showed identical activity, all are plotted as queens)

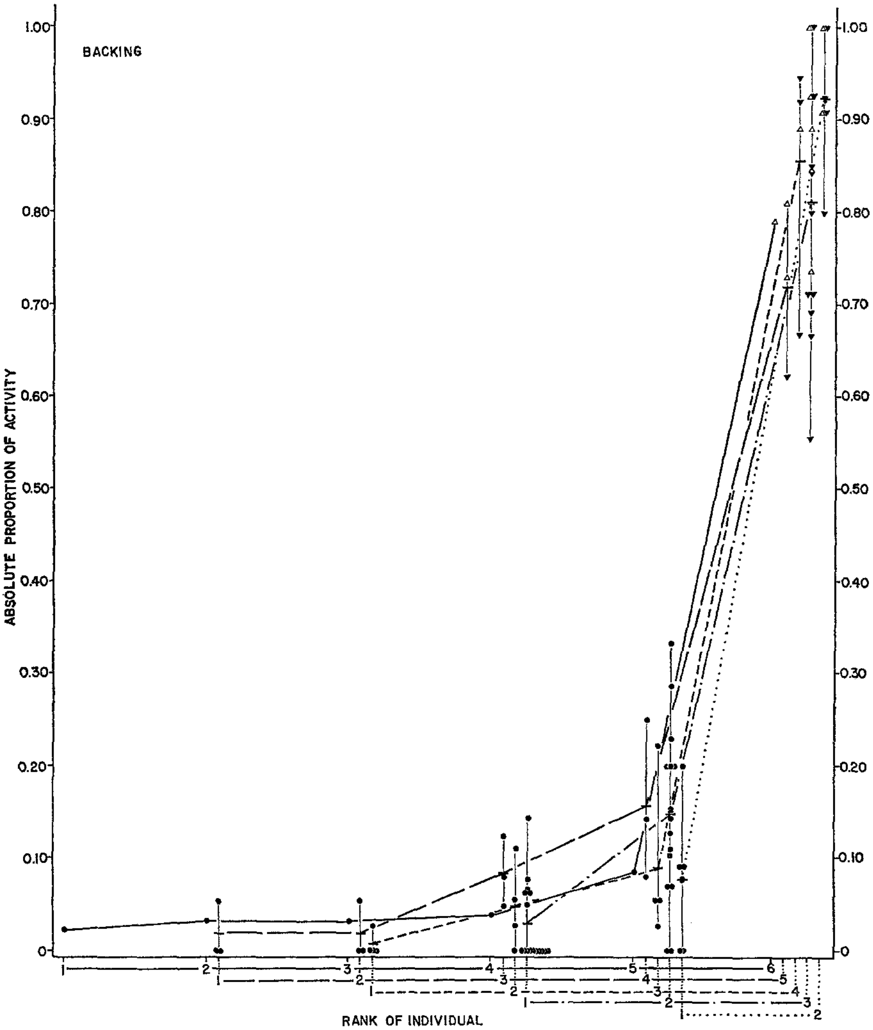

Fig. 3. Trends in backing (2a) for colonies of different sizes. (For explanation see Fig. 1.) ▼ Queen recognized by analogy with dissected queens (△△)

3a, 3b. Passing and Being Passed. For both of these behavioral categories, the comments for being nudged (1b) apply, except that the lines slope less steeply (Fig. 4).

4. Guarding. The lines connecting means are slightly concave (Fig. 5), possibly indicating a somewhat specialized class of individuals that guard for disproportionately long periods of time. However, the fre-

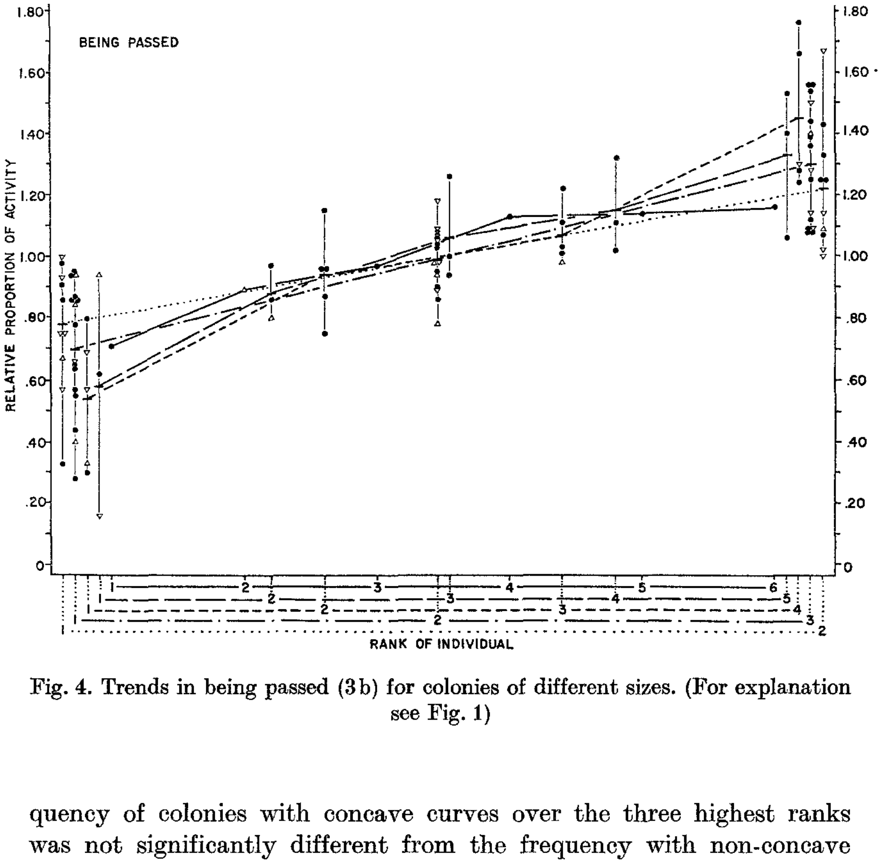

Fig. 4. Trends in being passed (3b) for colonies of different sizes. (For explanation see Fig. 1)

quency of colonies with concave curves over the three highest ranks was not significantly different from the frequency with non-concave curves ($p > 0.05$; $n = 25$).

5. *Pollen Collecting.* Seemingly there are two bees in each colony specialized as pollen collectors, one making on the average three times as many trips for pollen as the other (Fig. 6). All other bees do no pollen collecting or almost none. The lines connecting means are thus strongly concave, except for nests with two or three individuals, since the point at which the major departure from rectilinearity occurs is at the third highest rank. The total frequency of concave curves over the three highest ranks was not significantly different from that of non-concave curves ($p = 0.23$; $n = 9$) and low sample size prevented any test of this type over the fourth highest to second highest ranks.

6, 7. *Working on Burrows and on Cells.* For both of these behavioral categories, the lines connecting the means are not or scarcely concave, but the mean activity of individuals in the highest rank is several times that of those in rank 1, much as in Fig. 2. The total frequency of concave

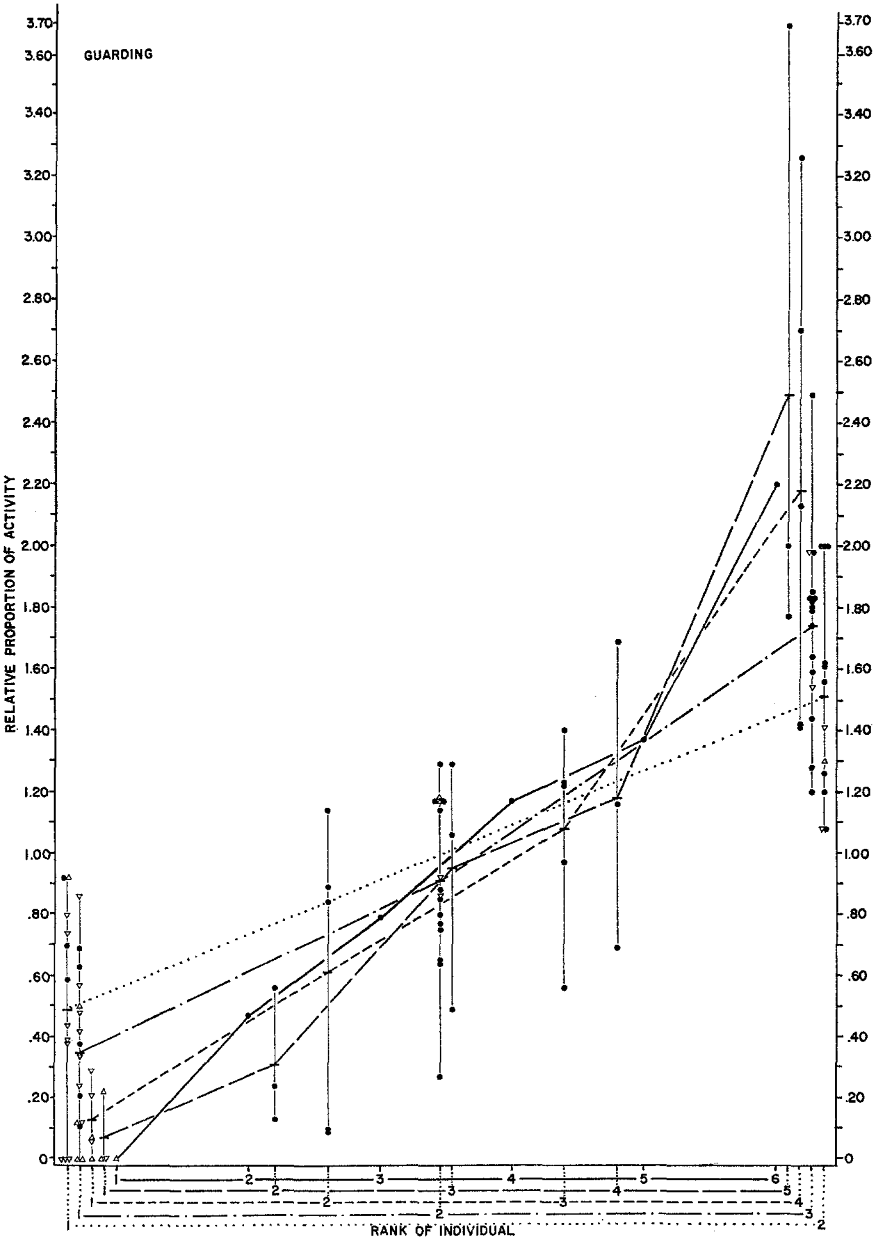

Fig. 5. Trends in guarding (4) for colonies of different sizes. (For explanation see Fig. 1)

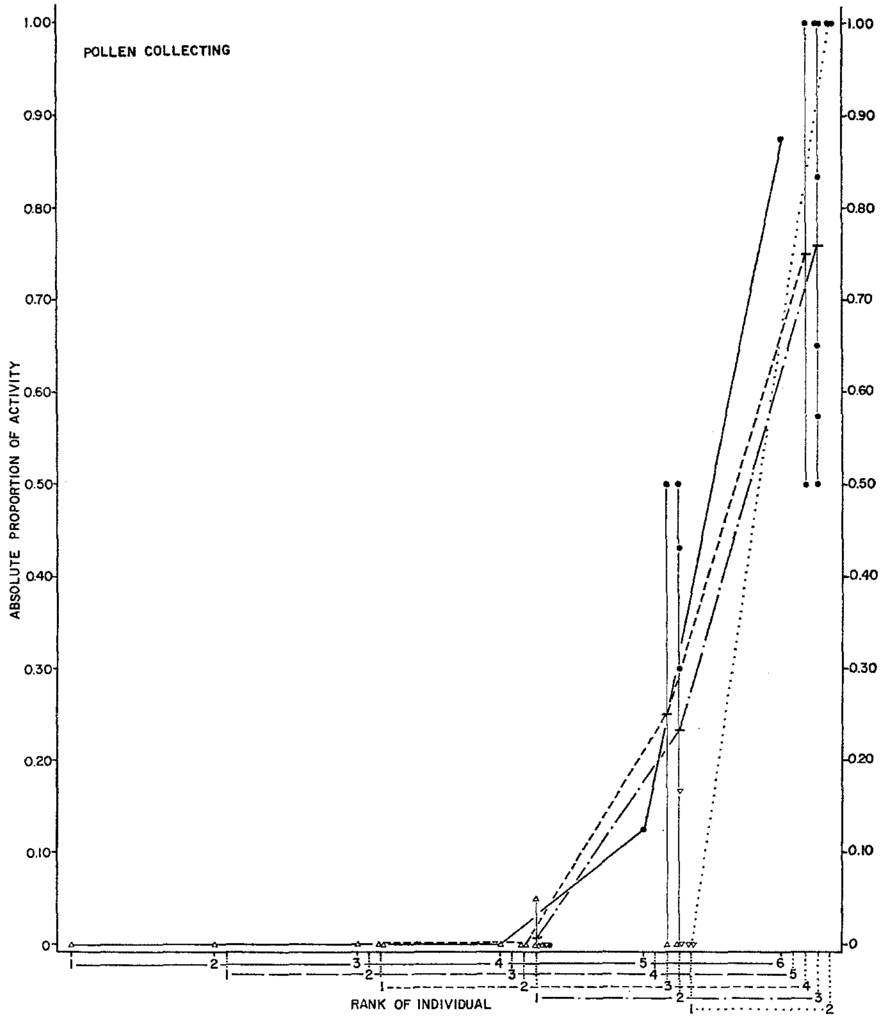

Fig. 6. Trends in pollen collecting (5) for colonies of different sizes. (For explanation see Fig. 1)

curves over the three highest ranks was not significantly different from that of non-concave curves ($p \gg 0.05$; $n = 23$ and 17 respectively).

In summary, caste distinctions are most strongly discernible in the categories of backing (2a) and pollen collecting (5) where the individuals showing maximal activity are equally differentiated from their nest mates independent of colony size, and to a lesser extent in nudging (1a) and probably guarding (4) where the degree of specialization is somewhat influenced by the number of individuals in the colony.

E. Relationships between Ovarian Development and Behavioral Categories

To investigate relationships between ovarian development and the behavioral types, we plotted each bee with respect to its ranks for ovarian state and for the behavioral category concerned. The sequence of ranks was from rank 1 (the least ovarian development or the least activity) to rank 2, 3, 4, 5, or 6 (greatest ovarian development or greatest activity, depending on colony size). In each colony the highest ranking individual ovarially is the queen. (See Section A for ovarian ranking procedure.) Ranks were used instead of frequencies or proportions for each activity because (1) our major interest was in the hierarchies among colony members—hierarchies could be obscured if all data were considered together because of variations in total activity between colonies independent of colony size—and (2) total activity and proportions of total activity vary greatly depending on the number of individuals in each colony.

Because of the use of ranks and also because the data were probably not normally distributed, conventional statistical tests for the significance of correlations were inapplicable (e.g., rank correlation tests such as Spearman's are restricted to paired series). Furthermore, complex relationships, involving both negative and positive correlations over different ranges of ranks, which probably have some biological significance, were often discerned; these could not be tested for by methods which consider simple correlations only. For these reasons, symbols in Table 3 describing particular relationships are predominantly based on impressions obtained from examination of the plots, and may or may not involve statistical significance.

An additional complication throughout is introduced by the use of colonies of different sizes. This leads to consideration of which ranks are equivalent in the various size classes. Since the highest ranking bee ovarially is the queen in each case, there is reason to regard the highest ranks as equivalent for ovarian development. For the behavioral categories there are no such *a priori* reasons for deciding on ranking equivalences. Thus, is it the highest ranking bees or the lowest for a particular behavioral attribute which are equivalent in terms of the other variables? Or, if the highest and lowest ranking bees are equivalent across all colony sizes, are the intermediate bees equivalent in an even distribution or not? We have attempted to solve these problems by equating ranks in such a way as to minimize the variation that could be attributed to colony size, thus maximizing clustering in the scatter diagrams. (Tied ranks were plotted midway between the ranks concerned, independent of the spacing involved.) If similar processes are operating in colonies of different sizes, qualitatively equivalent ranks should be associated by this method. (In the scatter diagrams some of the ranks have been lumped for the two largest colonies.) The results are presented in Figs. 7–12 and Table 3.

Table 3. Relationships of ovarian development to behavioral categories for colonies of two to six bees. Each symbol represents the type of correlation (+ = positive, − = negative, 0 = no correlation) as determined from scatter diagrams for each interval from left to right along the behavioral axis

Nudging[a]	Being nudged[b]	Backing[a]	Following[b]	Passing[c]	Being passed[a]	Guarding[b]	Pollen collecting[c]	Working on burrows[c]	Working on cells[c]
1a	1b	2a	2b	3a	3b	4	5	6	7
+++ (Fig. 7)	−++ (Fig. 8)	+++ (Fig. 9)	−++	−−− (Fig. 10)	000	−+0 (Fig. 11)	−−−	000	0+− (Fig. 12)

[a] Grouping of ranks as in Fig. 7.
[b] Grouping of ranks as in Fig. 8.
[c] Grouping of ranks as in Fig. 10.

F. Behavioral Differences among Colony Members

1. Behavioral Characteristics of Colony Members (Univariate). Using colonies of three to six bees, the observed distributions of the maximal backers (queens)[1], bees showing maximal guarding (guards) and those doing maximal pollen collecting (foragers) among the ranks for each behavioral category were compared with those theoretically expected had queens, guards, and foragers been uniformly distributed among the ranks, using chi-square tests. For each test the distribution was divided into three groups, bees showing maximal behavior (ranks 3, 4, 5, or 6 depending on colony size; Table 4, "max"), those with minimal behavior (rank 1; Table 4, "min") and intermediates (ranks 2, 2+3, 2+3+4 or 2+3+4+5, depending on colony size; Table 4, "int"). For each test involving foragers the distribution was also divided into two equal groups, bees with high activity (Table 4, "high") and those

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[1] Since maximal ovarian size, defining the queen, is invariably associated with maximal backing, all bees that were maximal backers were considered queens even if dissections were not made.

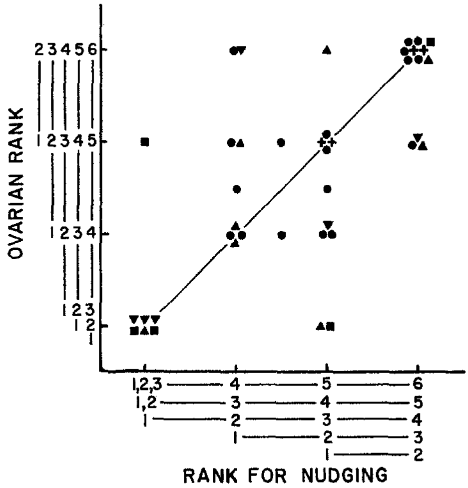

Fig. 7. Relationship between ovarian development and nudging (category 1a) for colonies of different sizes. All scatter diagrams (Figs. 7–16) show the ranks of each individual for each category. Ranks on each axis are segregated according to colony size. (For explanation of grouping, see text.) Rank 1 = minimal activity; ranks 2, 3, 4, 5, and 6 = maximal activity or ovarian development, depending on colony size as shown by the following symbols: + member of a colony of two bees; ● member of a colony of three bees; ▲ member of a colony of four bees; ■ member of a colony of five bees; ▼ member of a colony of six bees. Relationships are emphasized by lines connecting the major clusters

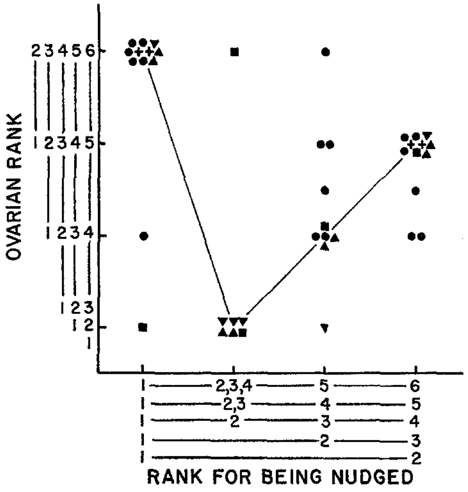

Fig. 8. Relationship between ovarian development and being nudged (1b) for colonies of different sizes. (For explanation see Fig. 7)

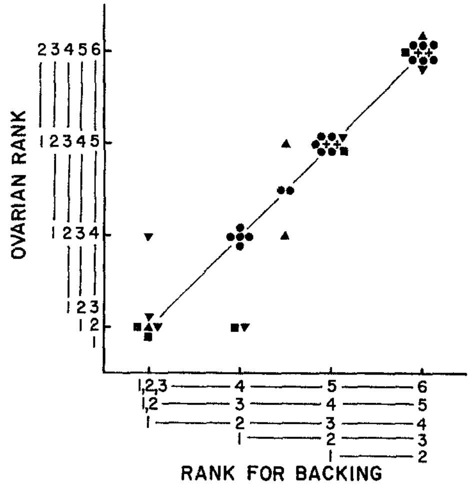

Fig. 9. Relationship between ovarian development and backing (2a) for colonies of different sizes. (For explanation see Fig. 7)

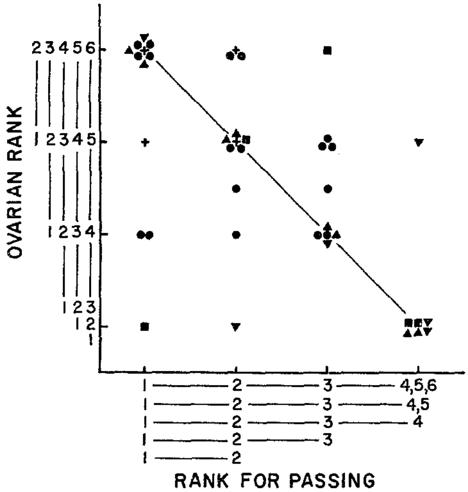

Fig. 10. Relationship between ovarian development and passing (3a) for colonies of different sizes. (For explanation see Fig. 7)

with low activity (Table 4, "low"), in an attempt to minimize the effects of low sample size. In Table 4, "var" (variable) signifies no significant deviation from a uniform distribution among the levels.

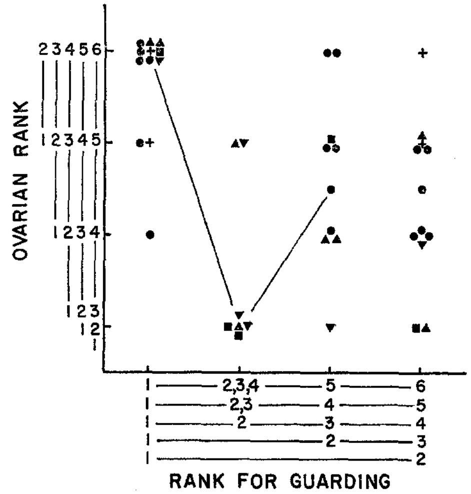

Fig. 11. Relationship between ovarian development and guarding (4) for colonies of different sizes. (For explanation see Fig. 7)

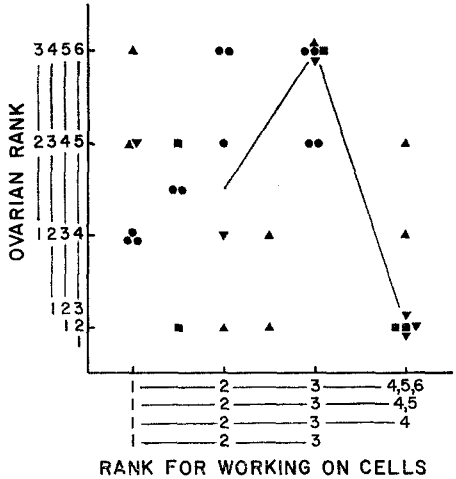

Fig. 12. Relationship between ovarian development and working on cells (7) for colonies of different sizes. (For explanation see Fig. 7)

2. Relationships between Behavioral Categories (Bivariate). To investigate relationships between the behavioral categories, we plotted each bee with respect to its rank for the two categories being compared, as in Section E. Colonies of only two bees were excluded because the

Table 4. Activity levels of Queens, Guards, and Foragers for ten behavioral categories. (* $0.05 > p > 0.01$; ** $p < 0.01$). Sample sizes (numbers of colonies) are in parentheses. For explanation, see text

	Queen	Guard	Forager
Nudging (1a)	max** (25)	var (25)	low[c] (9)
Being nudged (1b)	min** (25)	int[c] (25)	var (9)
Backing (2a)	max[a]	min* (24)	var (8)
Following (2b)	min** (24)	var (24)	var (8)
Passing (3a)	min** (25)	var (25)	high* (9)
Being passed (3b)	var (25)	var (25)	var (9)
Guarding (4)	min** (25)	max[a]	int[c] (9)
Pollen collecting (5)	min*[b] (9)	var (9)	max[a]
Working on burrows (6)	max[c] (23)	var (23)	var (9)
Working on cells (7)	var (17)	min* (17)	var (9)

[a] Position by definition.

[b] Tested by grouping the two highest ranks *vs.* the remainder.

[c] $0.1 > p > 0.05$.

unavoidable perfect negative correlation between the two components of each interaction (e.g., nudging and being nudged) would have biased the relationships seen. For colonies of three individuals a contingency table was prepared for each relationship investigated and the observed frequencies were tested against those expected on the basis of random association between the activities concerned (i.e., equal frequencies in each cell), using a chi-square test (3×3 table, $df = 8$). The small number of colonies with more than three members prevented adequate testing of these. Even using nests with three bees only, sample sizes varied widely, being 48, 42, 27, or 18. In at least the last two instances the number is probably too small to allow for adequate partitioning in a test involving eight degrees of freedom. The results from these tests have nevertheless been included as a rough guide. In many cases associative trends were seen best in colonies with more than three individuals and were not shown to be statistically significant when only nests with three bees were considered.

Passing and being passed are behavioral categories excluded from this analysis because they appear to be incidental and related only to other activities—they have no special importance in themselves—and because they appear possibly composite. They also show minimal differentiation among bees, as stated in Section D.

Table 5. Relationships between behavioral categories for colonies of three to six bees. Column headings are the same as for rows, as shown by corresponding numbers. Symbols in sets of three (lower left) represent the relationships (+ = positive, — = negative, 0 = no correlation) as determined from scatter diagrams for each interval from left to right along the abscissa. Each scatter diagram was made with the lower numbered behavioral category on the ordinate, the higher numbered one on the abscissa. When the relations for each interval were not obvious, the general relationship is shown in parentheses. Paired superscripts ($^{w, x, y, z}$) refer to the grouping of ranks in scatter diagrams; the first applies to the ordinate, the second to the abscissa. Symbols in upper right refer to the significance of deviation from a random association, considering only colonies of three bees (** = $p < 0.01$; * = $0.05 > p > 0.01$; n.s. = $p > 0.05$). Sample sizes are shown in parentheses

	1a	1b	2a	2b	4	5	6	7
1a. Nudging		** (48)	** (48)	* (48)	n.s. (48)	n.s.[a] (18)	n.s. (42)	n.s. (27)
1b. Being nudged	$-++^{yw}$ (Fig. 13)		** (48)	* (48)	n.s.[a] (48)	n.s.[a] (18)	n.s. (42)	n.s. (27)
2a. Backing	$+++^{zz}$ (Fig. 14)	$-++^{yz}$		* (48)	n.s.[b] (48)	n.s. (18)	n.s.[a] (42)	n.s.[a] (27)
2b. Following	$-+0^{xy}$	$+++^{zz}$	$-0+^{wz}$		n.s. (48)	n.s. (18)	n.s. (42)	n.s. (27)
4. Guarding	$-++^{yw}$	$++-^{xx}$ (Fig. 16)	$---^{xz}$	$+--^{zy}$		n.s. (18)	n.s.[a] (42)	n.s.[a] (27)
5. Pollen collecting	$(-)^{xz}$ (Fig. 15)	$(0)^{zz}$	$(-)^{xz}$	$(+)^{zz}$	$(0)^{yy}$		n.s. (18)	n.s. (18)
6. Working on burrows	000^{xx}	000^{zz}	$(+)^{zz}$	$00+^{zz}$	$(-)^{zx}$	$(0)^{yy}$		n.s.[a] (27)
7. Working on cells	000^{xz}	000^{xz}	000^{xx}	000^{xx}	$(-)^{zx}$	$(0)^{xx}$	$(+)^{xx}$	

[a] $0.5 > p > 0.1$. [b] $0.5 > p > 0.1$ for 3-bee colonies; $p < 0.05$ for all colonies, $n = 85$.

[w] Grouping of ranks as on abscissa in Fig. 13.

[x] Grouping of ranks as on ordinate in Fig. 15.

[y] Grouping of ranks as on ordinate in Fig. 13.

[z] Grouping of ranks as on abscissa in Fig. 15.

The results are shown in Figs. 13–16 and Table 5.

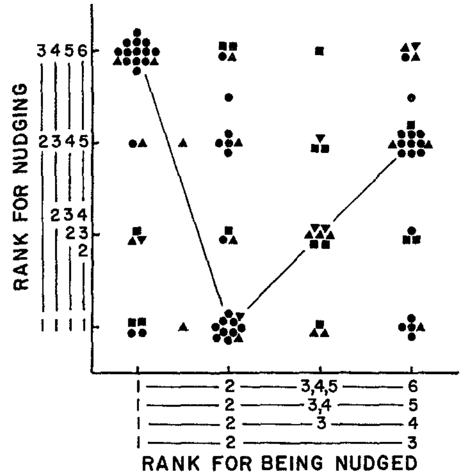

Fig. 13. Relationship between nudging (1a) and being nudged (1b) for colonies of different sizes. (For explanation see Fig. 7)

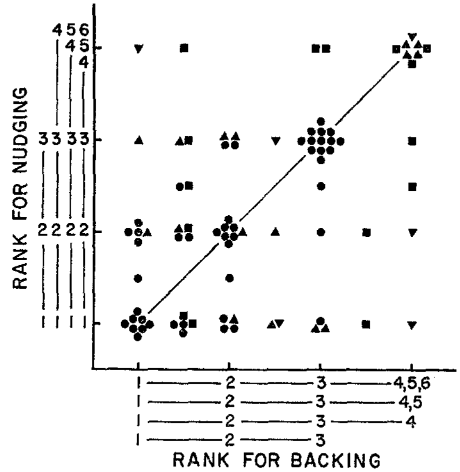

Fig. 14. Relationship between nudging (1a) and backing (2a) for colonies of different sizes. (For explanation see Fig. 7)

3. Conclusions Based on Univariate and Bivariate Studies. Table 6 shows typical ranks positions for the various members of colonies of three, four, five and six bees as deduced from the univariate and bivariate studies. Since the rank order for each behavioral category was

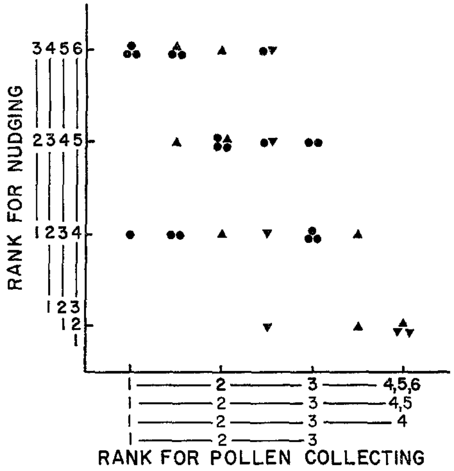

Fig. 15. Relationship between nudging (1a) and pollen collecting (5) for colonies of different sizes. (For explanation see Fig. 7)

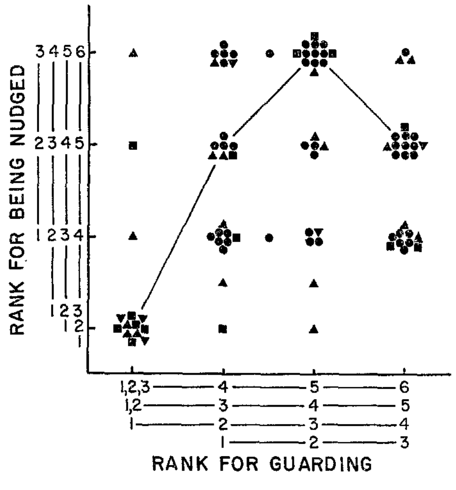

Fig. 16. Relationship between being nudged (1b) and guarding (4) for colonies of different sizes. (For explanation see Fig. 7)

determined independently by reference to the positions of queens, guards and foragers in that category (univariate), and since there is considerable variation in some of the data causing uncertainty as to the interrelationships of some behavioral categories (bivariate), there are some instances in which the relationships between categories derivable from

Table 6. Typical rank positions of particular females with respect to behavioral categories and ovarian development (see text for explanation)[a]

	Queen				Guard				Forager				Other worker(s)		
Bees per colony	3	4	5	6	3	4	5	6	3	4	5	6	4	5	6
Nudging (1a)	**3**	**4**	**5**	**6**	2	3	4	5	**1**	**1**	**1**	**1**	2	2, 3	2, 3, 4
Being nudged (1b)	1	1	1	1	2	3	4	5	3	4	5	6	2	2, 3	2, 3, 4
Backing (2a)	**3**	**4**	**5**	**6**	**1**	**1**	**1**	**1**	2	2	2	2	3	3, 4	3, 4, 5
Following (2b)	**1**	**1**	**1**	**1**	2	2	2	2	3	4	5	6	3	3, 4	3, 4, 5
Passing (3a)	**1**	**1**	**1**	**1**	2	2	2	2	**3**	**4**	**5**	**6**	3	3, 4	3, 4, 5
Being passed (3b)	2	2	2	2	3	4	5	6	1	1	1	1	3	3, 4	3, 4, 5
Guarding (4)	**1**	**1**	**1**	**1**	**3**	**4**	**5**	**6**	2	2	2	2	3	3, 4	3, 4, 5
Pollen collecting (5)	**1**	**1**	**1**	**1**	2	2	2	2	**3**	**4**	**5**	**6**	3	3, 4	3, 4, 5
Working on burrows (6)	**3**	**4**	**5**	**6**	1	1	1	1	2	3	4	5	2	2, 3	2, 3, 4
Working on cells (7)	2	3	4	5	**1**	**1**	**1**	**1**	3	4	5	6	2	2, 3	2, 3, 4
Ovarian development	**3**	**4**	**5**	**6**	2	3	4	5	**1**	**1**	**1**	**1**	2	2, 3	2, 3, 4

[a] See Table 4 for indications of significance levels. Ranks shown in bold type are those assigned with greatest confidence.

Table 6 do not agree with those presented in Tables 4 and 5. Such internal inconsistencies are not unexpected in data such as are considered here.

From Table 6 the general characteristics of colony members can be easily derived. Thus, the queen is generally the most active bee, as shown by its high ranking in those categories involving directed activities or the initiation of such activities. The queen's high incidence of nudging and backing (accompanied by following in response) promotes increased activity of other bees by drawing quiescent individuals down from the vicinity of the burrow entrance to the region where most of the work such as burrowing, cell construction and cell provisioning occurs. The low incidence of passing by the queen results from her tendency to back after encountering another bee rather than to pass it. The queen also exhibits a high level of activity by ranking high in work on burrows and cells.

By contrast the maximal guard is apparently the least active bee as shown by its low rankings in those categories on which the queen rates highly. The relatively high incidence of nudging exhibited by the guard results from its persistent stimulation of other bees which may have taken over as guards for short periods of time, when the guard returns to guarding. The high rank of the guard in being passed results from its being the individual most frequently at the burrow entrance, and thus the one which must be passed by bees leaving the burrow. There is also often a bee below the guard which may pass the guard, remain at the entrance for a short period and then return down the burrow.

The maximal forager exhibits moderate activity, mainly as a result of stimulation by the queen (as shown by the forager's high ranking in being nudged and following), resulting in the forager being "drawn" down to regions where work is required.

Any other bees present (in colonies of 4, 5, or 6 bees) occupy intermediate positions for each behavioral category. Such bees should thus probably be regarded as opportunists which contribute to the general activity of the colony but do not preferentially fulfill any particular function. These may be individuals which are available to take over the functions of the more specialized individuals should any of these be lost through some accident. There is evidence (section G) that it may be one of these "other" bees which becomes a replacement queen if the original queen is lost.

4. Multivariate Studies. Although the analyses described above indicated that colony members were specialized as queens, guards, foragers, and other workers, they provided no means of ascertaining the relative distinctness of each type of individual, nor did they provide any assurance that such distinctions were valid given the meagerness of some of the data and the difficulties in testing for relationships between behavioral categories.

Two multivariate techniques were used to obtain estimates of the legitimacy of recognizing specialized types of bees and the distinctness of such groups, a principal components analysis for the former and stepwise discriminant analyses for the latter. The absolute proportion of each activity contributed by each bee in its colony, rather than its rank, was used since the data were expected to deviate less from the assumptions involved in the techniques concerned than would the ranked data. The data sets used were somewhat limited because of the necessity for completeness for all behavioral categories in each colony. Thus data for pollen collecting could not be included, but all nine other categories were considered.

The principal components analysis of nine colonies of three bees (Fig. 17) showed that using the first and third principal components as the axes, those bees designated as queens formed a reasonably distinct cluster separated from the workers. Within the elongate worker cluster, those bees designated as guards all fell at one end. (The first three principal component axes respectively accounted for 42%, 16%, and 14% of the total variance.) We appear to be justified in distinguishing queens as a distinct group; moreover, workers could be logically divided into guards and a group that is designated, for convenience, as foragers.

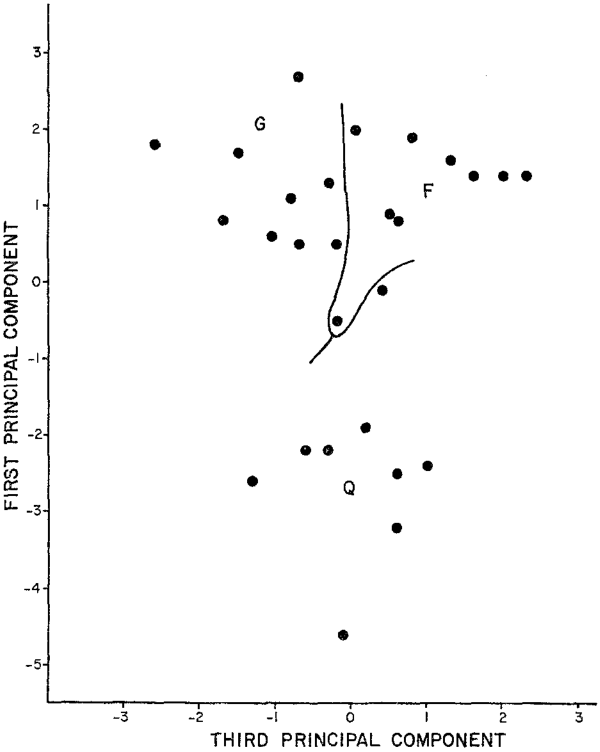

Fig. 17. Occupants of colonies of three bees plotted on the first and third principal component axes for nine behavioral variables. *G* guards, *F* foragers, *Q* queens

(The data support the impression that maximal guards are usually not maximal pollen collectors.)

On the basis of the principal components analysis, the occupants of each of the three-bee colonies were partitioned into three groups, queens, guards, and foragers. A stepwise discriminant analysis was done using these groups and the individuals were plotted on the first two canonical axes which accounted for 69% and 31% of the total dispersion, respectively (Fig. 18). The groups are completely separated with no overlap. It is evident that the first canonical variate reflects differences between queens and workers and the second variate shows discrimination among workers between guards and foragers. When this analysis was repeated using the data in the form of ranks, the results were essentially identical, with equivalent discrimination of the three groups. This confirmation both of the usefulness of ranked data and of the robustness of this multivariate technique is reassuring.

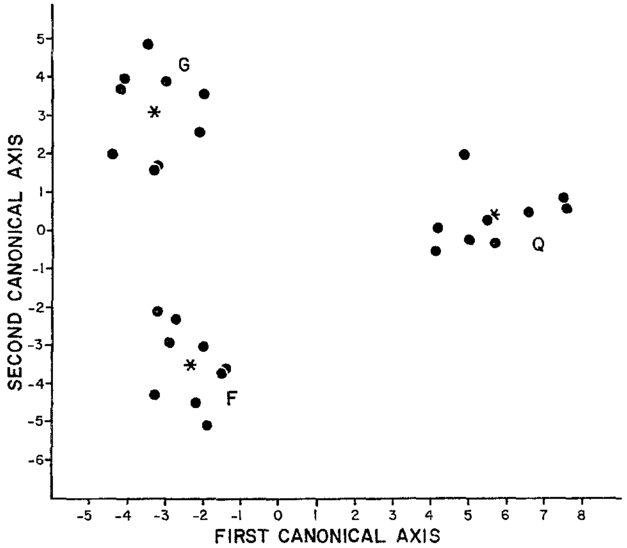

Fig. 18. Occupants of colonies of three bees allocated to three groups (queens, guards, foragers) and plotted on the first and second canonical axes for nine behavioral variables. * group mean

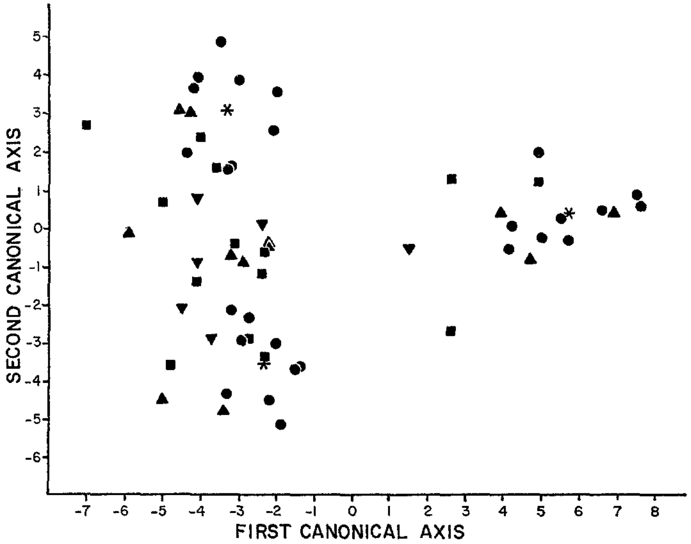

Fig. 19. Occupants of colonies of four (▲), five (■) and six (▼) bees plotted on the canonical axes derived from colonies of three (●) bees (see Fig. 18). * group mean for colonies of three bees

Using the three groups derived from the colonies of three bees as a basis, similar data on the 33 occupants of seven colonies of four to six bees were added to the discriminant analysis, each individual being plotted on the same axes as were those from the three-bee colonies (Fig. 19). In this case one bee from each colony fell clearly closest to the "queen" group and could be so classified. The remaining bees were fairly evenly distributed between the "guard" and "forager" groups and formed a continuum between these groups. Of these workers, 9 fell closer to the mean of the "guard" group and 17 closer to the mean of the "forager" group. In each colony one of the workers could be designated a guard and another a forager by reference to the plot; i.e., in no case did all the workers in a colony fall in the forager group or the guard group.

On this basis the occupants of colonies containing four to six bees were partitioned into four groups, as follows, for each colony: (1) the queen, (2) the bee falling at the guard end of the worker group, (3) the bee at the forager end, and (4) any other bees taken together. A stepwise discriminant analysis was run using these four groups and the results were plotted using the first vs. second canonical axes (Fig. 20) and the second vs. third axes (Fig. 21). These axes account for 86.9%, 10.6% and 2.4% of the dispersion respectively. The former plot shows essentially the same thing as did the comparison to colonies of three bees and confirms that the occupants may be designated as queens, and workers comprising guards, foragers, and others intermediate between guards and foragers. On the other hand, the third canonical axis seems to relate to worker attributes and discriminates most efficiently between the specialized workers (guards and foragers) and the generalized bees in the "other" group; the queens fall intermediate along this axis. This third axis thus seems best interpreted as reflecting degree of worker specialization, with the highly specialized guards and foragers at one extreme and the opportunists at the other.

These results of the multivariate analyses essentially substantiate the concepts underlying Table 6, in that they indicate the existence of three distinct classes of bees specialized for certain sets of activities (queens, guards, and foragers) and a fourth class which, although tending to fall in the general category of workers, is not specialized for particular activities.

G. Queen Replacement

Overwintered queens of *Lasioglossum zephyrum* seldom survive as long as their colonies; replacement of queens is therefore a normal part of the life cycle. Early in the investigation we learned that queens could be recognized behaviorally (invariably maximal backers, usually maximal

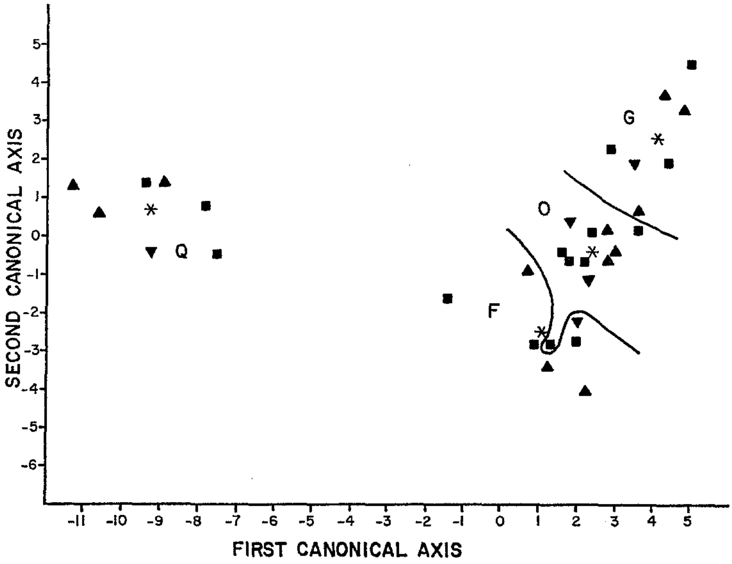

Fig. 20. Occupants of colonies of four (▲), five (■) and six (▼) bees allocated to four groups (queens, guards, foragers, others; see text for explanation), plotted on the first and second canonical axes for nine behavioral variables. * group mean

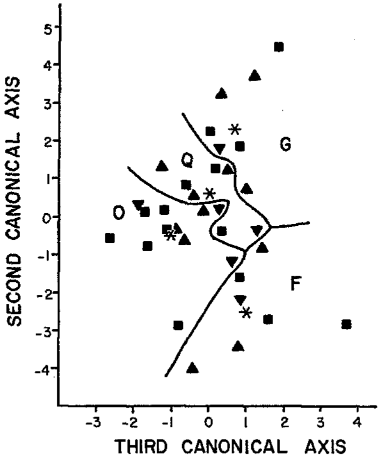

Fig. 21. Occupants of colonies of four (▲), five (■) and six (▼) bees allocated to four groups (queens, guards, foragers, others; see text for explanation), plotted on the second and third canonical axes for nine behavioral variables. * group mean

nudgers, etc.). As a result, the effect on the colony of removal of the queen could be studied. On 12 occasions the queen was removed from a colony and the interactions among the remaining bees were observed. The results are summarized in Table 7.

In 11 of the 12 cases one of the remaining bees showed queenlike behavioral characteristics shortly after the original queen had been removed. Such replacement occurred with adults up to at least 30 days old. Although differentiation was clearcut in eight instances, identification of the replacement queen was tentative in three, mainly because of the reduced activity of the bees involved so that there were few interactions. Because of the different times between queen removal and the first intensive subsequent observations, it is impossible to state the exact time required before a new queen becomes recognizable. In all cases it was almost certainly less than a day, and the one case showing less than four hours could have been typical. In any event it is apparent that removal of a queen usually causes another bee to take over as queen within a very short time. In the one case [colony 230W (2)] in which neither of the two remaining bees became a queen, their ovaries remained small and undeveloped, unlike those of replacement queens.

Frequently the bees which became replacement queens had previously exhibited considerable behavior characteristically not shown by queens, such as guarding and pollen collecting. In 9 out of the 11 cases of successful queen replacement, the new queen was probably not the bee which had had the second largest ovaries, as judged by the positive correlation of backing and nudging with ovarian development (Section F), but was most likely the bee with the third largest ovaries. The replacement queen was also not usually the bee which was originally nudged the most and which probably thus experienced the greatest behavioral difference due to removal of the queen, but on the average was the one which was nudged second most (mean rank of replacement queens for being nudged, before manipulation $= 0.8$ rank below the highest rank; $n = 11$). The reason for this may have been that the bee with second largest ovaries (nudged most frequently on the average) was subject to such intense traumatization by the activities of the queen that she was unable to enlarge her ovaries as quickly after removal of the queen as was the less traumatized bee with third largest ovaries. (There is evidence that the most nudged bee is nudged proportionately more by the queen than by other nest mates considered together.)

From the trends toward division of labor (Section F, Table 6), it seems probable that the bee with third largest ovaries (at least in colonies originally with four or more members) was one of those which exhibited intermediate activity for all behavioral categories. Such a bee would probably more easily adopt the behavioral patterns of more

Table 7. Queen replacement in colonies from which the queen was removed

Number of bees in colony	Colony number	Identity of bees in rank order before removal of queen[a]	Identity of replacement queen	Maximum time to queen replacement	Basis for recognition of replacement queen
5 reduced to 4	245E(1)	2a: *Y*(0); *B*(0); *O*(1); *U*(2); *W*(5) 1a: *Y*(5); *B*(8); *U*(8); *O*(17); *W*(24)	*O*	22 hrs 43 min	Backing, when colony first observed.
4 reduced to 3	230W(1)	2a: *W*(0); *O*(0); *B*(0); *U*(7) 1a: *W*(0); *O*(0); *B*(1); *U*(10)	*O*	5 hrs 42 min	Backing, when colony first observed.
	231W(1)	2a: *O*(1); *W*(1); *B*(1); *U*(34) 1a: *O*(1); *W*(4); *B*(9); *U*(134)	*W*	25 hrs 15 min	Relative activity, when colony first observed; later confirmed by backing.
	245E(2)	2a: *U*(0); *B*(0); *Y*(1); *O*(17) 1a: *Y*(5); *B*(6); *U*(11); *O*(12)	*B*	29 hrs 20 min	Relative guarding and activity, when colony first observed (for 150 min).
	248E(1)	2a: *W*(0); *O*(2); *U*(4); *Y*(12) 1a: *U*(1); *W*(5); *O*(11); *Y*(62)	*O*	16 hrs 19 min	Backing, when interactions first observed.
3 reduced to 2	230W(2)	2a: *W*(0); *B*(2); *O*(11) 1a: *W*(1); *B*(6); *O*(32)	None	—	Little behavioral differentiation after 148.5 hrs; ovaries of both undeveloped
	231W(4)	2a: *B*(0); *O*(0); *W*(7) 1a: *B* (0); *O*(1); *W*(5)	*B*	3 hrs 46 min	Backing, when colony first observed, confirmed by dissection.
	234E(2)	2a: *Y*(0); *W*(6); *U*(34) 1a: *W*(16); *Y*(23); *U*(81)	*Y*(?)	?	Relative guarding, over 267 min (16 days).
	245E(3)	2a: *U*(0); *Y*(0); *B*(9) 1a: *U*(7); *Y*(23); *B*(23)	*U*	?	Relative activity, and foraging by *Y*; confirmed by dissection; *but Y* guards less.
	247E(2)	2a: *Y*(1); *W*(3); *O*(5) 1a: *Y* (9); *W*(10); *O*(71)	*W*(?)	?	Relative guarding over 64 min (1 day), both bees inactive.

Table 7 (continued)

Number of bees in colony	Colony number	Identity of bees in rank order before removal of queen[a]	Identity of replacement queen	Maximum time to queen replacement	Basis for recognition of replacement queen
3 reduced to 2	248E(2)	2a: *W*(0); *U*(2); *O*(4) 1a: *W*(0); *U*(6); *O*(9)	*U*	?	Foraging by *W*; confirmed later by relative activity; *W* lethargic and vanished within 33 hours.
	249E(2)	2a: *G*(0); *B*(2); *W*(4) 1a: *G*(1); *B*(9); *W*(37)	*G*(?)	?	Relative guarding over 147 min; *but G* left nest to feed.

[a] Numbers indicate the totals of backing (2a) and nudging (1a) for the bees (designated by letters) in each colony. In all cases the original queen (subsequently removed) falls in the highest ranks for both activities.

specialized bees than would one of the already specialized individuals which would have to make a more radical change in behavior. The lack of such generalized supernumerary individuals in colonies with only three members may have contributed to the relative difficulty in the recognition of replacement queens in such small colonies.

The two cases in which it was presumably the bee with second largest ovaries that became the replacement queen were both unusual lethargic and apparently unable to function successfully (perhaps as a result of damage during manipulation of the nest when removing the previous queen). In the other [247E (2)], identification of caste was based solely on relative time spent guarding and both bees were very inactive; this identification was possibly incorrect.

Although the mechanisms whereby a queen suppresses queenlike behavior in nest mates are not yet definitely known, they may merely involve relative activity, especially frequency of nudging. It may be significant that all cases in which there was some uncertainty about the identity of the replacement queen involved colonies reduced to two members. Possibly the removal of the previous queen, which was most likely the most active bee, resulted in a drop in general activity in such small colonies to a level lower than that required for efficient functioning in other respects also. In one [248E(2)] the only other bee was extremely. Whatever the mechanism, removal of the queen has effects within a very short time—reminiscent of the time required for responses to loss of the queen in the highly eusocial honeybees, *Apis mellifera* (Butler, 1954).

Although Michener, Brothers, and Kamm (1971 b) implied that the presence of an overwintered queen does not influence ovarian development of associated workers, the ovaries of summer bees involved in our replacement experiments appear to have been partially inhibited by the presence of their queen. When bees were dissected a few days after becoming replacement queens, their ovaries were on the average as large as those of the original queens. This fact indicates that ovarian enlargement took place after removal of the original queen. If this reflects a true difference between overwintered queens and queens reared during the same season as their associated workers, it may be that an overwintered bee causes less inhibition of ovarian development in her daughters than does a summer female in associates of about the same age.

H. Oophagy

Since colony members other than the queen often have ovaries containing fully developed oocytes, such bees sometimes lay eggs. Of 20 instances of egg laying observed, 11 eggs were laid by bees other than the queen.

On three occasions (in colonies of two, three, and five bees) oophagy, each time by the queen, was observed. The queen showed increased activity while another bee was completing the pollen ball and laying an egg; she nudged the other colony members more often and ran along the burrows more agitatedly than at other times. She repeatedly investigated the cell containing the ovipositing bee and attempted to push into the cell. While the egg-layer was plugging the cell the queen typically nudged her repeatedly. In each case, shortly (5 to 20 min) after the cell had been plugged and the egg-layer had moved away, the queen re-opened the cell, entered and ate the egg. The queen then reworked the pollen ball and laid her own egg, after which she plugged the cell. Bees other than the queen were never seen to perform oophagy.

The process has previously been described for *L. zephyrum* and *L. versatum* by Batra (1964, 1968), but the identities of the "cannibalistic" individuals were not known to her. Also, in one of the two cases she described for *L. zephyrum*, the egg that was eaten was two days old.

I. Discussion

Although there are obviously females showing diverse reproductive capacities in colonies of *Lasioglossum zephyrum*, the use of the word "queen" for the bee with the largest ovaries has always been somewhat suspect. Indeed Batra (1964, 1966) largely avoided the term because of the great variability among the females. Michener, Brothers, and

Kamm (1972b), while using the word, suggested that its application was not appropriate. The present paper, however, shows that the queen is a more distinctive caste in this species than hitherto recognized. The behavioral categories for which trend curves are concave (Section D), and with queens as judged by ovarian development limited to or concentrated in a rank at either extreme, indicate that queens are distinct in a number of behavioral features. Most of the relationships of behavioral categories to ovarian development (Section E, Table 3), in which correlations are positive over one behavioral range and negative over another, have that feature because of the distinctiveness of the queens as a caste. The same applies to the relationships between behavioral categories (Section F, Table 5). The multivariate studies also indicate marked differentiation of queens from the other colony members. For these reasons we use the word "queen" to emphasize the distinctness of that caste even in this very primitively social bee. Since queens are better differentiated in size and longevity in some related species of the same subgenus (Michener and Wille, 1961; Wille and Orozco, 1970), it is quite probable that in such species the behavioral distinctiveness of queens is even more marked than in *L. zephyrum*. The differentiation of workers into guards, foragers, and others (Table 6) is less clear, as can be judged from that table as well as from Figs. 1 to 6 and 17 to 21, although the distinctions between guards and foragers are quite marked in colonies of three bees.

Previous work (Michener, Brothers, and Kamm, 1971a) indicated that caste determination occurs in the adult stage. This conclusion is verified in Section G above in which queens were removed from their colonies; another bee in each colony quickly became a queen both ovarially and behaviorally. From these observations it seems clear that the queen is in some way able to inhibit ovarian development and queen-like behavior in her nest mates, at least in the small colonies with which we worked.

Michener, Brothers, and Kamm (1971a) speculated on the mechanism by which queens of *L. zephyrum* inhibit their nest mates and suggested that nest guarding by the queen in early stages of colony formation might play a significant role. Records of contacts between bees did not seem to show any particular bees contacting one another more often than others. We now know that this was an error; certain bees do more frequently contact others and when the types of interactions are segregated, meaningful specialization in contacting behavior is noted. We have not yet studied in detail the stages in caste differentiation but in our nests the queen rarely guarded and our earlier suggestion seems unlikely. We still see no evidence of transfer of materials among bees in a colony and are inclined to the theory that the physical rather than chemical effects of contacts partly or fully inhibit queenlike development among the workers.

Since the behavior in the nest rarely looks overtly aggressive, it is difficult to think of one bee as dominant and another subordinate. Nudging is the closest thing to the behavior of dominants in other social Hymenoptera, but it rarely if ever involves open jaws, biting, etc. It is for this reason that dominance-subordinance patterns were not discerned in earlier studies of behavior inside the nests (Batra, 1964, 1968; Michener, Brothers, and Kamm, 1971a, b). Indeed the type of behavior most diagnostic of the queen is backing *away* from another bee that has just been nudged. As shown in Section E, the bees that follow such backing bees most, tend to have the smallest or second smallest ovaries. The backing and following behavior has no obvious parallel among the wasps and other bees.

As indicated in Section F queens are usually the principal nudgers. Nudging not only suggests the dominance behavior seen in other primitively eusocial wasps and bees but there are associated similarities as well. Except for the queen, there is a positive correlation between nudging and being nudged. This is largely in agreement with the dominance behavior noted below for wasps and bumblebees, and as with those forms, implies an ability on the part of the queen and others to recognize individuals with enlarged ovaries.

Although the queen preferentially nudges others with ovaries most nearly like her own, she is followed principally by bees with the least enlarged ovaries. This seems to mean that the queen's nudging often merely disturbs the other bees with enlarged ovaries; these bees usually remain facing the entrance if they are in that part of the nest and continue to guard or remain behind the guard. They are often nudged repeatedly, while continuing to face upward and away from the nudger. The nudging may inhibit further ovarian enlargement of these bees but fails to elicit turning and following. When the queen nudges a bee with slender ovaries, the latter is more likely to turn; the queen then backs down the burrow and is followed.

The nudging-backing sequence should probably be considered as a ritualization of agonistic behavior. It is not uncommon for aggressive encounters in other bees and wasps and even in termites to involve two phases of activity, a forward lunging movement followed by a rapid withdrawal (R. Jander, personal communication). A similar sequence is observable in nudging encounters not followed by backing, and the backing activity may thus be a further elaboration of the withdrawal phase.

Thus it appears that queen behavior may not only inhibit ovarian development of workers but may play a role in causing division of labor among workers, those with somewhat enlarged ovaries tending to remain in the nest and guard (although they do sometimes forage) and those with more slender ovaries tending to work on cells and to forage to provision the cells. [Interestingly, in *Lasioglossum* (*Evylaeus*) *calceatum*, Bonelli (1965) recognized the oldest worker, with largest ovaries, as the principal guard and as further specialized, at least in longevity.]

There appear to be some remarkable parallelisms in caste determination among groups of adults in various social Hymenoptera. In a group of overwintered adult *Polistes* (Vespidae), a dominant individual, which will become the queen, rises above subordinates or if challenged, strikes, bites, or even stings (Eberhard, 1969). The subordinates are gynes (potential queens), but become auxiliaries, that is, overwintered workers. Similar dominance-subordinance relations are known also in another vespid, *Mischocyttarus* (Jeanne, 1972). In bumblebees, *Bombus*, fighting likewise occurs in queenless groups of workers or artificial groups of queens (Free, 1955). Moreover, fighting in normal colonies may occur between the queen and certain of her workers, or among particular workers (Cumber, 1963). Again, in queenless colonies of the honey bee, *Apis mellifera*, fighting among certain workers may occur until one establishes itself as a "false queen" (Sakagami, 1954, 1958).

In all the cases, involving at least two and probably three separate origins of social behavior, the aggression of the dominant seems directed particularly toward one or a few other individuals, not indiscriminately toward all other colony members. In all cases the dominant individual has the largest ovaries, and her aggression is directed toward others that have partial ovarian enlargement. In the wasps, dominance apparently needs to be exercised over a given subordinate for relatively limited periods (10 or 15 days) after which the subordinate will ordinarily remain as a worker. In the bumblebees permanent ovarian suppression may not occur but fighting may likewise be limited, as in the wasps, to a rather brief period. From these observations one must conclude that nest mates can distinguish individuals with ovarian enlargement from those with slender or regressing ovaries.

It is not known how such recognition occurs. Pardi (1947) suggested for *Polistes* that either odor or behavior, perceived visually, might provide the cues. For the bees, living in dark nests, odor or possibly contact pheromones seem the more probable. For *Apis* it is likely that ovarian development in workers is associated with production of queen pheromones (queen substance) (Velthuis, Verheijen, and Gottenbos, 1965). In any event a "false queen" of *Apis* is treated much like a real queen; once such a bee is established, fighting subsides.

It may seem surprising that such similar and complex mechanisms, involving recognition of individuals with enlarging ovaries, would arise independently in unrelated groups of social Hymenoptera. However, the basis for such behavior is doubtless inherited from solitary ancestors. In *Augochlora pura*, a solitary bee, groups of adult female nest mates sometimes remain together during a more or less prolonged preoviposition period, especially during and immediately after hibernation. As soon as ovarian development begins, fighting ensues and the excess females are ejected from the nest; this seemingly assures the solitary nesting behavior of this species (Stockhammer, 1966). Thus solitary Hymenoptera can probably also recognize the ovarian condition of other females, their competitors. With little modification, such behavior might serve as the basis of caste control in related social bees. Even if *Augochlora pura* arose from social ancestors, as seems quite possible, similar behavior probably assures the isolation of solitary bees.

The instances of egg eating observed in *Lasioglossum zephyrum* may represent "differential oophagy" such as has been described in temperate species of *Polistes* (Gervet, 1964; Eberhard, 1969). In *P. fuscatus* Eberhard found that only egg-layers were oophagous, that egg-eating was usually followed immediately by oviposition, that all eaten eggs were newly laid and that wasps never ate their own eggs; furthermore, the dominant female destroyed the eggs of subordinates with much greater efficiency than the opposite. Although in *Bombus* eggs laid by both queens and workers are sometimes eaten, Sakagami and Zucchi (1963) record differential eating of workers' eggs by queens. The similarities to the situation in *L. zephyrum* are striking.

By contrast, Jeanne (1972) found in *Mischocyttarus drewseni* that females sometimes ate their own eggs, usually immediately after laying them. Also, eggs in all stages of development were eaten. Jeanne concluded that egg-eating in this wasp was probably "nutritional egg-eating" rather than the type of "differential oophagy" seen in *Polistes, Bombus,* and perhaps now in *L. zephyrum.*

It is of some interest to note that in *Polistes* the most dominant worker is the individual which becomes the replacement egg-layer when necessary (Eberhard, 1969). Although it is difficult to characterize a "most dominant" worker in a colony of *L. zephyrum*, it seems reasonable that it would be the worker showing maximal nudging (and largest ovaries). In this case, it is often not the most dominant worker which becomes the replacement queen, but the bee which could be considered the second most dominant. This difference between *Polistes* spp. and *L. zephyrum* may be linked with the different methods of feeding and differences imposed by nest architecture leading to greater specialization by certain of the workers in *L. zephyrum.*

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Denis J. Brothers
Department of Entomology
University of Natal
Pietermaritzburg
Republic of South Africa

Charles D. Michener
Department of Entomology
The University of Kansas
Lawrence, Kansas 66045
U.S.A.