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The Behavioral Genetics of Colony Defense in Honeybees: Genetic Variability for Guarding Behavior

Michael D. Breed[1,2] and Kevin B. Rogers[1]

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Guard honeybees stand at the entrance of colonies and facilitate the exclusion of nonnestmates from the colony. In this study, we examined the hypothesis that genetic variability among individuals in colonies might explain variability in guarding activity. To do this, we cross-fostered honey bees between colonies with high-defensive responses and colonies with low-defensive responses in alarm pheromone tests. Individuals from high-defensive colonies were more likely to guard in their own colonies (controls) than cross-fostered bees from low-defensive colonies. Cross-fostered high-defensive bees also were more likely to guard in low-defense colonies. These results support the hypothesis that interindividual differences in guarding behavior are at least partially under genetic control. A positive correlation between number of guards and response to alarm pheromone demonstrates a link between behaviorally separated components of the overall defensive response.

KEY WORDS: honeybees; *Apis mellifera*; colony defense; guarding behavior; genetic variability.

INTRODUCTION

The purpose of this paper is to test the hypothesis that nest guarding behavior in honeybees has a genetic basis. The behavior of guard ho-

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[1] Department of Environmental, Population, and Organismic Biology, The University of Colorado, Boulder, Colorado 80309-0334.
[2] Institute for Behavioral Genetics, The University of Colorado, Boulder, Colorado 80309-0447.

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neybees first was characterized in detail by Moore *et al.* (1987), although Maschwitz (1964) presented a detailed account of pheromonal communication involved in colony alarm. Moore *et al.* (1987) found that a small number of bees in each colony specialized in this task, but the turnover rate of guards was very high; guarding is apparently a brief occupation of a few bees in the transition between within-hive activities and foraging. Subsequently, Breed *et al.* (1989) showed that the persistence of guards at a colony entrance is correlated with colony responsiveness in alarm pheromone tests (Collins and Kubasek, 1982). As responsiveness in alarm pheromone tests has a significant heritability (Collins, 1979), the correlation between the two characters suggested a genetic basis for guarding.

Quantitative genetic analyses of behavioral traits in honeybees are complicated by the haplodiploid sex-determining mechanism and by social interactions among individuals. Collins *et al.* (1984) estimated heritability for a number of social traits, including response to alarm pheromone, by using colony means and calculating sire-queen and dam-queen variances. Later, Moritz (1986) proposed a technique for measuring heritabilities of social traits by calculating genetic variances of small groups within colonies. Brandes (1988) used a technique developed by Moritz and Klepsch (1985) to calculate heritabilities of learning behavior using a partially parthenogenetic subspecies of honeybee.

Robinson and Page (1988) and Frumhoff and Baker (1988) tested whether genetic background (patriline membership) could influence worker honeybee activity in colonies. Their results provide conclusive evidence that some behavioral variability among workers can be explained by genetic variability among workers. Robinson and Page (1988) used colonies in which different worker patrilineal subfamilies carried electrophoretically distinguishable markers. Frumhoff and Baker (1988) used color morphs from the progeny of doubly inseminated queens to address the issue of such differentiation. The use of artificially inseminated queens with semen from a relatively limited number of drones in each of these studies could have influenced their results. Robinson and Page (1988) found that there are genotypic differences in the tendency to guard the entrance or remove corpses, two roles played by workers of approximately the same age. Their data also suggest that guards constitute a genetically specialized group with respect to the colony population as a whole, but definitive proof is lacking.

In our experiment we classified pairs of colonies as high defensive (HD) or low defensive (LD), based on their response to alarm pheromone (Collins and Kubasek, 1982). It is important to note the HD and LD colonies are not from lines selected for defensiveness; they are repre-

sentative of the normal variation in this character. Reciprocal transfers of marked, newly emerged, adult bees were made between these colony pairs and then guarding activity was observed. Our experimental design does not directly distinguish between preimaginal environmental effects and genetic effects. Other experiments have found no preimaginal effects on a wide range of honeybee behavior, including kin recognition (Breed, 1983), division of labor (Robinson and Page, 1988), and alarm behavior (Moritz *et al.*, 1987). While our experiment is focused narrowly on guarding, rather than a broad range of activities, it provides a supporting test for genetic influences on defensive behavior by using a cross-fostering design with colonies headed by naturally mated queens.

MATERIALS AND METHODS

Open mated honeybee colonies were maintained in and near Boulder, Colorado. Colonies were assayed for responsiveness to alarm pheromone using methods modified from those of Collins and Kubasek (1982). A cotton swab with 50 μl of isopentyl acetate was placed at the center of the landing board. The number of bees in Polaroid pictures of the colony entrances taken before treatment (control) and 90 s after treatment were counted; the control was subtracted from the treatment value to yield a measure of colony response. Six pairs of colonies then were selected so that one member of the pair had a substantially higher defensive response (HD) than the other member (LD). Within each pair of colonies, there was a substantial difference. Colonies in each pair were separated by several kilometers.

Once pairs were established, two brood frames of pupae were removed from each colony and brought into the laboratory. As the pupae eclosed and emerged as adults, they were collected and marked with Testor's enamel for identification of age and colony membership. Half of the workers were returned to their natal colony and half were placed in the paired colony. The number of bees marked from a colony ranged from 1516 to 4278. If transferred bees were less viable than returned bees, then consistently smaller numbers of transferred bees should guard; there is no evidence for this in the data (Table I). Our experiment assumes equal viability of HD and LD bees in both environments. When conducting observations, the observer was blind with respect to interpretation of the marks.

Colony entrances then were observed for 3 h each day. When a marked guard was seen its color code was recorded for later analysis. An additional mark (plastic number tags) were added so that guards could be distinguished if observed repeatedly; this prevented multiple counts

Table I. Colony Pairings, with Pheromone Test Results, Number of Bees Introduced or Returned to Each Colony, Mean Age of Initiation of Guarding, and Number of Guards from Each Marked Group

				No. of bees		No. of guards	
		Mean age	Pheromone test	Returned	Introduced	Returned	Introduced
1	HD	17.3	140	1085	1475	3	9
	LD	17	55	1475	1085	16	23
2	HD	17	83	1135	1557	34	25
	LD	18.4	18	1557	1135	26	47
3	HD	20.6	165	758	893	15	3
	LD	18.8	10	893	758	6	14
4	HD	20.1	241	1057	993	36	27
	LD	21.6	83	993	1057	15	16
5	HD	16	52	1069	2139	1	0
	LD	19.2	3	2139	1069	21	7
6	HD	20.3	161	1117	1338	18	1
	LD	18.8	15	1338	1117	6	39

of a single individual and allowed documentation of the number of days each individual guarded. Observations of colonies were terminated when the youngest marked bees were 25 days old; guards older than this are very rare (Moore *et al.*, 1987).

Statistical analyses were performed using the Statview II statistical package on a Macintosh II computer. Because of possible problems of independence (the behavior of one bee inhibiting or stimulating another), each colony is treated as a single replicate in the analysis.

RESULTS

Mean Age at Initiation of Guarding

Guarding was initiated by bees in HD colonies at a mean age (mean of the colony means) of 18.6 days (SE = 0.82, n = 6) and in LD colonies at a mean age of 19.0 days (SE = 0.57, n = 6). There was no significant difference between these means (F = .234, df = 1,10, p = .64). If age at initiation of bees with HD parentage is compared with the age at initiation of bees with LD parentage, there is also no significant difference (mean, HD = 18.9, SE = 0.19; mean, LD = 18.8, SE = 0.23).

Number of Guards

Table I shows the number of bees marked, the number of guards observed, and the results of pheromone response tests for each colony.

Two-way analysis of variance (Table II) of the number of guards from the HD and LD colonies when placed in each environment (HD and LD) was performed after correcting by dividing by the number of marked bees available and then applying an arcsine transformation because of the use of frequencies. There was a significant effect of genetic source ($p = .03$) but no significant effect of adult environment ($p > .05$). There was no significant interaction effect between rearing environment and genetic source ($p > .05$).

The lack of an environmental effect in the ANOVA was interesting, and we pursued testing for environmental effects using other statistical approaches. A non-parametric analysis for directional changes in propensity to guard in different environments yielded positive results. Examination of colony pairs reveals that, in 10 of 12 cases, bees from HD genetic sources guarded more than bees from LD genetic sources ($p = .038$, two-tailed binomial test). Pooled g tests comparing the frequencies of guarding in HD and LD environments revealed that HD bees guard more in LD environments ($G = 6.16, p = .01$) and that LD bees guard less in HD environments ($G = 4.01, p < .05$). Six of the twelve possible individual g tests were significant (Table III). HD bees appear to be more responsive to their environment than do LD bees, with four of the six HD tests showing significance. The HD bees displayed a 32% increase in the number of guards in the LD environment (mean of HD bees in HD environment = 17.3 ± 5.5, LD environment mean = 22.9 ± 5.2) and LD bees show a 16% decrease in the number of guards in the HD environment (mean of LD bees in LD environment = 10.6 ± 1.9, HD environment mean = 8.9 ± 4.4). Thus, this further analysis indicates that expression of guarding is affected by the colony environment.

Persistence

Based on previous experiments (Moore *et al.*, 1987), we hypothesized that guards from HD colonies would be more persistent than guards

Table II. Two-Way Analysis of Variance of Number of Guards Observed from Each Genetic Group Under Each Rearing Condition

	df	MS[a]	*F* test	*p*
Environment	1	0.008	0.66	.4261
Genetic effects	1	0.068	5.273	.0326
Interaction	1	0.003	0.205	.6558
Error	20	0.013		

[a] Mean squares.

Table III. Analysis of Frequency of Guarding in the Two Environments[a]

	HD		LD	
Replicate	*G*	*p*	*G*	*p*
1	17.6	.0001	2.0	ns
2	2.1	ns	0.0	ns
3	0.0	ns	1.0	ns
4	8.1	.005	3.6	ns
5	5.1	.03	21.1	.0001
6	8.1	.005	4.0	.05
Total	41.0		31.7	
Pooled	6.2	.01	4.0	.05

[a] Comparisons are between the frequency of guarding in the HD and that in the LD environment. The columns indicate the genetic source of the bees tested. ns, not significant.

from LD colonies. Persistence was measured for each guard by counting the number of consecutive days it guarded. Bees from HD sources were significantly more persistent in guarding than bees from LD sources (one-tail test, $F = 3.274$, df = 1,21, $p = .042$). The mean number of days guarded by HD bees was 1.27 (SE = 0.082, $n = 12$ colonies) and the mean for LD bees was 1.10 (SE = 0.038, $n = 11$ colonies). Of 223 guards from HD sources, 29 (13%) were observed to guard on more than 1 day, while 13 of 153 (8%) guards from LD sources guarded on more than 1 day.

DISCUSSION

These findings address two areas of concern. First, we present an independent test, using methodologies different from those of previous studies, that demonstrates a genetic basis of guarding behavior. Second, we place the magnitude of the colony's guarding response in a correlational context with other defensive behaviors. In addition, this information contributes to the overall model being developed (see, e.g., Collins *et al.*, 1980; Breed *et al.*, 1990) for honeybee colony defense.

Our most important finding is that whether or not a worker honey bee serves as a guard either is genetically correlated or is influenced by preimaginal factors, although preimaginal factors are unlikely. This is consistent with the conclusion of Robinson and Page (1988, 1989) that task performance frequencies differ among patrilines. Environmental interactions with these genetic effects would be expected. Calderone and Page (1991) found that high- and low-pollen collecting lines in colonies

interacted so that the presence of high-pollen collection bees inhibited the activities of the low line. This is the expected result in a threshold stimulus model such as that proposed by Robinson *et al.* (1989). Our results also suggest environmental effects of the same type. LD bees in HD colonies appear to be inhibited from performing the task, while HD bees in LD colonies are stimulated.

We also conclude that these genetic effects can be distinguished as a complex of rather different, but correlated, behavioral patterns [(Breed *et al.*, 1989); see also Collins *et al.* (1980) for a general model of honeybee defensiveness]. Response to alarm pheromone is a heritable characteristic (Collins, 1979), and there is a genetic basis for guarding (Robinson and Page, 1988; this study). Breed *et al.* (1989) showed a correlation between persistence in guarding and response to alarm pheromone. While Breed *et al.* (1990) point out that guards may be adapted for a quite different role (defense against robbing) than other defensive bees (protection against vertebrate predators), the pattern of correlations among guard behavior, response to alarm pheromone, stinging behavior, and flight in response to major disturbances suggests a unified mechanism for regulating the intensity of defensive responses.

Quantitative analyses have the advantage of generating a number, representing heritability, which can be tested for statistical significance. Unfortunately, social traits are subject to nonlinear and nonadditive effects due to interactions among individuals in the social group (Moritz and Southwick, 1987); such effects greatly complicate calculations of heritability. In the present experiments we can conclude that there is a significant genetic effect underlying the expression of guarding behavior, but we cannot calculate a heritability for this trait.

In our experiments we cannot distinguish matrilineal (chromosomal assortment) from patrilineal (multiple-mating) effects. Robinson and Page (1988) tested for, and found, patrilinear effects. It seems likely that both the paternally and the maternally contributed genomes affect defensive behavior. Our data considers matrilineal and patrilineal contributions together.

The genetic control of defensive behavior in honeybees is consistent with our knowledge of subspecies differences in defensiveness (Winston, 1987). Unfortunately, little is understood of the comparative biology of colony defense among the various subspecies of *Apis mellifera*. Species of honey bees that more commonly nest in the open (*Apis florea* and *Apis dorsata*) have larger numbers of workers that continuously engage in colony defense (Seeley *et al.* 1982). The Africanized honeybee, a subspecific variant of *Apis mellifera* that is now spreading into North America, has at least some aspects of its defensive responses dramatically

heightened. As Breed *et al.* (1990) point out, it will be interesting to determine if all phases of the defensive response are elevated, or if certain phases are unlinked and independently amplified in the Africanized bees.

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REFERENCES

Brandes, Ch. (1988). Estimation of heritability of learning behavior in honeybees (*Apis mellifera capensis*). *Behav. Genet.* **18:**119–132.

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

Breed, M. D., Robinson, G. E., and Page, R. E. (1990). Division of labor during honey bee colony defense. *Behav Ecol. Sociobiol.* **27:**395–401.

Breed, M. D., Rogers, K. B., Hunley, J. A., and Moore, A. J. (1989). A correlation between guard behaviour and defensive response in the honeybee, *Apis mellifera. Anim. Behav.* **37:**515–516.

Calderone, N., and Page, R. E. (1991). A genotype × colony-environment interaction effect on pollen-hoarding behavior of worker honeybees (*Apis mellifera*) (manuscript in preparation).

Collins, A. M. (1979). Genetics of the response of the honeybee to an alarm pheromone, isopentyl acetate. *J. Apic. Res.* **18:**285–291.

Collins, A. M., and Kubasek, K. J. (1982). Field test of honeybee colony defensive behavior. *Ann. Entomol. Soc. Am.* **75:**383–387.

Collins, A. M., Rinderer, T. E., Tucker, K. W., Sylvester, H. A., and Lackett, J. J. (1980). A model of honeybee defensive behavior. *J. Apic. Res.* **19:**224–231.

Collins, A. M., Rinderer, T. E., Harbo, J. R., and Brown, M. A. (1984). Heritabilities and correlations for several characters in the honeybee. *J. Hered.* **75:**135–140.

Frumhoff, P. C., and Baker, J. (1988). A genetic component to division of labour with honeybee hives. *Nature* **333:**358–361.

Maschwitz, U. (1964). Gefahrenalarmstoffe und Gefahrenalrmierung bei sozialen Hymenopteren. *Z. Vergl. Physiol.* **47:**596–655.

Moore, A. J., Breed, M. D., and Moor, M. J. (1987). Characterization of guard behavior in honeybees, *Apis mellifera. Anim. Behav.* **35:**1159–1167.

Moritz, R. F. A. (1986). Estimating the genetic variance of group characters: Social behaviour of honeybees (*Apis mellifera* L.). *Theor Appl. Genet.* **72:**513–517.

Moritz, R. F. A., and Klepsch, A. (1985). Estimation of heritabilities of worker characters: A new approach using laying workers of the cape honeybee (*Apis mellifera* capensis Esch.). *Apidologie* **16:**47–56.

Moritz, R. F. A., and Southwick, E. E. (1987). Phenotype interactions in group behavior of honeybee workers (Apis mellifera L.). *Behav. Ecol. Sociobiol.* **21:**53–57.

Moritz, R. F. A., Southwick, E. E., and Harbo, J. R. (1987). Maternal and pre eclosional factors affecting alarm behaviour in adult honeybees (*Apis mellifera* L.). *Insectes Soc.* **34:**298–307.

Robinson, G. E., and Page, R. E. (1988). Genetic determination of guarding and undertaking in honeybee colonies. *Nature* **333:**356–358.

Robinson, G. E., and Page, R. E. (1989). Genetic determination of nectar foraging, pollen foraging, and nest-site scouting in honeybee colonies. *Behav. Ecol. Sociobiol.* **24:**317–323.

Robinson, G. E., Page, R. E., Strambi, C., and Strambi, A. (1989). Hormonal and genetic control of behavioral integration in honeybee colonies. *Science* **246:**109–112.

Seeley, T. D., Seeley, R. H., and Akratanakul, P. (1982). Colony defense strategies of the honeybees in Thailand. *Ecol. Monogr.* **52:**43–63.

Winston, M. L. (1987). *The Biology of the Honeybee*, Harvard University Press, Cambridge, Mass.

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