Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera*

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To test the hypothesis that colonies of honey bees composed of workers with faster rates of adult behavioral development are more defensive than colonies composed of workers with slower behavioral development, we determined whether there is a correlation between genetic variation in worker temporal polyethism and colony defensiveness. There was a positive correlation for these two traits, both for European and Africanized honey bees. The correlation was larger for Africanized bees, due to differences between Africanized and European bees, differences in experimental design, or both. Consistent with these results was the finding that colonies with a higher proportion of older bees were more defensive than colonies of the same size that had a lower proportion of older bees. There also was a positive correlation between rate of individual behavioral development and the intensity of colony flight activity, and a negative correlation between colony defensiveness and flight activity. This suggests that the relationship between temporal polyethism and colony defensiveness may vary with the manner in which foraging and defense duties are allocated among a colony's older workers. These results indicate that genotypic differences in rates of worker behavioral development can influence the phenotype of a honey bee colony in a variety of ways. *Key words: Apis mellifera,* behavioral development, defense, foraging, honey bees, life history. *[Behav Ecol 11:44–55 (2000)]*

Colonies of higher social insects function in many respects as well-integrated units with their own distinctive patterns of growth, development, and behavior (Hölldobler and Wilson, 1990; Oster and Wilson, 1978; Seeley, 1995; Wilson, 1971). Understanding how the activities of individual colony members give rise to these colony patterns is a key question in insect sociobiology.

Genotypic variability among workers influences the division of labor in colonies of the European honey bee *Apis mellifera* (reviewed by Page and Robinson, 1991), the dwarf honey bee *Apis florea* (Oldroyd et al., 1994), and several ant species (Carlin et al., 1993; Stuart and Page, 1991; Snyder, 1992, 1993). Effects of genotypic differences for a particular worker behavior on the manifestation of that same behavior at the colony level have been studied for hygienic behavior (Rothenbuhler, 1964), pollen collection (Calderone and Page, 1988), and corpse removal (Robinson and Page, 1995). For example, artificial selection for a colony-level trait, the amount of pollen stored in the hive (reviewed by Page, 1997), resulted in individual foragers from the high line being more likely to collect pollen than foragers from the low line (Calderone and Page, 1988; Page and Fondrk, 1995; Page et al., 1995b). However, there have been no studies of the effects of genotypic differences for a particular worker behavior on the manifestation of a different behavior at the colony level. In this paper we explore the relationship between variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera.*

Worker honey bees of some genotypes start foraging at younger ages than do workers of other genotypes (Calderone and Page, 1988, 1991; Giray and Robinson, 1994; Kolmes et al., 1989; Page et al., 1992; Robinson et al., 1989; Winston and Katz, 1982). Age at onset of foraging is a good indicator of the rate of behavioral development because the transition from working in the hive to foraging is particularly sharp. We hypothesized that genotypic variation in rate of behavioral development influences the expression of colony defensive behavior because older bees are engaged in both foraging and colony defense. Soldiers, behaviorally and genotypically distinct from other foraging-age bees (Breed et al., 1990), defend the nest by stinging large intruders such as mammals. Older workers produce more venom (Whiffler et al., 1988) and alarm pheromones (Boch and Shearer, 1966; Crewe and Hastings, 1976; Robinson, 1985) than do younger workers. They also show a lower threshold of response to alarm pheromones (Allan et al., 1987; Collins, 1980; Robinson, 1987) and to mechanical stimuli that elicit stinging behavior (Kolmes and Fergusson-Kolmes, 1989; Paxton et al., 1994). Variation in honey bee colony defensive behavior has a genetic component (Boch and Rothenbuhler, 1974; Collins et al., 1982, Guzman-Novoa and Page, 1993, 1994), but the effects of variation in worker behavioral development on colony defense are not known.

Implicit in the hypothesis that genotypic variation in the rate of behavioral development influences the expression of colony defensive behavior is the assumption that colonies composed of faster developing workers have an age structure skewed toward physiologically and behaviorally more advanced workers. Theoretical results (Giray T, unpublished data) suggest that, given equilibrium conditions for birth rates, death rates, and the rate at which bees mature from hive bees to foragers, an increase in maturation rate will result in a colony with a higher proportion of older bees. How rates of individual behavioral development influence the functional

demography of a bee colony may depend on several factors that govern honey bee mortality (Neukirch, 1982; Seeley, 1995; Visscher and Dukas, 1997; Wolf and Schmid-Hempel, 1989).

We also determined the effects of genoytpic variation in worker behavioral development on colony foraging activity. Foraging and defense may be linked in honey bee colonies because these activities are performed by the oldest workers (Breed et al., 1990). A higher proportion of workers devoted to defense may mean fewer bees available for foraging; soldiers may forage less or not at all (Breed et al., 1990), and stinging a fleshy vertebrate is a fatal act, due to sting autotomy. Likewise, foragers are away from the hive for long periods of time and are not always available to defend the colony against an intruder. As is the case for defensive behavior, genetic effects on foraging activity have been shown (e.g., Guzman-Novoa et al., 1994). We hypothesized that faster individual behavioral development also may result in a larger force of foragers, resulting in greater colony foraging activity. We also investigated whether there is a trade-off between colony foraging activity and colony defensiveness.

General methods

Experiments were performed with both European and Africanized bees. European bees are the descendants of several races of bees introduced from Europe into the Western hemisphere beginning in the 1600s; Africanized bees are the descendants of tropical African bees (*Apis mellifera scutellata*) introduced into Latin America in 1956. Numerous studies have demonstrated that colonies of Africanized bees are much more defensive than those of European bees (e.g., Collins et al., 1982; Guzmán-Novoa and Page, 1993, 1994; Villa, 1988). Africanized bees also have been reported to exhibit relatively faster behavioral development (Winston and Katz, 1982). Studying both tropical and temperate bees also provided the opportunity to begin considering the ecological correlates of any differences in the relationship between individual behavioral development and colony defense.

Experimental bees

Experiments 1, 2, and 4 were performed in the summer of, 1995 at the Bee Research Facility of the University of Illinois at Urbana-Champaign. Colonies were maintained according to standard commercial procedures. Six colonies were used in experiment 1, each headed by a naturally mated queen that was more than 1 year old at the time of the study. Fourteen ''source colonies'' (defined below) were used in experiments 2 and 4, each headed by a queen that was instrumentally inseminated with semen from a single, different drone.

Experiment 3 was performed in 1994 at Miel Vita Real, a commercial beekeeping operation in Ixtapan de la Sal, Mexico (19° N, 99° W), 150 km southwest of Mexico City. Nine Africanized and eight European source colonies were used; two of them (one Africanized and one European) were headed by naturally mated queens, and the rest were headed by singly (instrumentally) inseminated queens. In all cases, we used samples of newly emerged adult workers ($n = 20$) to determine whether each colony was of European or Africanized descent. Morphometric measurements of wings (Sylvester and Rinderer, 1987) and mitochondrial DNA typing (Hall and Smith, 1991) were performed. Wing measurements were made in Guzmán-Novoa's laboratory and mitochondrial DNA typing was performed in the laboratory of R. E. Page, University of California, Davis.

We labeled queens with colored, numbered tags (Graze KG, Weinstadt, Germany) and clipped their right forewings to pre-vent mating flights (which are sometimes taken by instrumentally inseminated queens; see Kaftanoglu and Peng, 1982). We inspected colonies frequently to make sure that these queens were not lost or superseded.

Experiment 1: differences in defensive behavior between colonies composed of young versus old bees

Methods

To test the hypothesis that colonies with a higher proportion of older individuals are more defensive than colonies with a higher proportion of younger individuals, we asymmetrically divided colonies into old and young colony halves and compared their defensiveness.

The following procedure (modified from Laidlaw HH, personal communication) was used to asymmetrically divide two-story colonies of 40,000–50,000 bees each into two fragments with equal numbers of bees, one with mostly older bees and one with mostly younger bees. The technique is based on the premise that older bees tend to congregate nearest the entrance, while younger bees tend to be found closer to the brood (Seeley, 1985, Winston, 1987). One day before splitting a colony in two, we replaced the original queen with two caged, unrelated queens, one in the bottom and one in the top story. On the next day, day 0, we moved all but one of the frames of brood and their adhering bees to the top story, and all other frames with honey or empty comb, one frame of older brood, and their adhering bees to the bottom story, nearest the entrance. To monitor the efficacy of this technique, we marked 500 foragers and 500 brood-tending nurse bees on the thorax with a paint spot (Testor's enamel paint) before rearranging the frames of comb (nurses and foragers identified according to established criteria; e.g., Robinson, 1987). Nurse bees were also marked on the abdomen to facilitate observing them later (Seeley and Kolmes, 1991).

Before sunrise on day 1, each colony was split by sealing the bottom and top stories of the hive and taking both fragments to another apiary. The apiary was located >12 km away from the original site to prevent older bees from returning to their original nest site if they recognized the landscape (Dyer, 1994). The two colony fragments were placed about 15 m apart facing away from each other. This was done to minimize worker drifting from one fragment to the other, especially when the bees were orienting to the new location of their hive. We were careful not to place the two fragments too far apart or in different apiaries to minimize the possibility of different environmental influences on defensive behavior (Paxton et al., 1994; Winston, 1987). The colony fragments were left undisturbed for the rest of the day.

Colony fragments were opened on day 2 and population sizes estimated by counting the number of frames covered with bees (to the nearest half side of a frame) in each hive (~2000 bees per two-sided frame completely covered with bees; Guzmán-Novoa and Page, 1994). When necessary, populations were equalized by removing bees from the more populous colony fragment (population estimates provided in Figure 1 legend). Then we collected all the marked bees we could find with a vacuum device (Bioquip); collecting them at this point means that effects of drifting should be detectable. To facilitate collecting the marked older bees, many of them foragers, we closed the hive entrances with a wire mesh screen and collected them as they returned. Counts of marked bees were used to estimate the age demography of each colony fragment. All the marked bees were later returned to the fragment they were collected from, except for the colony 2 fragments (by accident). These counts indicated that we did produce fragments with markedly different age demographies: for each pair of colony fragments Fisher's Ex-
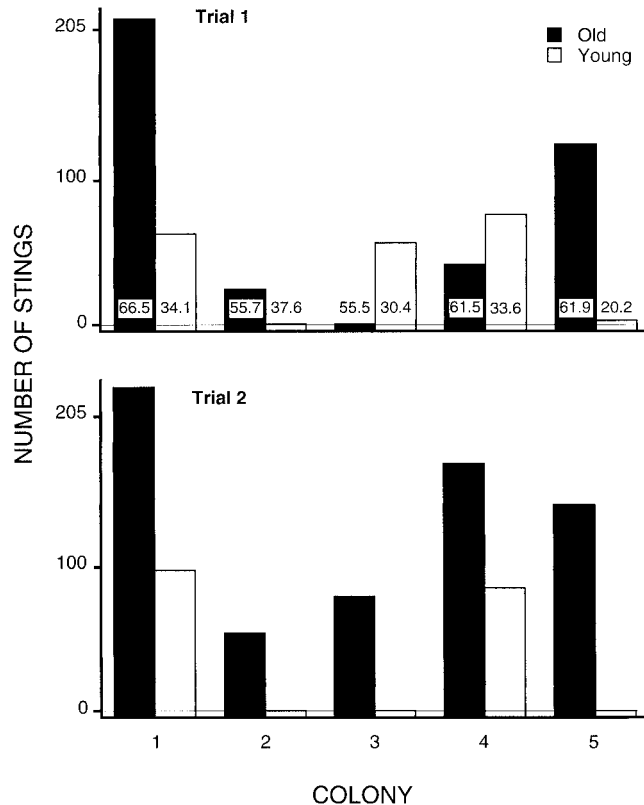
Figure 1
Intensity of stinging behavior for colony fragments with mostly old or young bees. The colony 2 young-bee fragment did not sting the patch (made more apparent on the graph by elevating the 0-response line). In each pair of colony fragments, 260–350 marked old bees and 350–500 marked young bees were recovered. Recovered marked bees were used to calculate the percentage of old bees in each colony fragment. For instance, in pair 1, the young colony fragment contained 93 marked old and 319 marked young bees, the old colony fragment contained 175 marked old and 157 marked young bees. The percentage of old bees in a colony fragment is calculated as the proportion of marked old bees in that colony fragment divided by proportion of all marked bees in that colony fragment. The estimated percentage of old bees in each colony fragment is indicated in the bars in trial 1. Each colony fragment in each pair contained equal numbers of frames covered with bees: pair 1, 12; pair 2, 8; pair 3 and 4, 10; pair 5, 9 frames (~2000 bees per frame). Results of statistical analyses in text.

act test p values were <.0001 for distribution of young and old bees; old colony fragments each contained 56–67% of the marked older bees, whereas young colony fragments contained only 20–38% of the marked older bees (see Figure 1).

Colony fragments were left undisturbed for the remainder of day 2 and the next day. On day 4, a well-established sting assay (Guzmán-Novoa and Page, 1994) was used to measure colony defensiveness. After dropping a brick on the top of the hive (from a height of 30 cm), a patch (7 × 8 cm) of dark brown suede leather attached to a 90 cm dowel was waved in front of the hive entrance; we recorded the time elapsed until the first bee stung the patch. If no bee stung the patch in the first 2 min, the trial was recorded as "no response". We also counted the total number of stings embedded in the patch 1 min after the first sting. This was repeated 2 days later, using new leather patches. Both colony fragments were always assayed simultaneously. Two pairs of people performed these assays, one pair per colony fragment. The pairs alternated be-

tween old-bee and young-bee fragments from trial to trial to minimize the effects of observer bias.

Results
Old colony fragments responded significantly more intensely to the disturbance. The median number of stings for old colony fragments was 95, compared to 31.5 for young colony fragments ($p < 0.03$, $z = -2.2$; Wilcoxon signed rank test; range 0–218 and 0–94 for old and young colony fragments, respectively). Bees in the old colony fragment stung the leather patch more frequently than did bees in the young colony fragments in 8 of 10 trials (5 colony pairs, 2 trials each; see Figure 1). There was a significant positive correlation between the percentage of old bees and the number of stings ($p < .03$, $r = .7$).

Old colony fragments responded only marginally more quickly than did young colony fragments to the disturbance. The median time to first sting for old colony fragments was 4.75 s compared to 10.25 s for young colony fragments ($p = .05$, $z = -2.0$; range 1–103 s and 2–120 s for old and young colony fragments, respectively). But time to first sting and number of stings were significantly negatively correlated ($p < .04$, $r = -0.74$, $n = 9$; $\log(x)$ transformed average scores), as in previous studies (Guzmán-Novoa and Page, 1993, 1994). One young colony fragment did not sting the patch in either trial.

Across all colony fragments, the number of stings on the leather patch in the first and the second trials was significantly correlated ($p < .02$; $r = .74$; $n = 10$). Time to first sting was not similarly correlated ($p = 0.09$, $r = 0.57$; $n = 6$), but it was not significantly different from trial to trial ($p = 0.9$, $z = -0.14$, $n = 6$; Wilcoxon signed rank test on $\log(x)$ transformed data; the smaller sample size reflects the fact that only 6 out of 10 colonies responded in both trials).

Experiment 2: correlation between worker behavioral development and colony defensiveness in European bees

We tested the hypothesis that genetic variation in worker behavioral development is correlated with genetic variation in colony defensiveness.

Methods
Experiment 2A: measuring rate of worker behavioral development.
One-day-old adult bees were obtained from 14 unrelated source colonies, each colony headed by a queen instrumentally inseminated with semen from a single, different drone. The offspring of each queen made up a "genotype group." Seven single-cohort-composite (SCC) colonies were established (Giray and Robinson, 1994), each containing only 1-day-old bees from three to six genotype groups. Some of the same genotype groups were used in more than one SCC colony. This enabled us to infer relative behavioral development rankings for each genotype group by comparing the same genotype group in different SCC colonies. Adult bees in composite colonies experienced a common environment, but we did not rear them in the same environment before adult emergence (as did Calderone and Page, 1988). This is because there are consistent differences in rate of behavioral development between genotype groups that are reared together only during adulthood (Giray and Robinson, 1994).

SCC colonies were created as follows. Frames of sealed brood were removed from source colonies and placed in an incubator (34°C). Approximately 500 1-day old (0–24 h old) adult bees from each genotype group were marked on the thorax with a genotype group-specific spot of paint (Testor's enamel) and held in a small hive in an incubator until all marking was completed for each colony. Each colony was com-

posed of 1500–2500 1-day-old bees (i.e., 6 or fewer 500-bee genotype groups). A caged queen (unrelated to any of the genotype groups), one frame of honey and pollen, one frame of honey, and one frame of empty comb (for the queen to lay eggs) were placed into the hive. The colony was placed outside in the morning after spending one night in the incubator. The queen was released from the cage and the hive entrance opened in the afternoon.

We determined differences in behavioral development by quantifying precocious foraging, which occurs in single-cohort colonies due to the lack of older bees (Huang and Robinson, 1992, 1996). Precocious foraging typically starts when bees are 7–10 days of age, about 2 weeks earlier than the onset of foraging under more normal conditions (Giray and Robinson, 1994; Robinson et al., 1989). Observations at the hive entrance started when the bees were 4 days of age to observe the first precocious foragers. We conducted two 1-h observation sessions daily, 1 h in the morning (900–1100 h) and 1 h in the afternoon (1500–1700 h). Hive entrances were equipped with a small door to facilitate vacuum collection of returning foragers. Foragers were identified either as bees with pollen loads on the hind legs (pollen foragers), or bees with distended abdomens (water or nectar foragers). Bees with distended abdomens were dissected to verify if they contained nectar in their crops; only bees with thin fluid in the crop (i.e., not honey) were counted. The number of presumed water or nectar foragers that were discarded according to this criterion was <5%. Sampling of precocious foragers continued until about 150 bees were collected or until the bees in the colony reached 12 days of age, whichever came first.

We used two-way *G* tests (Sokal and Rohlf, 1995) to compare the representation of each genotype group in the precocious forager sample with its initial representation in each SCC colony (Figure 2). To determine differences between genotype groups, unplanned tests of homogeneity were used (Sokal and Rohlf, 1995). We compared performance of genotype groups in different SCC colonies to rank them from the slowest to the fastest. Results from all SCC colonies were used to assign ranks for the 14 genotype groups according to their relative rates of behavioral development (Table 1).

We also calculated indices of relative rate of behavioral development for each genotype group each time it was tested in a different SCC colony (Table 1). The index is the proportion of bees from genotype group X in the precocious forager sample (Figure 2) divided by the proportion of bees from genotype group X in the whole colony (Giray and Robinson, 1994). The greater the value of this index, the faster the rate of behavioral development. These indices were used to aid comparison of genotype groups within a SCC colony and in correlative analyses described below. The numbers used to generate these proportions are found in the Figure 2 legend.

A census of all bees present in each SCC colony was made at the conclusion of the experiment. Censuses were performed after freeze-killing all remaining bees. Censuses were used to determine whether there were differences in mortality between genotype groups during the experiment.

Experiment 2B: measuring colony defensiveness. We measured the defensive behavior of the 14 source colonies. These were the source colonies for the 14 ranked genotype groups used to make the SCC colonies studied in experiment 2A. Measurements were made with the sting assay described in the methods for experiment 1, with the following modifications. The brick was not dropped on top of the hive because there were height differences between the source colony hives. Instead, a mechanical disturbance was produced by tying a brick to a 30-cm rope, lifting the brick to a horizontal position rel-

ative to the end of the rope, and then releasing it; the brick then squarely hit the side of the bottom box of the hive. The assay was performed on each colony by a pair of researchers, after practicing on dummy hives; one person released the brick while the other kept time and waved the leather patch. Assays were conducted blind with respect to source colony identity.

The 14 source colonies were located in two apiaries due to space constraints. All source colonies in each apiary were tested simultaneously to minimize environmental differences and to eliminate the possibility of bees from a previously agitated colony stinging a leather patch ''belonging to'' another colony. The two apiaries were assayed 30 min apart, which was as close in time as possible due to the distance between the apiaries. Each colony was tested twice, at 2-day intervals, using new leather patches each time. The weather on both days was stable and sunny. Each colony received a defensiveness score equal to the average number of stings deposited on the leather patch in the first minute following the first sting.

Correlation analysis was performed on the behavioral development rankings of genotype groups obtained from the SCC colonies and the defensiveness scores of the corresponding source colonies. Because colony population size is known to be positively correlated with defensiveness (see Guzmán-Novoa and Page, 1993, 1994), the effect of population size was controlled for by partial correlation analysis. Colony population size estimates were obtained as in experiment 1.

Results

There was no significant correlation between rate of behavioral development and colony defensiveness when all colonies were included in the analysis (Figure 3; $p > .9$; $\rho = 0.01$, $n = 14$; Spearman partial rank order correlation, but see experiment 4 and Table 4). However, when the five colonies that showed no response in the defensiveness assay were excluded, the correlation improved dramatically and was significant (Figure 3; $p < .03$, $\rho = 0.665$, $n = 9$; Sokal and Rohlf, 1995). The five colonies that never stung the patches also did not respond to repeated and even stronger disturbances (unpublished observations), but they appeared normal in other respects, including foraging activity (see Table 2 and experiment 4).

Behavioral development rankings, defensiveness, and population sizes (and flight activity; experiment 4) for each genotype group are presented in Table 2. Colony population size was not significantly correlated with defensiveness ($p > .4$, $r = 0.24$, $n = 14$). This is not consistent with previous studies (see Guzmán-Novoa and Page, 1993, 1994), but probably is attributable to the fact that colony sizes were similar to one another in this experiment. There was not much size variation among the 14 source colonies; only 3 deviated > 1 SD from the mean. Despite these results, partial correlation analysis was used to be consistent with the analyses for experiments 3 and 4.

Time to first sting (data not shown) and number of stings in the first minute were negatively correlated, as in experiment 1 ($P < .03$; $r = -.71$, $n = 9$; $\log(x)$ transformed average scores). Defensiveness scores for each colony from trial to trial were significantly correlated ($p < .05$, $\rho = 0.56$, $n = 14$).

Experiment 3: Correlation between worker behavioral development and colony defensiveness in Africanized and European bees

We tested the same hypothesis as in experiment 2, this time with both European and Africanized honey bees.
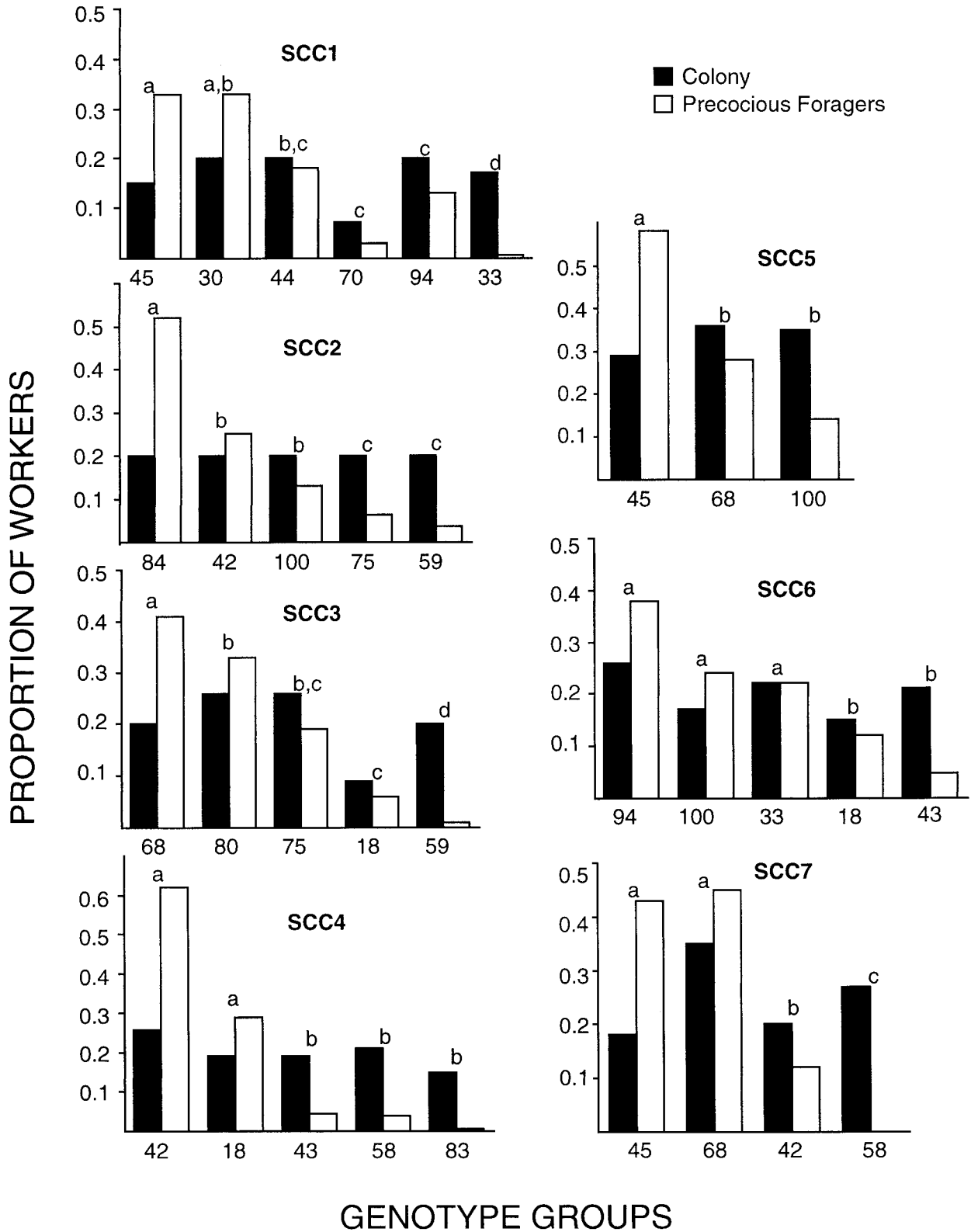
Figure 2
Differences between genotype groups in behavioral development. The graph depicts the proportion of workers from each genotype group sampled as precocious foragers and in the whole colony. There was significant heterogeneity in these distributions ($p < .0001$) in all eight single-cohort composite colonies (SCC; using actual frequencies). Different letters over the bars show genotype groups that differ significantly from each other (see text for details of statistical analysis). Three source colony queens 84, 59, and 30 were lost during the experiment, so those genotype groups were eliminated from the study. Total number of precocious foragers and colony population for each SCC were: SCC1, 160 and 2482; SCC2, 144 and 2560; SCC3, 203 and 1950; SCC4, 134 and 2083; SCC5, 64 and 1522, SCC6, 165 and 2418; SCC7, 51 and 1612.

Table 1

Ranks for rate of individual behavioral development (BD) for genotype groups from European bee source colonies in Illinois

Genotype group	Index of relative rate of behavioral development							
	SCC1	SCC2	SCC3	SCC4	SCC5	SCC6	SCC7	BD rank
45	2.20				2.00		2.39	14
68			2.05		0.78		1.29	13
42		1.25		2.38	0.40		0.60	11
44	0.88							11
80			1.27					11
70	0.43							8
94	0.65					1.46		8
100		0.66				1.41		8
33	0.04					1.05		5.5
75		0.31	0.73					5.5
18			0.64	1.53		0.80		4
43				0.24		0.24		2
58				0.18			0.01[a]	2
83				0.05				2

Different indices for the same genotype group are based on tests in different single-cohort composite (SCC) colonies. A higher index and rank indicates a faster rate of behavioral development.

[a] Score assigned arbitrarily; no bees foraged from this genotype group in this SCC.

Methods
Experiment 3A: measuring rate of worker behavioral development.
We compared rate of behavioral development of genotype groups of Africanized and European bees in nine pairs. SCC colonies were used as in experiment 2, with the following differences. Because we specifically wanted to compare Africanized and European bees, we tested only two genotype groups in each SCC colony, one Africanized and one European. Each SCC colony was composed of 1500 bees, about 750 bees from each genotype group (and precisely determined for each SCC colony). To determine differences in rates of behavioral development, we collected the first 50 precocious foragers; this

Figure 3
Correlation between individual worker behavioral development and colony defensiveness for European bees in Illinois. The analysis was performed two ways: with all colonies (broken line) and with the five colonies that did not exhibit defensive behavior excluded (solid line).

sample size is sufficient to determine differences between two genotype groups (Giray and Robinson, 1994). One European genotype group was used in two different SCC colonies, each time paired with a different Africanized genotype group.

We could not generate rankings of relative rates of behavioral development as in experiment 2 because each genotype group was compared with only one other genotype group. Instead, we calculated differences in the rate of worker behavioral development for each pair of genotype groups in such a way that we could compare the magnitude of these differences between different pairs. We calculated pairwise differences as follows. The first 50 foragers were typically collected over a 3-day period. Of these 50, all bees observed to initiate foraging from a colony on each day received the same score regardless of genotype group. The score represents the day on which they were first observed. Note that, unlike experiment 2, a higher score denotes a slower rate of behavioral
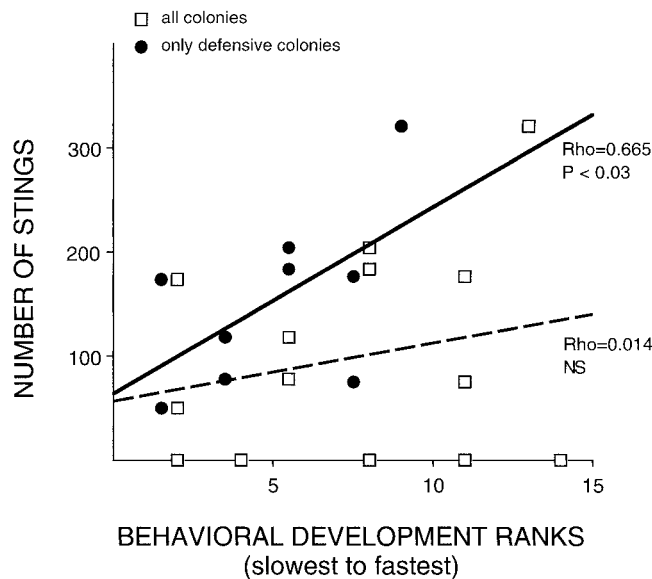
Table 2

Behavioral development rank, defensiveness, flight activity, and population size for genotype groups of European bees in Illinois

Genotype group	BD rank	Defensive-ness	Flight activity	Population size
45	14	0	211	15.75
68	13	642	96.5	10.8
42	11	0	271	17
44	11	151	198	10.75
80	11	354	119.5	11
70	8	408	31	11.5
94	8	368	39.5	19
100	8	0	132	10.75
33	5.5	235	85.5	21.5
75	5.5	156	77.5	9.5
18	4	0	77.5	9.7
43	2	99	129	11.75
58	2	346	50.5	12.62
83	2	0	74.5	9.35

BD rank from Table 1; defensiveness = total no. stings summed over 2 assays; flight activity = total no. bees exiting colony summed over 1-min observations conducted on 5 days (see experiment 4).

Table 3

Rate of behavioral development, defensiveness, and population sizes for genotype groups of Africanized and European bees in Mexico

SCC	Genotype group (n)		Rate of BD Score	Rate of BD Difference	Defensiveness Score	Defensiveness Difference	Size Score	Size Difference
	TE-2	(38)	23.97		13.5		13	
1	3-29	(14)	33.36	−0.180	4	9.5	9.75	3.25
	4-14	(33)	24.05		12		11.5	
2	3-8	(15)	25.50	−0.030	6	6	10	1.5
	TE-9	(39)	26.46		11.5		13.5	
3	3-6	(15)	30.20	−0.069	3.5	8	8.75	4.75
	C-16	(21)	29.81		13.5		13.25	
4	8-7	(30)	23.33	0.127	11	2.5	10	3.25
	6-10	(20)	20.13		12		12	
5	7-28	(26)	26.10	−0.130	4.5	7.5	7.5	4.5
	2-22	(17)	17.06		12		11	
6	8-27	(35)	31.09	−0.270	3.5	8.5	7.5	3.5
	TE-6	(42)	24.93		8		8.5	
7	7-28	(8)	28.50	−0.071	4.5	3.5	7.5	1
	NC-7	(12)	8.17		9.5		7.5	
8	NC-2	(38)	27.86	−0.394	3	6.5	6	1.5

For each single-cohort composite (SCC) colony, European genotype group is the bottom line, Africanized genotype group is the top line.

development. For each pair, we subtracted the mean score for the Africanized bee genotype group from that of the European bee genotype group. This value was standardized by dividing it by the total number of foragers collected from the SCC colony because there were slight differences in this number. This "behavioral development difference" (Table 3) value was used to correlate worker behavioral development with colony defensiveness.

Experiment 3B: measuring colony defensiveness. The defensiveness of Africanized and European bee source colonies for eight of the nine pairs was measured. The defensiveness measurement method was different from the one used in experiment 2 because the colonies could not all be tested at once in Mexico due to the unavailability of enough volunteers. An alternative method was chosen for which interference between colonies (i.e.; bees stinging the patch of another colony) is less of an issue.

A defensiveness score was assigned to each colony based on observations made while opening and manipulating the colony in a standard manner. The top cover of the hive was removed and three puffs of smoke were applied to the bees (smoke calms bees and is used in virtually all honey bee colony manipulations; see Visscher et al., 1995); then with no further use of smoke a center frame in the top box was removed and replaced, and the top cover replaced. During this manipulation the observer made a subjective assessment of the tendency of bees to sting, hang from the combs, run on the combs, and fly up from the combs towards the observer, assigning a value from 1 to 4 for each trait, with 4 denoting the highest intensity. A colony that scores 4 for all measures would receive a maximum total defensiveness score of 16. Stinging and flight behavior are obviously related to colony defense (Breed et al., 1990), and hanging and running behavior are more likely to be exhibited by the more defensive Africanized bees (Guzman-Novoa E, unpublished data). We tested each colony twice, at 2-day intervals and left colonies undisturbed between tests. For each trial, all colonies were tested in one day, and all within 2 h. All tests were conducted by the same person, who did not know the results of the behavioral development assays.

We calculated average defensiveness scores for the two trials

for each source colony and then calculated a "defensiveness difference" in the same way as behavioral development difference. Population sizes of source colonies were also estimated (as in experiment 2), and these data were used to calculate a "population size difference" for each pairing in the SCC colonies. We again used partial correlation analysis to examine the relationship between behavioral development difference and defensiveness difference, independent of population size.

Results

There was a significant positive correlation between behavioral development differences and colony defensiveness differences for Africanized and European bees (Figure 4; $p < .03$,
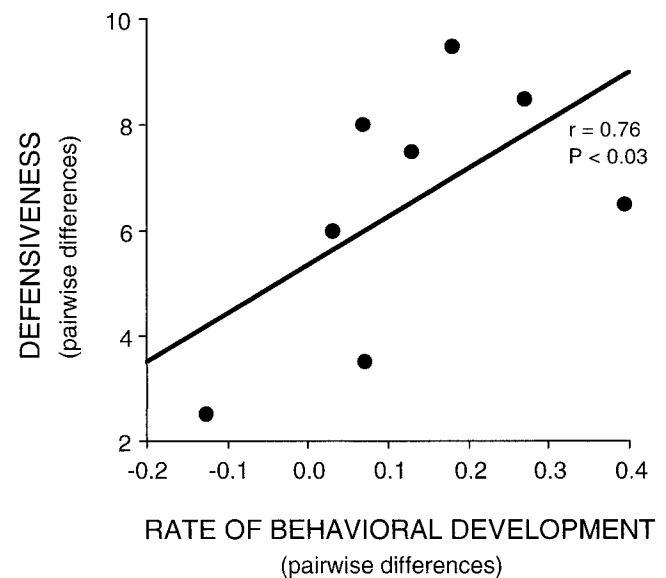

r = 0.76
P < 0.03

DEFENSIVENESS (pairwise differences)

RATE OF BEHAVIORAL DEVELOPMENT
(pairwise differences)

Figure 4
Correlation between differences in individual worker behavioral development and differences in colony defensive behavior for Africanized and European bees in Mexico.

$r = .76$, $n = 8$). Population size differences also were significantly positively correlated with defensiveness differences ($p < .05$, $r = 0.70$, $n = 8$).

Africanized bees showed faster rates of behavioral development than did European bees in SCC colonies ($p = .03$, $n = 9$; Wilcoxon signed-rank test). Bees in the Africanized group started foraging earlier than bees in the European group in eight out of nine pairs, which is indicated by the lower behavioral development scores for the Africanized genotype groups in all pairs except pair 4 (Table 3).

The source colonies of Africanized bees were more defensive than the source colonies of European bees ($p < .01$, $U' = 54$, $n = 8$ Africanized and 7 European colonies; Mann-Whitney U test; Table 3). The only European colony with a defensiveness score comparable to those of the Africanized colonies was colony 8–7; workers from this colony also were the only ones to show faster behavioral development than an Africanized genotype group in a SCC colony (pair 4). Intertrial defensiveness scores for each colony were significantly correlated ($p < 0.001$, $\rho = 0.93$, $n = 15$, Spearman rank order correlation).

Experiment 4: correlation between rates of worker behavioral development and colony flight activity in European bees

As mentioned in the Introduction, older bees in a colony may be at least partially specialized for either defense or foraging (Breed et al., 1990). We therefore tested the two hypotheses that genotypic differences in worker behavioral development are correlated with genotypic differences in colony foraging activity, and that there is a trade-off between colony defensiveness and colony foraging activity.

Methods

We measured colony flight activity, a reliable indicator of colony foraging intensity (Gary, 1967, Marceau et al., 1990), because it is much easier to measure than foraging intensity per se. We measured flight activity for all 14 source colonies used in experiment 2 on 5 different days, at a time of the day with high foraging activity. Flight activity was measured according to the method of Gary (1967). A large wire-mesh funnel was placed temporarily over the entrance of each hive; the bottom of the funnel completely covered the hive entrance, and the top of the funnel tapered to a 2 cm × 2 cm opening. We recorded both the time until the first bee left the hive and flew out of the funnel and the number of bees flying out of the funnel in 1 min. Two observers counted the number of bees leaving from each hive and compared their counts for reliability. Typically the counts were identical; if not they were averaged. On each test day, all source colonies were measured within 1 h of each other. On the first 2 days each source colony was tested twice and the counts averaged. On later days only one measurement was made per colony to decrease the time interval between the measurements of different colonies. Flight activity was measured for 3 days consecutively, followed by a 1-week interval during which defensiveness assays were conducted. The final two measurements of flight activity were made 2 days after the last defensiveness assays were conducted. Flight and defensiveness assays could have not been carried out simultaneously; for defensiveness measurements the colonies need to be undisturbed for at least 2 days before being assayed. Comparison of flight activity data before and after the defensiveness tests suggested no variation due to defensiveness testing on the intervening days (see Results below).

Friedman tests and Kendall coefficient of concordance analyses (Sokal and Rohlf, 1995) were conducted to determine
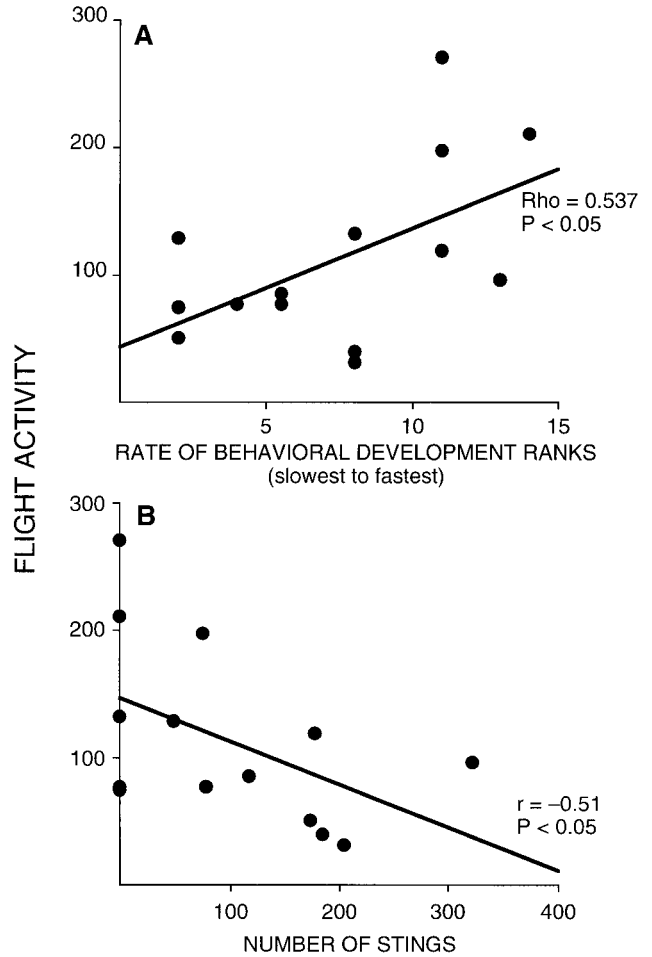

Figure 5
(A) Correlation between individual worker behavioral development and colony flight activity for European bees in Illinois. (B) Correlation between colony flight activity and defensiveness for European bees in Illinois.

whether there were differences in flight activity between source colonies or on different days for each source colony. The 5 days of flight activity data were summed (as in Page et al., 1995b), and these scores were used in correlation analyses. We used estimates of colony population size in partial correlation analyses to control for the effect of this variable on colony flight activity.

Results

There was only a marginally significant positive correlation between individual rate of behavioral development and colony flight activity when all colonies were included in the analysis ($p = 0.053$, $\rho = 0.537$, $n = 14$; Figure 5A). In contrast to the results of experiment 2, removing the five colonies that did not respond in the defensiveness assay reduced this correlation ($p > 0.5$, $\rho = 0.23$, $n = 9$).

There was a significant negative correlation between colony defensiveness and flight activity, even though the assays were conducted on different days ($p < .05$, $r = -.51$, $n = 14$; Figure 5B). When the analysis was repeated excluding the five colonies that did not respond in the defensiveness assay, a slightly lower correlation was obtained ($r = -.45$, $n = 9$), but it was not significantly different from the result of the first analysis.

Table 4

Results of partial correlation analyses for rate of worker behavioral development (BD), colony defensiveness, colony flight activity, and colony population size (size) for data from all 14 source colonies of European bees and single-cohort colonies derived from them

	Defensiveness	Flight activity	Size
Rate of BD	**0.035** (0.595)	**0.009** (0.728)	0.68 (−0.034)[a]
Defensiveness		**0.007** (−0.743)	0.21 (0.294)
Flight activity			0.32 (0.205)

[a] Numbers in parentheses are Spearman's ρ.

Colony flight activity was consistent from day to day ($p < .01$, $W = 0.48$, df = 13, Kendall coefficient of concordance); this suggests that there was no effect of conducting defensiveness testing on the intervening days. There also were significant differences between colonies (Friedman test; $p < .01$, $\chi^2 = 30.9$, df = 14). Colony population size was not correlated with flight activity ($p > .5$, $r = .15$, $n = 14$). This result is not consistent with previous findings showing that larger colonies have greater flight activity (e.g.; Gary, 1967). This discrepancy is again probably related to the fact that colony population sizes were similar to one another in this experiment.

The results from experiments 2 and 4 (a trade-off between colony flight activity and defensiveness and weak correlations between these two measures and the rate of worker behavioral development) prompted us to perform an additional statistical analysis. Partial correlation analyses were performed on the data in experiments 2 and 4 to further examine the relationships between worker behavioral development, colony defensiveness, and colony flight activity. A partial correlation analysis for all three factors and colony size was performed on data from all 14 source colonies and the SCC colonies (Table 4). There were significant correlations between colony defensiveness and rate of worker behavioral development and between colony flight activity and rate of worker behavioral development when other factors were statistically controlled.

Table 5

Differences in rate of behavioral development (BD), defensiveness, and population sizes for pairs of genotype groups of European bees in Illinois

Pair	Genotype group	Differences in		
		Rate of BD	Defensiveness	Size
1	68			
	75	1.32	0.61	1.30
2	44			
	70	0.45	−0.46	−0.75
3	94			
	33	0.61	0.22	−2.50
4	43			
	58	0.06	−0.56	−0.81
5	68			
	80	0.78	0.29	−0.20
6	80			
	75	0.54	0.39	1.50
7	94			
	43	1.17	0.58	7.25
8	70			
	33	0.39	0.27	−10.00

For each pair, differences were calculated from the data in Tables 1 and 2 by subtracting the value for the fast genotype group (top lines) from the value for the slow genotype group.

Colony flight activity and defensiveness showed a significant negative correlation when rate of worker behavioral development and colony size were statistically controlled. There were no significant correlations associated with colony size; power analyses indicated that much larger samples would have been required for correlations of these magnitudes to be statistically significant (analyses not shown).

Correlation between rates of worker behavioral development and colony defensiveness in European bees: an alternative analysis

There was a significant positive correlation between individual behavioral development and colony defensiveness in experiment 3, whereas in experiment 2 the correlation was marginal unless variation in flight activity was accounted for in the analysis. In experiment 2, genotype groups were tested in groups of three to six rather than in pairs as in experiment 3. We therefore renalyzed the data from experiment 2 in a manner similar to the analysis performed in experiment 3 to explore the possibility that this difference in results was related to differences in method of analysis. We used the data from experiment 2 to calculate differences in worker behavioral development and colony defensiveness between selected pairs of genotype groups and their corresponding source colonies. Pairs of genotype groups were selected from among the various groupings only if they were tested in the same SCC colony and if their respective source colonies responded in the defensiveness assay.

Relative rates of worker behavioral development were calculated as in experiment 2: the proportion of precocious foragers from genotype group X divided by the proportion of bees from genotype group X in the colony (Giray and Robinson, 1994). Bees from genotype groups with faster behavioral development received higher values of this measure. We could not calculate mean behavioral development scores as in experiment 3 because there were too few precocious foragers for some genotype groups (the sample of precocious foragers was drawn from three to six genotype groups). Differences between genotype groups were then calculated by subtracting the slower genotype group's score from the faster one's, as in experiment 3. Differences in defensive behavior and population size were calculated similarly. With this method there was a significant positive correlation between individual behavioral development and colony defensiveness in experiment 2 ($p < .02$, $r = .82$, $n = 8$; Table 5 and Figure 6), concurring with the results of the partial correlation analysis in experiment 4.

DISCUSSION

These results indicate that genotypic variation in rate of worker individual behavioral development is one factor that influences variation in colony defensiveness. Results of experiment 1 suggest that the correlation between individual behavioral
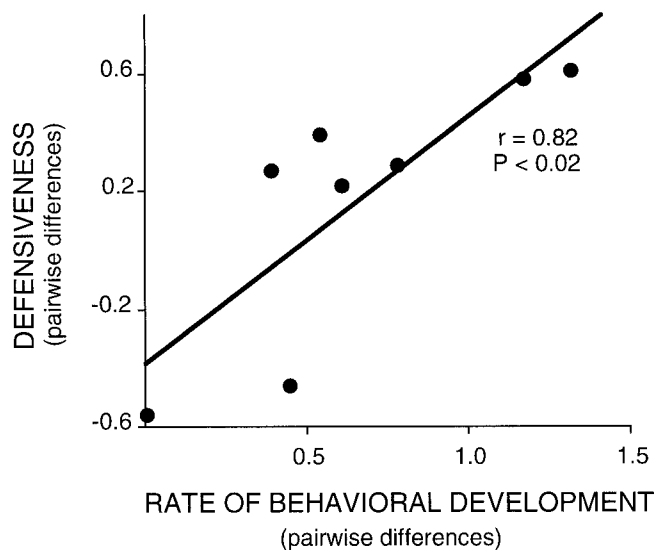
Figure 6
Correlation between pairwise differences in individual worker behavioral development and differences in colony defensive behavior for European bees in Illinois (alternative analysis).

development and colony defensiveness is due to colonies with faster maturing bees being composed of a higher proportion of individuals inclined to perform defensive behavior. As discussed in the Introduction, a faster developmental rate may result in colonies that contain more bees with relatively high amounts of venom (Whiffler et al., 1988) and alarm pheromones (Boch and Shearer, 1966; Crewe and Hastings, 1976; Robinson, 1985), and a relatively low threshold of response to alarm pheromones (Allan et al., 1987; Collins, 1980; Robinson, 1987) and mechanical stimuli that elicit stinging behavior (Kolmes and Fergusson-Kolmes, 1989; Paxton et al., 1994). The significant, but weak, correlations we detected, however, are consistent with previous studies (see Guzmán-Novoa and Page, 1993, 1994), showing that there are other mechanisms governing defensive behavior in addition to the mechanism based on individual rate of behavioral development examined in this study.

The higher correlation between individual behavioral development and colony defensiveness in experiment 3 relative to experiment 2 may have been because half of the colonies studied were of Africanized descent, while in experiment 2 all the colonies were of European descent. It is possible that colony foraging activity is affected more by differences in individual behavioral development than is colony defensiveness in European bees, and the converse is true for Africanized bees. That foraging and defense are at least partially mutually exclusive is supported by our finding of a negative correlation between colony flight activity and defensive behavior in experiments 2 and 4 (foraging activity was not measured in experiment 3). Perhaps there is a difference between Africanized and European bees in the allocation of labor to foraging versus defensive behavior. This is plausible in light of our confirming that Africanized bees start foraging at younger ages than do European bees (Winston and Katz, 1982). This difference, if it exists, may be a selective response for more intense defensive behavior in Africanized bees due to more intense vertebrate predation (reviewed in Roubik, 1989; Winston, 1995). Or it may be an adaptation for more intense foraging behavior in European bees to gather enough resources during the relatively short period resources are available in the native habitat, at the expense of a lower level of defensive

behavior (Winston, 1995). Artificial selection for increased foraging and decreased defensiveness in European bees also has occurred extensively in Europe and North America over the past several hundred years of apiculture.

Differences between the results of experiments 2 and 3 also may have been a consequence of methodological differences. Results of the alternative analysis for the data from experiment 2 suggest that pairwise analyses are more powerful than the way the data were initially analyzed. It probably is easier to precisely determine differences in rate of individual behavioral development between two genotype groups in the same colony rather than infer the rankings of genotype groups based on their performance in several different colonies.

Because our experiments were conducted with colonies with relatively narrow genotypic variance, it is appropriate to question whether the results are relevant to the situation in nature: if colonies are composed of bees with different rates of behavioral development due to multiple mating, are there discernible effects on colony defensiveness and foraging activity? The consistent differences between European and Africanized bees shown in this study suggest that genetic differences in rate of individual behavioral development can contribute to differences in colony traits, at least when comparing different populations of colonies. Colony differences in foraging and defensiveness within populations of European bees have also been reported (Ruttner, 1988), suggesting that similar influences can occur within a population of colonies, despite extreme polyandry.

Our results suggest that the timing of worker maturation (and its hormonal underpinnings; see Fahrbach and Robinson, 1996) may be important in shaping various aspects of colony life history in honey bees. This may be analogous to the role played by the timing of sexual maturation (and its hormonal underpinnings) in shaping life history in noncolonial organisms. Within an organism, variance in a physiological process can influence several traits (Finch and Rose, 1995), leading to trade-offs in life-history strategies (Finch and Rose, 1995, Ketterson et al., 1996). Within a honey bee colony, faster rates of worker behavioral development may be associated more in some cases with increased colony defensive behavior, while in other cases with increased colony flight activity. It is possible that polyandry and the consequent high worker genotypic diversity it engenders ameliorates the severity of these trade-offs in honey bee colonies because bees of different genotypes can partition labor within the same colony, allowing phenotypic plasticity to emerge at the colony level (see Crozier and Consul, 1976; Giray and Robinson, 1994; Page et al., 1995a). In contrast, life-history trade-offs within an individual organism may be more pronounced because phenotypic plasticity can be costly (Lessels, 1991; Roff, 1992; Stearns, 1989). Using a life-history perspective to guide further studies on the effects of individual behavioral development on different aspects of the colony phenotype may contribute toward our understanding of the mechanisms and evolution of insect colony organization.

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