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Behavior Genetics of Honey Bee Alarm Communication

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Communication of alarm by Apis mellifera involves the release of alarm pheromones associated with the sting and accompanying structures. The Committee on the African Honey Bee (Michener, 1972) suggested that the increased aggressiveness of the Africanized bee might be due to an enhanced responsiveness to pheromones, or the release of greater quantities of pheromone.

In order to investigate these possibilities, a measurement procedure for responsiveness to pheromones was developed and used to evaluate this character in several populations. The patterns of inheritance for the measured aspects of alarm response were also examined, as genetic manipulation has been suggested as a feasible way to cope with the Africanized bee.

MATERIALS AND METHODS*

A bioassay procedure to evaluate the response of caged honey bee workers was developed for use under controlled laboratory conditions (Collins & Rothenbuhler, 1978). Frames of emerging worker brood from individual queens were caged separately and held in a 35°C incubator for 24 h. The newly emerged workers were transferred in single-source groups of 30 bees to glass-fronted wooden cages (Kulinčević & Rothenbuhler, 1973) and arranged on shelves in a 35°C walk-in incubator for testing. After at least 24 h for acclimation and aging, testing was initiated using a double-blind identification system. Each group/cage of bees was tested several times per day for three days using a single pheromone, or a mix of pheromones, diluted in paraffin oil 1:9 v/v. At least 1 h elapsed between successive test sessions in the incubator.

The test was initiated by removing the paper liner from the glass front and counting the no. of bees on the front, top, sides, and floor of the cage (initial activity level - IAL). The pheromone was then presented to the bees by placing .03 ml on a slice of cork and holding it under the wire cage floor for 1 m. The time at which the bees began to flicker their wings and increase their locomotion was noted (speed of response - SR), and the intensity of that response (IR) was quantified. Occasionally the bees did not respond at all (none), otherwise a response was barely discernible (weak),

*In cooperation with Louisiana Agricultural Experiment Station.

clearly given by a few of the bees (medium), simultaneously given by most of the bees (strong), or explosive in appearance (very strong). The time of cessation of the response was noted and the duration (DR) calculated. For analysis, SR and DR were adjusted for IAL using a least-squares analysis of covariance. IR was analyzed by chi-square.

RESULTS

1. Variation in inbred European lines

In 1973 four inbred lines of bees available at The Ohio State University were chosen for bioassay on the basis of their behavior in the field (Collins & Rothenbuhler, 1978). Two, Van Scoy and Susceptible, were relatively non-defensive; Brown-Caucasian (Br-Cau) and Resistant were defensive. Isopentyl acetate (IPA), (the only sting alarm pheromone identified at that time, Boch et al., 1962) was used as a stimulus.

Br-Cau and Resistant were not significantly different in SR or DR, but the Br-Cau had more intense responses (Table 1). The Susceptible bees were slower to react, but had similar DR. The Van Scoy bees were slowest, reacted for shorter periods of time, and had the greatest percentage of none responses.

Table 1. -- Response to IPA by bees from four inbred lines. Means (± std. dev.) followed by different letters are significantly different.

	Line			
	Van Scoy	Susceptible	Resistant	Br-Cau
SR(s)	30.8 ± 23.5a	22.0 ± 21.3b	15.9 ± 19.9c	16.0 ± 20.6c
	$F = 6.05$	$d.f. = 3, 357$	$P < 0.01$	
DR(s)	21.1 ± 24.1g	43.2 ± 21.7h	43.3 ± 24.3h	37.0 ± 20.1h
	$F = 5.57$	$d.f. = 3, 357$	$P < 0.01$	
IR(% obs.)				
none	25	18	12	12
weak	15	26	25	9
medium	25	35	27	16
strong	32	21	35	25
very strong	3	0	1	38
	$\chi^2 = 295.67$	$d.f. = 16$	$P < 0.01$	

2. Mode of inheritance in inbred European lines

The Br-Cau line and another nondefensive line, YD, were used as parents for F_1 and backcross matings following the scheme of Rothenbuhler (1960) (Collins, 1979). Worker offspring of inbred queens inseminated by a single drone were assayed using the cage test procedure (Table 2). DR was not measured. A genetic analysis of the data indicated that a more responsive phenotype was partially

dominant to a less responsive phenotype.

Table 2. -- Response to IPA by workers from YD, Br-Cau, F_1, and backcross matings. All means significantly different but those *.

	YD	Br-Cau	F_1	Backcross to: YD	Backcross to: Br-Cau
SR(s)	12.6	4.8*	6.3	8.7	5.2*
IR(% obs.)				In comparison to the parental patterns, the colonies tested were:	
none	8	2	1		
weak	34	3	6	7 ≃ Br-Cau	10 ≃ Br-Cau
medium	45	18	25	12 inter-	5 inter-
strong	11	50	49	mediate	mediate
very strong	2	27	19	7 ≃ YD	

Using the segregation pattern in the backcrosses, it was estimated that 2 or 3 genes controlled each of the behavioral components.

3. Variation in free-mated European stocks.

Compounds derived from the honey bee sting were identified by gas chromatography and mass spectrometry (Blum et al., 1978, in prep). The stimulation of caged worker bees was used to bioassay synthetic forms of these compounds and many did show alarm pheromone activity (Collins & Blum, in press, in prep). Among the several colonies used as sources for worker bees, differences in response were observed. Some colonies were significantly slower to respond to a particular pheromone. However, response to one compound did not always indicate the level of response to another. Table 3 shows an example of 3 colonies, with #9 slowest to react to isopentyl acetate and benzyl acetate, but fastest to respond to isopentyl alcohol.

Table 3. -- Significant colony differences in speed of response (SR) (s) to alarm pheromone by caged worker bees.

Pheromone	Colony number 7	8	9
isopentyl acetate	5.08	4.20	7.54*
isopentyl alcohol	5.63	7.58*	4.93
benzyl acetate	5.65	5.56	11.17*

* Significantly different from means in column and row, $P < 0.05$.

4. Population differences between European and Africanized bees.

Ten field colonies each of European (multiple-drone inseminations) and Africanized (free-mated) stock from a single apiary in Venezuela were used as sources for worker bees. Bees were emerged, caged, and tested as described. The pheromone used was a mixture of butyl acetate (6.74%), isopentyl acetate (13.47%), isopentyl alcohol (6.74%), 2-heptanol (0.01%), hexyl acetate (6.74%), 2-heptyl acetate (0.05%), 2-nonanol (42.66%), benzyl acetate (0.01%), octanol (13.47%), and 2-nonyl acetate (10.11%) diluted 1:9 v/v in paraffin oil. The two populations were similar in how quickly they responded, but the

Africanized workers reacted more strongly and for longer periods of time (Table 4).

Table 4. -- Response to an alarm pheromone mixture by worker bees of European or Africanized genotype (means ± std. error).

	European	Africanized	
SR(s)	3.4 ± .3	4.1 ± .3	
	F = 2.07	d.f. = 1, 17	P = 0.17
DR(s)	73.5 ± 3.3	100.8 ± 3.6	
	F = 32.03	d.f. = 1, 17	P < 0.01
IR(% obs.)			
none	2	0	
weak	11	6	
medium	55	37	
strong	30	48	
very strong	2	9	
	χ^2 = 34.65	d.f. = 4	P < 0.01

5. Estimates of heritability

Drones from each of six European colonies (Louisiana, USA) and six Africanized colonies (Monagas, Venezuela) were used to singly inseminate three queens each from three inbred lines. Workers from each of the matings were tested for response to IPA. Data for IAL and SR were analyzed by a mixed model least-squares analysis of variance and the variance components calculated were used to estimate heritability, a genetic parameter indicating the ease of modification of a character by selection (Collins et al., in prep). Duration was not measured due to time limitations, and the intensity observations were in inappropriate form for such analysis. Estimates were made for the European population alone, the Africanized alone, and the combined population. The values in Table 5 indicate that SR could be readily altered by selection based on the lab test results, but not IAL. However, the high correlations between the two must be considered. A selection program reducing the response to alarm pheromones, which occurs early in the defensive behavior sequence (Collins et al., 1980) could be used to reduce the defensiveness shown by Africanized bees. However, if a bee results that shows reduced activity levels as well, other economically important traits such as honey production could be adversely affected.

Table 5. -- Estimates of heritability and phenotypic and genetic correlations for two behaviors of caged worker bees.

	Heritability		Phenotypic
Population	IAL	SR	correlation
European	.05	1.28	-.57
Africanized	.12	.31	-.26
Combined	.04	.83	-.49

DISCUSSION

If genetic selection is to be a viable way to combat the defensiveness of the Africanized bee, there must be existing variation in the behavior and we must have an adequate system to measure that behavior. The assay procedure described does in fact show that considerable variation in aspects of alarm communication exists in populations of the honey bee.

The genetic analyses indicate that the parameters measured are probably inherited in a quantitative manner, i.e. several genes are involved in the control of each aspect, and that at least some of them are sufficiently heritable that a selection program might significantly alter the behavior.

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