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GENETICS OF THE RESPONSE OF THE HONEYBEE TO AN ALARM CHEMICAL, ISOPENTYL ACETATE *

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Summary

In laboratory tests, two inbred lines of honeybees (*Apis mellifera*) different in colony defensive behaviour were shown to differ in their initial general activity level, the time to react to isopentyl acetate, and the initial intensity of the reaction. Evaluation of an F_1 and backcrosses to both lines indicated that a more responsive phenotype was dominant to a less responsive phenotype in all three characteristics. The degree of dominance for time to react was estimated as 0·502 and the heritability as 0·681. It was estimated that 2 or 3 loci controlled each of the three components of behaviour.

Introduction

The variability of expression of defensive behaviour by the honeybee (*Apis mellifera*) is in part due to genetic variability. Several researchers have investigated the differences between races (Stort, 1970; 1974; 1975*a*; 1975*b*; 1975*c*; 1976) and between inbred lines (Rothenbuhler, 1964; Boch & Rothenbuhler, 1974). All these observations of the behaviour were made on entire colonies. To reduce environmental variability below that present under these field conditions, a laboratory test was developed (Collins & Rothenbuhler, in press) utilizing isopentyl acetate (IPA), a major active component of honeybee sting alarm pheromone (Boch et al., 1962). Stocks of bees that consistently showed differences in defensive behaviour during colony management procedures in the field showed comparable differences in response to IPA during the laboratory tests. This laboratory testing procedure was used here to study the genetics of the differences in response between two inbred lines.

Materials and Methods

Two inbred lines of bees were chosen for their difference in expression of defensive behaviour. The Brown-Caucasian (*Br-Cau*) line, from The Ohio State University Bee Laboratory, was characterized as 'cross'. A 'gentle' line (*YD*) was obtained from the USDA Bee Breeding and Stock Center Laboratory, Baton Rouge, LA. Following the technique of Rothenbuhler (1960), inbred matings were made between a queen and a single drone, to provide F_1 and backcross generations. Worker bees from each parental line, the F_1, and the backcrosses to each parental line, were evaluated using the laboratory testing procedure.

Mature brood emerged in an incubator during 24 h, and 25 of the young bees were placed in a small glass-fronted wooden cage, described by Kulinčević and Rothenbuhler (1973). Water and sugar syrup were always available in gravity feeder vials. The test cages were arranged several cm apart on shelves in an observation-hive shelter, converted to serve as a walk-in incubator, maintained at 35°C. Testing took place in the shelter on day 2, 3 and 4 after caging, at 10h, 13h and 16h, under subdued lighting. Two, three or four cages were stocked from each colony tested.

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The test consisted of:

1. Evaluation of the activity of the bees prior to testing as :
   quiet—all bees on the comb at the cage back;
   slightly active—fewer than 10 bees moving about the sides and top of the cage; or
   active—more than 10 bees on the sides and top of the cage.
2. Presentation of the control stimulus, paraffin oil, for 60 s. Two drops of oil on a slice of No. 2 cork were inserted under the wire floor of the cage using a dissecting needle. A reaction to the control was never seen.
3. Presentation of the test stimulus, 1 part IPA (mixed isomers, J. T. Baker Co.) in 9 parts paraffin oil, by volume, for 60 s, in the same manner as the control.
4. Measurement of the period after presentation of the stimulus before a definite reaction was observed. The bees mainly responded in two ways. The more apparent was a flickering movement of the wings, usually as part of the initial response and continuing intermittently thereafter. The other was a general increase in locomotion, not oriented in direction. Bees made short, jerky runs on the cage surface, ending in brief confrontations with other bees. It was often difficult to ascertain if a specific bee had begun to respond, but the simultaneous response of several or most of the bees was quite distinctive. If the bees did not respond within 60 s, 'no reaction' was the observation recorded.
5. Evaluation of the initial intensity of the reaction as *none* (if no reaction was seen) or as *weak, medium, strong,* or *very strong,* based on the degree of change in activity and number of bees involved.

Following each complete test of all bees, the cages were rearranged within the same shelf positions. This ensured that the investigator carried out the testing with no knowledge of the source or previous response of the bees in a particular cage; it also diminished any effect of shelf position.

Results

Initial activity

For the character of initial activity level, the parental lines were significantly different ($\chi^2=53{\cdot}07$, $P<0{\cdot}01$). The *YD* were most often scored as quiet or slightly active, and the *Br-Cau* as active (Fig. 1.). The F_1 showed heterosis, being more frequently scored as

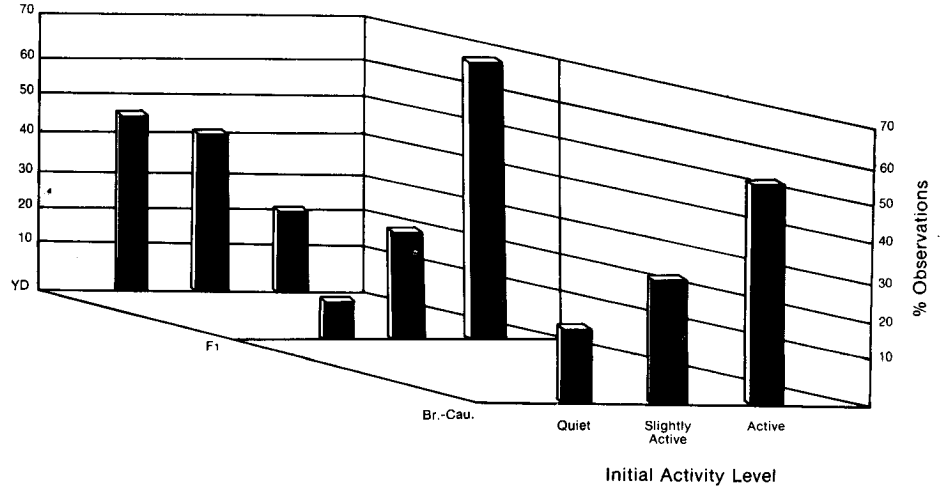

FIG. 1. Percentage of observations of initial activity level in each of three categories (quiet, slightly active, active) for the parental lines and for F_1.

active then the *Br-Cau* ($\chi^2=11\cdot19$, $P<0\cdot01$). Of 15 backcrosses to *Br-Cau* queens, 11 were similar to the *Br-Cau*; the remaining 4 differed from both parents. The backcrosses to *YD* queens showed wide variation: 7 similar to *Br-Cau*, 6 intermediate, 13 similar to *YD* (χ^2 significant at $P<0\cdot05$ or $<0\cdot01$). The relationships of the F_1 and backcrosses to the parental lines indicate that an active phenotype showed overdominance to a quiet phenotype.

Frequency of no reaction

In a number of tests the bees did not react to IPA. Rather than use an arbitrary period of 60 s in calculations of time to react, the frequency of no reaction was analysed as a separate aspect of the character. Table 1 shows that *YD*, and several of the backcrosses to *YD* queens, had significantly greater frequency of no reaction than the other bees (χ^2 significant at $P<0\cdot01$). These groups were also the slowest to react.

TABLE 1. Number of tests with isopentyl acetate resulting in no reaction, in the parentals, F_1 and backcrosses. In the last two columns ** indicates $P<0\cdot01$; NS indicates no significant difference.

	No reaction	*Reaction*	χ^2 *evaluation, compared to:* *Br-Cau*	*YD*
Br-Cau	2	178		
YD	12	142	**	
F_1	1	257	NS	**
backcrosses to:				
Br-Cau colony 3640	1	23	NS	NS
14 other colonies	0	18 or 24	NS	NS
total	1	329		
back crosses to *YD*:				
colony 3629	7	17	**	**
colony 3637	2	20	**	NS
colony 3649	3	14	**	NS
colony 3660	2	16	**	NS
22 other colonies	0 or 1	15 to 39	NS	NS
total	21	537		

Time to react

Observations of the time to react (in seconds) were categorized by initial activity level and genetic population, transformed to logarithms, and analysed by a two-way least squares analysis of variance and a least significant difference test (Table 2). Active bees reacted more quickly than quiet ones, but this did not account for all differences found between the five genetic groups. The 'cross' parents (*Br-Cau*) reacted more quickly than did the 'gentle' parents (*YD*). The F_1 generation was intermediate, and significantly different from both parents, but it more closely resembled the *Br-Cau* parent. All but one of the backcrosses to *Br-Cau* queens reacted as quickly as the *Br-Cau* parent. The backcrosses to *YD* queens were highly variable; 3 similar to *Br-Cau*, 14 intermediate, and 9 similar to *YD*.

TABLE 2. Least-squares means of time in seconds taken to react to isopentyl acetate by bees of the three activity levels in each genetic population, with the number of observations used in calculation (in brackets).

All total population means, and all total activity level means, are significantly different ($P<0\cdot01$) unless marked NS. The interaction of population and activity level was also significant ($P<0\cdot01$).

Population	*No. colonies tested*	*Quiet*	*Slightly active*	*Active*	*Total*
Br-Cau	5	5·2 (27)	5·3 (55)	4·0 (96)	4·8NS (178)
YD	3	17·8 (59)	12·5 (58)	7·4 (25)	12·6 (142)
F_1	5	7·6 (17)	6·6 (62)	4·8 (178)	6·3 (257)
backcrosses to:					
Br-Cau	15	6·6 (26)	5·0 (151)	3·8 (149)	5·2NS (326)
YD	26	12·8 (123)	8·0 (265)	5·9 (148)	8·7 (536)
Total		10·0 (252)	7·5 (591)	5·2 (596)	7·5 (1439)

Again, the pattern of segregation indicated dominance of the phenotype of the 'cross' parent. Four estimates of the degree of dominance were made, using the logarithm of the mean number of seconds to react, from the five populations. The mean estimate of degree of dominance was 0·502. That is, a fast-reacting phenotype was partially dominant over a slow-reacting one.

The regression of offspring on midparent was utilized to estimate the heritability of the time to react. For this purpose, a 'family' was designated as the offspring of all matings that involved drones that were brothers and queens that were sisters. The phenotypic values of parents (queen or drone) were calculated from observations on worker bees that were sisters of the individual. Offspring values were the mean from tests on workers from all matings in a family. The estimate of heritability, using the logarithm of the number of seconds to react, was 0·681.

Initial intensity

Initial intensity of the reaction was also influenced by the initial activity level (χ^2 significant at $P < 0\cdot01$). Active bees were more likely to react strongly or very strongly, quieter bees to react less intensely (Table 3).

TABLE 3. Relationship between intensity of reaction to isopentyl acetate and initial activity level. Entries are percentages (of the total number of observations) that fall into each category.

	Initial activity level			
Intensity of reaction	*Quiet*	*Slightly active*	*Active*	*Total*
none	0·9	1·2	0·8	2·9
weak	4·7	3·5	2·1	10·3
medium	7·6	17·5	9·2	34·3
strong	4·4	16·2	14·8	35·4
very strong	0·4	3·2	13·4	17·1
total	18·0	41·6	40·4	100·0

$\chi^2 = 46\cdot39$ $\quad P < 0\cdot01$

For this character, also, there were differences between the parental lines as analysed by a χ^2 test. *Br-Cau* bees reacted with strong or very strong intensity most often, *YD* bees with weak or medium intensity (Fig. 2). The F_1 was similar to *Br-Cau*. Ten backcrosses to *Br-Cau* queens were similar to *Br-Cau*, the remaining 5 were intermediate. Of the backcrosses to *YD* queens, 7 were similar to *Br-Cau*, 12 intermediate, and 7 similar to *YD* (χ^2 significant at $P < 0{\cdot}05$ or $< 0{\cdot}01$). As with the two other characters, the phenotype indicated as dominant by the segregation patterns was that associated with the 'cross' parent.

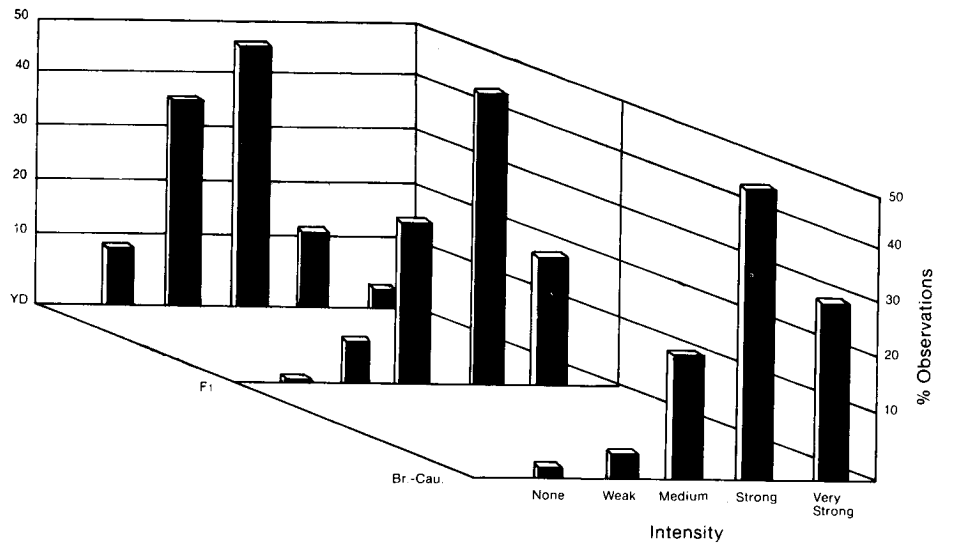

FIG. 2. Percentage of observations of initial intensity of reaction in each of five categories for the parental lines and for F_1.

It might be expected that the intensity of the reaction would be related to its speed, and this proved to be so. Five genetic populations whose intensity of reaction was classified respectively as none, weak, medium, strong, very strong, took mean times of 60·0, 15·1, 7·8, 5·7, 3·7 s to react; all means except the last two were significantly different; $F = 60{\cdot}22$ ($P < 0{\cdot}01$), df = 3 and 1438.

Number of genes

The proportion of colonies from the backcrosses to the *YD* parent whose character was similar to, or more extreme than, either parent was used to estimate the number of genes controlling expression of the character. For initial activity level the estimate was one or two genes; for time to react, two or three genes; and for intensity of reaction, two genes. Comparison of the segregation patterns of the three measures in the backcross colonies indicates that different genes probably affect each character.

Discussion

In the interpretation of these laboratory test results, in comparison with whole-colony measurements of defensive behaviour, two possible criticisms need to be considered. Defensive behaviour is normally exhibited in or at the periphery of the colony (queen,

workers, brood, combs, etc.), by guard bees 2 to 4 weeks old (Free, 1965), but in our tests, the bees are not in a colony, and they were younger. Subsequent work in our laboratory, as yet unpublished, shows that the behaviour seen in the cages was identical to the response of bees in small observation hives to alarm chemicals (including IPA) in the absence of other stimuli such as motion. In addition, marked bees that were cage-tested when 4 and 6 weeks old showed the same response levels as their sisters, tested in the manner of this paper (Collins, unpublished).

Our results do not seem consistent with those of Boch and Rothenbuhler (1974), who found that, in the colony, the 'gentle' phenotype was dominant to the 'aggressive' phenotype for response (in number of bees) to IPA, and to human breath administered at hive entrance. Response to disturbance (removing the hive cover) showed no dominance.

A comparison of our work with that of Stort (1975*c*, 1976, 1978) is first directed to his two measurements of defensive behaviour that correspond most closely to our measurement of the time to react to IPA: the speed of response to alarm stimuli. Stort measured the time before the first sting, and before the bees became 'aggressive'. Stort stated that these were correlated, probably having some genes in common, and that the African (aggressive) phenotype was at least partially dominant over the Italian. Our results lead to similar conclusions. In any case, the wide variation observed among the backcross colonies was also seen in backcrosses to the *Br-Cau* and *YD*.

Stort's other measurements, the number of stings in a leather ball near the hive entrance, the number of stings in the gloves of the observer, and the distance through which the bees followed him, might correspond to intensity of the reaction. For the number of stings in the ball, the African phenotype was dominant, but the gentle Italian phenotype was dominant for the other two characters. Stort estimated that 8 genes controlled the 5 characters he measured, and our estimates are similar.

The various studies used different populations of bees, except that the *Br-Cau* line was used by Boch and Rothenbuhler (1974) and by us. Dissimilar alleles, or loci, may be responsible for the variation observed in each instance. Also, since the three sets of experiments measure defensive behaviour in different ways, they may deal with slightly different components of a character controlled by distinct genetic systems.

We have demonstrated that at least two major components of response to alarm pheromone, the speed of reaction and the strength of reaction, are variables on which selection can be focused. The results presented also indicate that both components of defensive behaviour are enhanced in bees of a generally active phenotype. This factor should certainly be taken into account in attempts to select for less defensiveness; if such selection substantially reduced activity in general, it could reduce foraging, and hence economic productivity.

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