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RESPONSE TO ALARM PHEROMONE BY EUROPEAN AND AFRICANIZED HONEYBEES

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Summary

Workers of two honeybee (*Apis mellifera* L.) geographical types, European and Africanized, in Venezuela were assayed for response to sting-associated alarm pheromone. Groups of young bees were exposed to either isopentyl acetate (IPA) or a mixture of 10 alarm chemicals, including IPA, at five concentrations. Africanized bees were more active before exposure to the pheromones, responded with greater intensity and in greater numbers, and continued to respond for a longer time. Europeans bees responded more quickly for concentrations of 1/100 and 1/1000; otherwise, the speed of response was the same as for Africanized bees. It was concluded that relatively more intense colony defence by Africanized bees is caused, in part, by greater responsiveness to alarm pheromones.

Introduction

In the model of honeybee colony defence proposed by Collins et al. (1980) the first step is one of alerting, in which a worker bee detects a disturbance. For many worker bees, the major stimuli involved in the initiation of this step are alarm pheromones associated with the sting apparatus of their hivemates. An alerted bee may extrude her sting and move about, releasing pheromones that stimulate defensive behaviour by other bees. This communication of alarm is of special interest today because of its possible role in the extreme defensive behaviour demonstrated by the Africanized honeybee (Collins et al., 1982).

Levels of production of alarm pheromones by honeybees have been compared with differences in defensive behavior. Boch and Rothenbuhler (1974) studied two inbred lines of European bees, one more defensive than the other and found one third more isopentyl acetate (IPA), the first sting alarm pheromone identified (Boch et al., 1962), in the more defensive line. Kerr et al. (1974) recorded amounts of IPA in one colony of Africanized bees and four F_1 colonies that had resulted from crossing with an Italian strain. These are similar to amounts reported elsewhere for European colonies (Boch et al., 1962). There was no correlation of production with measures of intensity of colony defence. Crewe (1976) reported that African bees produced IPA in the same quantities as European bees, although their defensive behaviour was quite different.

Boch and Rothenbuhler (1974) and Crewe (1976) speculated that the differences in defensive behaviour seen between European and African bees were due more to different thresholds of response than to the quantity of alarm pheromone produced. Collins (1979) demonstrated differences in response to equal amounts of IPA by defensive and non-defensive stocks of European honeybees. Bees of a highly defensive line reacted more quickly and more vigorously than the non-defensive line.

The experiments presented here were done to determine whether or not Africanized bees respond differently from European bees to alarm pheromones and to determine if there are major differences in thresholds of response to the pheromones.

Materials and Methods

The European honeybees in these studies were of various commercial varieties from North America which have in their ancestry representatives of several European subspecies. Queens were taken to Venezuela and used to establish European colonies. Africanized colonies were established from locally-caught swarms and represent the feral population. As neither the European nor the Africanized bees can correctly be called race, subspecies, stock, or line representatives, we use the term 'geographical type' to indicate that the bees we studied showed major characteristics typical of descriptions for temperately- or tropically-adapted bees.

The methods for assaying the response to alarm pheromone were those developed by Collins and Rothenbuhler (1978). Mature worker brood was caged separately by colony source and emerged in an incubator within a period of 24 h. Thirty newly-emerged workers from a single colony were introduced into each of several small cages (Kulinčević & Rothenbuhler, 1973). For Experiment 2 we used the glass-fronted wooden cages described; for Experiment 1, cardboard and plastic replicas. Water and 50% (wt/wt) sucrose solution were freely available in gravity-feeder vials. The cages were then arranged several centimeters apart on shelves in an incubator room maintained at 35 °C. Newly-emerged bees were used because they had not yet begun to produce alarm pheromone (Boch & Shearer, 1966) which could interfere with the assay. Although bees of this age are not normally involved in colony defence, in cages they respond with the same, though less intense, behaviour as older bees (Collins, 1980). The bees were allowed to adjust to the cages in the dark for at least 36 h. Testing was done on the following three days under indirect lighting from a 60-W bulb.

Observations made during the test included the following.

(1) Initial activity level: the number of bees moving about the sides, top or front of each cage prior to testing.

(2) Speed of response: the time (s) following presentation of an alarm pheromone under the floor of a cage until a response was initiated. Onset of the response was observed as a group reaction expressed as flickering movements of the wings and a general increase in locomotion. If the bees did not respond within 30 s, the observation 'no reaction' was recorded.

(3) Intensity of the response: none, if no reaction was seen, or weak, moderate, strong or very strong, based on the number (1–5, 6–15, 16–24 or 25–30 and vigour of responding bees.

(4) Duration of the response: the time from onset of a reaction until the bees returned to their initial level of activity.

After each test of all caged bees, the cages were randomly re-arranged to ensure that shelf position within the incubator did not have a significant effect. Cage numbers were not visible to the observer during testing.

Experiment 1

Ten European and 10 Africanized colonies from apiaries near Acarigua, Venezuela, were used as sources of worker bees. Three to five cages per colony were prepared as above and were tested five times over a period of three consecutive days. The alarm pheromone used for testing was IPA in paraffin oil (medicinal paraffin) diluted 1:9 (vol/vol). Paraffin oil by itself does not elicit a response (Collins, 1979). Data were transformed to logs and analysed by bee type using least-squares analysis of covariance, with speed of response and duration as dependent variables and initial activity as the independent variable (SAS, 1982). Intensity data were analysed by contingency chi-square, deleting categories with less than five expected observations (Freund et al., 1960).

Experiment 2

Workers from 12 colonies of each geographical type were tested using five concentrations of an alarm pheromone mixture based on naturally occurring levels of 10 compounds previously shown to stimulate alarm responses in worker honeybees (Collins & Blum, 1982, 1983). The mixture contained 6·7% butyl acetate, 13·8% isopentyl acetate, 6·7% isopentyl alcohol, 0·02% 2-heptyl alcohol, 6·7% hexyl acetate, 0·06% 2-heptyl acetate, 42·7% 2-nonyl alcohol, 0·02% benzyl acetate, 13·8% *n*-octyl alcohol, and 10% 2-nonyl acetate (Blum, personal communication). Relative quantities for the components other than IPA were not known for Africanized workers; however, our experience with various mixtures showed no evidence of the ratios of components affecting responses as can be seen in some lepidopteran sex-pheromones (Roelofs, 1978).

The pheromone solution was serially diluted in medicinal paraffin (vol/vol) at levels of 1:10, 1:100, 1:1000, 1:10 000 and 1:100 000. Three cages per colony per concentration were tested a total of six times during three days, with observations on initial activity level, speed of response, and intensity of response as done in Experiment 1. Duration was not measured because of limitations of facilities and time. Analysis was by least-squares analysis of

TABLE 1. Speed and duration of response (mean ± SE) to alarm pheromone, IPA, by newly emerged European and Africanized caged honeybees.

Honeybee type	*No. bees active initially*	*Time (s) to response*	*Duration (s) of response*
European	4·51±1·34 *ns*	3·2±0·3 *ns*	73·5±3·3 *
Africanized	5·74±1·29	4·1±0·4	100·8±3·6

ns = Non-significant.
* = Difference between means significant at $P<0·0001$.

covariance and contingency chi-square (as in Experiment 1), and a least-significant difference test to separate means.

Results

In Experiment 1, the initial activity level of the caged workers and the speed with which they responded to the IPA were not significantly different for the two bee types (Table 1). Differences were found in the intensity with which the bees responded and the duration of response. In the Africanized type we observed a greater proportion of strong and very strong responses (Fig. 1), with more bees in the cage responding and the level of individual response being more vigorous. The very strong response was almost 'explosive' in appearance, a description that has been used for instances of colony defence by this type of bee (Stort, 1976). Africanized workers continued to respond for a longer period than the European workers (Table 1).

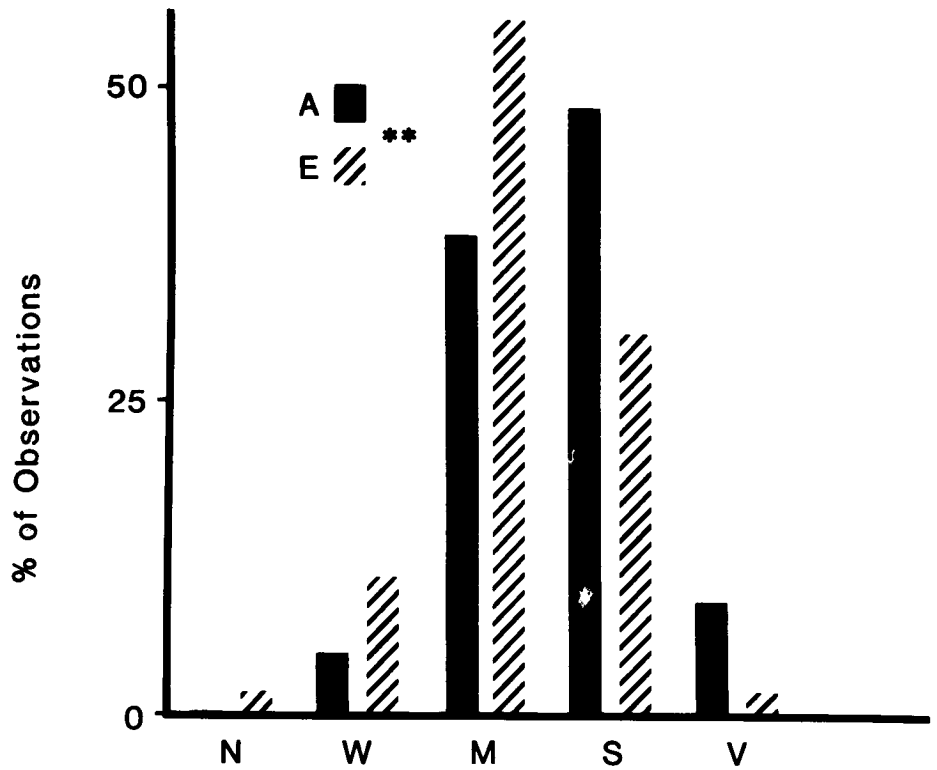

FIG. 1. Intensity of response to alarm pheromone (IPA) by European (E) and Africanized (A) honeybees. N = no response; W = weak; M = moderate; S = strong; V = very strong.
$\chi^2 = 34·65$ (df = 4, $P<0·01$).

TABLE 2. Response (mean ± SE) of newly-emerged European and Africanized caged honeybees to five concentrations of alarm pheromone components.
Pheromone concentration: A = 1/10; B = 1/100; C = 1/1000; D = 1/10 000; E = 1/100 000.
Initial activity level is expressed in number of bees and speed of response in seconds.
Means for both lines for speed of response followed by the same letter are not significantly different at $P<0.01$ as determined by the least significant difference.

Honeybee type and test	*No. bees active initially and speed of response for concentration:* A	B	C	D	E	*Mean*
Initial activity level						
European	4·5±0·3	4·9±0·3	4·5±0·3	4·8±0·2	5·1±0·3	4·8±0·2*
Africanized	5·5±0·3	5·6±0·2	5·6±0·2	6·3±0·2	6·1±0·3	5·8±0·2
Speed of response						
European	5·7±0·4*a*	6·5±0·5*b*	8·5±0·6*c*	8·4±0·8*c*	11·6±1·0	
Africanized	5·0±0·4*a*	8·5±0·4*c*	10·5±0·6*d*	10·3±0·7*cd*	9·7±0·8*cd*	

	Initial activity level		*Speed of response*	
	F	*P*	*F*	*P*
Concentration	1·85	0·09	32·95	0·0001
Honeybee type	9·02	0·0001	3·50	0·004

* Means significantly different at $P<0.01$.

In Experiment 2, the Africanized bees were slightly more active, initially, than the European ($P<0.01$, Table 2). Also, the two bee types were significantly different in the speed of the reaction. When the concentration of the alarm pheromone was reduced from 1/10 to 1/100 or 1/1000, the European bees responded about 2s faster than the Africanized bees. For the two lowest concentrations, the speed of response was the same for both types. As concentrations were increased, faster responses were observed in both bee types.

The slight difference in speed of response to the stimulus at 1/100 and 1/1000 concentrations did not affect the difference in intensity of the response (Fig. 2). The Africanized bees continued to have more bees responding more vigorously to pheromone concentrations of 1/10, 1/100, and 1/100 000 but were similar to the European for 1/1000 and 1/10 000. The significant heterogeneity chi-square ($\chi^2 = 20.35$, df = 8) confirms that there were more strong responses for higher concentrations of pheromone.

The covariance of initial activity level and time to react was significant in both experiments (1, $F = 38.7$, $P<0.001$; 2, $F = 68.34$, $P<0.001$). Bees that were initially more active, responded more quickly. This was also true for the two European lines tested by Collins (1979).

Discussion

The behaviour being measured by the test for response to alarm pheromone encompasses both portions of the stimulus–response pathway, perception of the stimulus and response to it. While it would be necessary to do physiological assessments such as electro-antennagraphs to measure actual perception of the alarm pheromone, it is useful to consider the data on speed of response as an indication of how readily the pheromone is perceived. The data reported here are the first showing that the European bee type is slightly more sensitive to alarm pheromone, as evidenced by the differences in speed of response to the 1/100 and 1/1000 concentrations. However, since many of the sting alarm-pheromones are produced in greater quantity in the Africanized geographical type (Collins et al., 1987), the Africanized bees might require less sensory sensitivity. This idea also has been proposed by Gonçalves and Stort (1978).

The major differences found between the two geographical types were in the magnitude of the active responses by the bee: the intensity of the response and its duration. The Africanized bees were consistently more responsive than the European. This type of difference is also seen in studies of the complete sequence of colony defence in which Africanized colonies repond more quickly and in greater numbers, inflict more stings on 'intruders' and pursue the attack for longer periods and over longer distances (Michener, 1972; Stort, 1980; Collins et al., 1982).

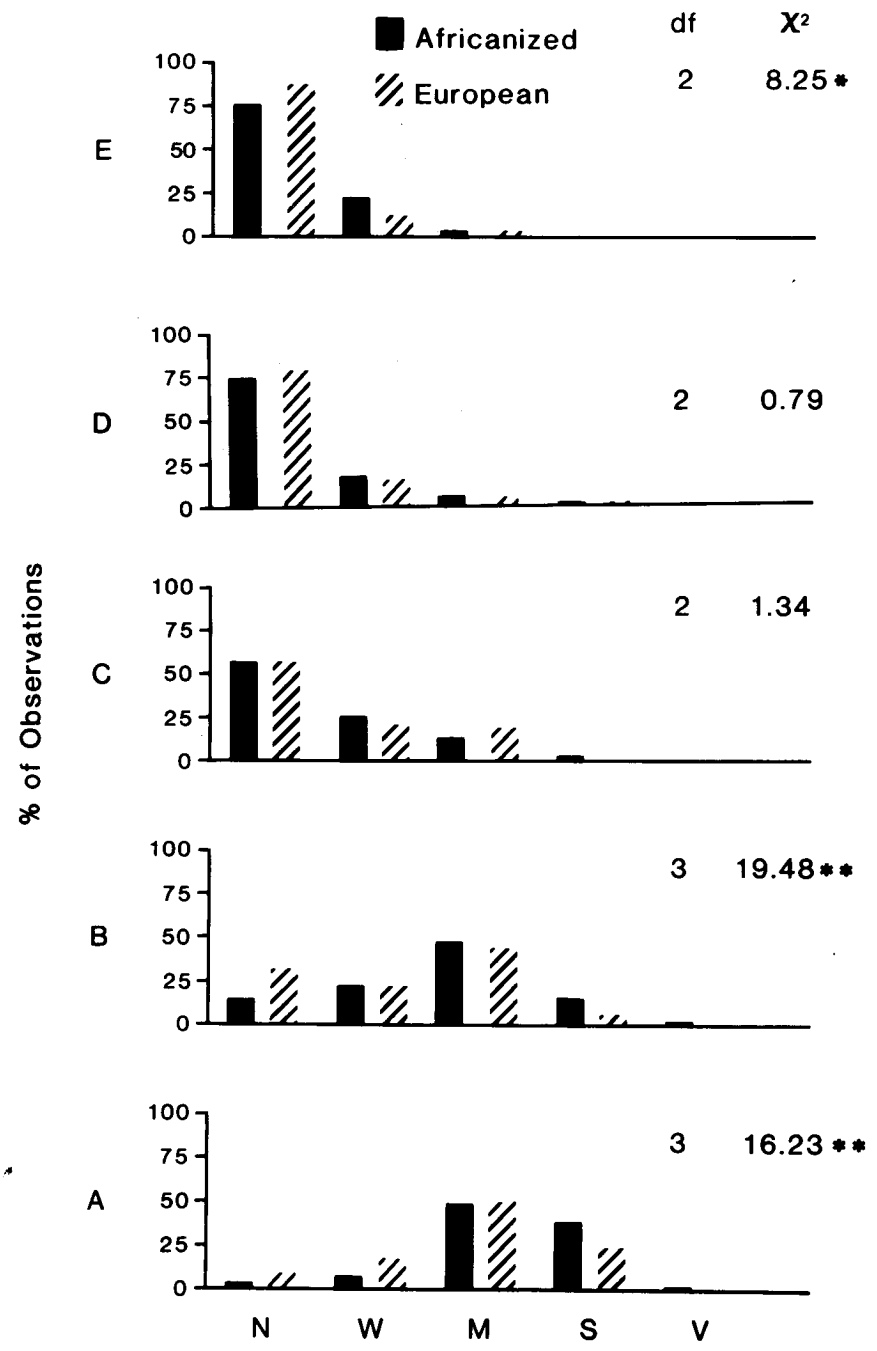

FIG. 2. Intensity of response to five concentrations of alarm pheromone (mixture of 10 components) by European (E) and Africanized (A) honeybees.
Concentrations of pheromone: A = 1/10, B = 1/100, C = 1/1000, D = 1/10 000, E = 1/100 000; other abbreviations as for Fig. 1.
* Significant at $P<0{\cdot}05$; ** Significant at $P<0{\cdot}01$.

There are two hypotheses to explain these dissimilarities: first the two types have different thresholds of excitation, or second, the thresholds are the same but the Africanized type generally exists at a higher level of nervous excitation (closer to the threshold) and needs less stimulation to raise it to the threshold. The data on initial activity level give weak support to the second hypothesis. The Africanized bees showed a higher level of activity prior to stimulation in Experiment 2. The same was true for the European lines studied by Collins (1979), the more defensive line being more active prior to testing. This can also be seen in comparisons of general activity within the hive; European bees are very quiet on the combs during colony manipulation; Africanized bees, both workers and queens, are more active and prone to fly off the combs.

The data presented here indicate that the high level of colony defense exhibited by Africanized bees can be attributed in part to their response to alarm pheromone. More Africanized workers respond to the chemical signal than European workers, and the response is more energetic and of longer duration. In addition to demonstrating differences between the two honeybee types in response to the same level of pheromonal stimulation, investigation into the effect of a greater concentration of some sting alarm-pheromone components in the Africanized type (Collins et al., 1987) is necessary.

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