Effect of Age on the Response to Alarm Pheromones by Caged Honey Bees[1,2]

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ABSTRACT

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The response of newly emerged, 4 and 6-wk-old honey bees (*Apis mellifera* L.), to alarm chemicals was compared in a laboratory test. Older bees were significantly more active before testing. The 4-wk-old bees reacted faster than the newly emerged and the 6-wk-old groups, but there were no differences in the duration of their reactions. Younger bees were more likely to engage in fanning, often with exposure of the Nasonov gland.

After development of a laboratory test of the response of honey bees, *Apis mellifera* L., to alarm chemicals (Collins and Rothenbuhler 1978) a concern arose that the newly emerged bees used for the test might have been an inappropriate age. Under normal conditions in a colony the bees that are involved in alarm and defense behavior do so at an age when others are beginning to forage. However, researchers report guarding activity or the beginning of foraging at various ages, as early as 8 days and as late as 41 days (Boch and Shearer 1963, Free 1965, Lindauer 1961, Ribbands 1952). In each instance the bees identified as being engaged in guarding behavior were older than those used in the original investigations. My purpose was to determine whether response to alarm chemicals in laboratory test cages was different for bees of different ages.

Materials and Methods

Caged mature brood from 4 colonies was allowed to emerge in an incubator during a 24-h period. Each newly emerged bee was marked (McDonald and Levin 1965) according to colony by putting a spot of paint on the thorax. Some of the marked bees were then caged in glass-fronted wooden cages (Kulinčevic and Rothenbuhler 1973) 30 bees/cage, 6 cages/colony, and cages were placed in a 35° C walk-in incubator for testing. The remaining marked bees were returned to the original colonies and allowed free flight until they were 4 wk old. At that time they were collected with a vacuum collection device designed by Jaycox (1970), placed in the test cages in the walk-in incubator, and tested. After this test, bees were left in cages in the incubator until they were 6 wk old and were then tested again.

The test procedure was that used by Collins and Rothenbuhler (1978). Bees in each cage were stimulated by 1 of 2 alarm pheromones——isopentyl acetate (IPA), a component of the sting alarm pheromone (Boch and Shearer 1962), or 2-heptanone (2HPT), a component of the mandibular gland alarm pheromone (Shearer and Boch 1965). These were diluted in paraffin oil to a proportion of 1 part alarm pheromone in 9 parts oil.

The observations made were the following: 1. Initial activity level — the number of bees moving about the cage before stimulation by a chemical. 2. Seconds to react — the time from presentation of stimulus until a distinct wing flicker and increase in locomotion was seen. 3. Intensity of the reaction — a subjective estimate of the strength of the reaction recorded as none, weak, medium, strong, or very strong. 4. Duration of the reaction — time from onset of the response until bees returned to ca. their initial level of activity. Finally, after all tests, the percent of stimulus presentations resulting in no reaction was determined. In addition, the recording of a 5th observation was initiated for the present test. The new character, fanning behavior, consisted of fanning wings or sometimes fanning accompanied by exposure of the Nasonov gland. At no time was a bee seen to fan with a protracted sting.

Data were analyzed by using analysis of covariance with initial activity level as the dependent variable, a least significant difference test, and the chi-square test.

Results

The analysis indicated no significant differences between the 2 chemicals used for testing except for fanning behavior. Therefore, results for all other observations are combined in the following tables.

Measurements for newly emerged bees and for 4- and 6-wk-old bees are compared in Table 1. Initial activity level and seconds to react differed significantly at the 3 ages with initial activity level increasing with age. However, 4-wk-old bees responded faster than either newly emerged or 6-wk-old bees. When data on seconds to react were examined for individual colonies (Table 2), all had the same pattern though there were slight differences between

Table 1.——Test results for the 3 age groups: initial activity level; seconds to react; and duration of reaction. Values are least squares means from the analysis of covariance.

Age of bees (wk)	N	Initial Activity level (no. bees)	Time to react (sec)	No. of non-reactors (%)	Duration of reaction (sec)
0	288	6.5a[a]	3.6d[b]	18 (7)	51.64
4	144	14.7b	2.2e	9 (7)	46.34
6	77	18.9c	3.5d	2 (3)	41.03

[a] Means followed by different letters are significantly different at $P<0.05$, $F = 5.39$, d. f. 1 and 15.

[b] Means followed by different letters are significantly different at $P<0.01$, $F = 11.34$, d. f. 1 and 15.

Table 2.——Comparison of 4 colonies evaluated for time to react (raw means).

Age of bees (wk)	Colony number	Time to react (sec)[a]	Rank
0	1394	3.83 ac	1
	1395	4.84 a	4
	1396	3.84 ac	2
	1399	3.95 a	3
4	1394	1.59 b	1
	1395	1.86 b	3
	1396	1.76 b	2
	1399	2.02 bc	4
6	1394	2.08 bc	1
	1395	3.31 ac	4
	1396	2.47 c	2
	1399	2.81 c	3

colonies. Finally, there were no differences among the 3 age groups in duration of the reaction or in frequency of nonreactors.

The intensity of the reaction of the 3 age groups is compared in Table 3. There was a significant difference here ($X^2 = 23.74$, $P < 0.01$). The oldest bees appeared to react with slightly less intensity than the younger 2 groups; that is, the majority of the ratings for the 6-wk-old bees fell in the category of medium intensity.

The results of observations of number of bees engaged in fanning behavior following presentation of the chemical stimulus are summarized in Table 4. There were significant differences in the amount of fanning done by the 3 age groups as well as in response to the 2 chemical stimuli ($X^2 = 36.22$, $P < 0.01$). At all ages, fanning occurred more frequently following stimulation by 2-heptanone than after stimulation by isopentyl acetate. However, as bees aged they engaged in this behavior less and less.

Discussion

The increasing level of activity seen as bees aged was not unexpected. Previous observations of bees confined in these cages over a period of weeks have shown increasing activity, often involving "running," a photopositive orientation that may represent attempts to get out and fly, which interfered with testing (Collins and Rothenbuhler 1978). By allowing the bees free flight for 4 wk, we hoped to remove this interference.

The faster response of 4-wk-old bees, which are closest to the usual age of onset of foraging and guarding, could be due to some physiological development associated with age or experience of alarm situations. With newly emerged bees, the response tended to be faster and clearer after they had been tested once or twice though the reaction time was not significantly different. The 6-wk-old bees had remained caged for 2-wk during which they were provided with sugar syrup but not a protein source. Probably the resulting weakened or stressed condition contributed to their slower, less intense responses.

The original purpose of the laboratory testing with alarm pheromones was to provide a controlled method of quantifying defensive behavior to study the modes of inheritance. The procedure is being refined as one part of a series of tests that will be used in achieving a selection index for honey bee breeding. This will involve laboratory and field evaluations of several characters including honey production, disease resistance, pollination effectiveness and defensive behavior. Aged, free-flying bees are hard to handle for such evaluation since there is considerable extra work and, therefore, the generation time is extended by one mo. The preferred method for selection is the test with newly emerged bees. A reexamination of Table 2 shows that the ranking of the 4 colonies was quite similar for all 3 age groups though 4-wk-old bees reacted faster. Thus, it should be possible to use newly emerged bees to test colonies in the selection procedure.

The difference in the occurrence of fanning behavior was interesting. Several young caged bees frequently fanned their wings, often in conjunction with exposure of the Nasonov gland. Older bees, particularly those 6-wk old, did not often exhibit this behavior. Fanning and Nasonov scenting at the hive entrance have been mentioned by several observers (Sladen 1902, Renner 1960) and have been seen by me when colonies are stimulated by

Table 3.—Test results for the 3 age groups - intensity of the reaction. Analysis of age x intensity, $x^2 = 23.74$, d. f. 6, $P < 0.01$.

Age of bees (wk)	No. (%) of bees showing indicated intensity				
	Slight	Medium	Strong	Very Strong	Total
0	24 (9)	92 (34)	133 (49)	21 (8)	270 (100)
4	4 (3)	46 (34)	74 (55)	11 (8)	135 (100)
6	4 (5)	45 (60)	26 (35)	0 (0)	75 (100)

Table 4—Test results for the 3 age groups - fanning. Analysis of age x chemical, $x^2 = 36.22$, d. f. 1, $P<0.01$.

Caging age (wk)	No. (%) of bees fanning after stimulation by	
	IPA	2HPT
0	105 (2.43)	171 (3.95)
4	6 (0.27)	94 (4.35)
6	0 (0.00)	0 (0.00)

alarm pheromones. Perhaps this behavior is limited to younger house bees who are not actively engaged in colony defense but are acting to provide cohesiveness for the colony, both during a defensive encounter and after the intruder has departed when the defenders are summoned back. However, Butler and Calam (1969) stated that young bees do not expose the Nasonov gland, and at least one component (geraniol) of the pheromone released from the gland is not produced by the young bees (Boch and Shearer 1963). The function of Nasonov fanning by young bees in relation to alarm response certainly requires more investigation.

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Footnotes

[1] Hymenoptera: Apidae.

[2] In cooperation with Louisiana Agric. Exp. Stn. Received for publication Oct. 4, 1979.

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