

Defensive behaviour and the division of labour in the African honeybee (*Apis mellifera scutellata*)

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Summary. 1. Venom protein release and alarm pheromone production by African worker honeybees (*Apis mellifera scutellata*) were documented with respect to adult age. Behavioural assays were used to explore relationships among venom protein release, the alarm pheromone bouquets and the recruitability of bees to colony defence as a function of age.

2. The concentration of venom protein released by young worker honeybees is low (10–30 µg/bee), gradually increasing with age, reaching a plateau of 60 µg/bee at 17 days and remaining at this value in older bees.

3. Nine alarm pheromones from the sting were identified and analysed gas chromatographically. Of these, only isopentyl acetate, isopentyl alcohol, benzyl alcohol and n-hexyl acetate showed concentration profiles that clearly differed with age. The mandibular gland alarm pheromone 2-heptanone, had a reasonably well-defined concentration profile as related to age.

4. Behavioural assays showed: (1) more bees of all ages responded to an isolated sting (sting alarm pheromones) than to (2) an isolated pair of mandibular glands (2-heptanone). A response equivalent to (1) occurred when both a sting and a pair of mandibular glands were presented together. Older bees (15–25 days old) responded more readily to the donor stings, mandibular glands and stings and mandibular glands together of 15–25 day old bees than did younger bees. The responses of older bees (25 days old) were more rapid and of longer duration than of younger bees.

5. Our combined data on the production of venom protein, alarm signals and their efficacy in

recruitment; and the latency and duration of worker responses can be correlated with age. The defensive capacity of African worker bees is one which develops in relation to age and is consistent with defence being yet another element associated with the division of labour in honeybees.

Introduction

Defence in honeybees involves both stinging attacks on aggressors by individual bees as well as the recruitment of nest mates. Recruitment depends upon the receptivity of bees to alarm signals (Boch et al. 1962; Boch and Shearer 1965) that are synthesized at rates which vary widely with age (Boch and Shearer 1966, 1967; Crewe and Hastings 1976). Moreover, the olfactory capacities and hence receptivity of bees also vary with age (Masson and Arnold 1984). The delivery of venom is highly variable (Hoffman and Jacobson 1984) but anatomical changes in the venom apparatus are correlated with age (Owen 1983). Finally, these observations, coupled with recent evidence for the hormonal regulation of pheromone production and perception (Robinson 1987), strongly suggest that defensive behaviour in honeybees may well be intimately related to the division of labour.

On a priori grounds, an effective defensive system should comprise the temporal coincidence of venom production, the elaboration of alarm signals and the ability to respond to and to elicit responses of other bees. Yet, the precise roles of all of the constituents of the alarm signals in colony defence remains equivocal. For example, Boch et al. (1970) found isopentyl acetate to be at least

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20-fold more potent than 2-heptanone in eliciting guarding behaviour; but recruitment could not be correlated with isopentyl acetate alone. More detailed analyses of the pheromones of the sting apparatus (Blum et al. 1978) and of the responsiveness of bees to them (Collins and Rothenbuhler 1978; Collins and Blum 1982, 1983) have shown that several additional compounds of the sting apparatus elicited defensive behaviour and that others did not.

We now report the results of chemical and behavioural assays of worker honeybees in order to systematically describe how venom protein and alarm production, hence pheromonal bouquet change with age and, in turn, how the ability of worker bees both to recruit other bees and themselves to respond to recruitment vary. Finally, that defensive behaviour is orchestrated ontogenetically as a reasonably well defined aspect of the division of labour in honeybees is assessed.

Methods and materials

Livestock. All assays were performed on worker honeybees, *Apis mellifera scutellata*, taken from queenright colonies in apiaries in either Johannesburg or Pretoria. The bees were collected at eclosion from brood frames incubated at 30 °C. Individual bees were colour-coded with paint for recognition as to age and then replaced in apiary colonies or observation hives until needed.

Venom protein. Individual bees were induced to sting through a rubber membrane (finger tips of surgical gloves) into a test tube containing 1 ml of distilled water. The onset of a sting was measured from the actual penetration through the membrane (zero time). The bee was then pulled away leaving the intact sting in the membrane. After a fixed interval, the sting was carefully removed so as not to squeeze the venom sac. The test tubes were then inverted a few times to wash the venom droplet into the water. The venom protein collected was measured as total protein using the method of Lowry et al. (1951) as modified by Hartree (1972) using bovine serum albumin as a standard.

Pheromones. Bees from 15 apiary colonies were collected individually in vials and placed on ice for transport to the laboratory. For each sample, the stings and associated glands were dissected from 10 bees, pooled, and placed in 1 ml of dichloromethane. Five such samples were collected for each age group assayed. Mandibles with their glands were treated in the same way, but 20 mandibular glands were pooled to comprise a single sample in 1 ml dichloromethane. All of the samples were stored at -20 °C for four months prior to gas chromatographic analysis.

Each sample was concentrated to 60% with nitrogen gas. The sting samples were analyzed with a Hewlett Packard 5890A gas chromatograph fitted with a bonded methyl silicone fused silica capillary column, diameter 0.32 mm × 25 m. The signals from the flame ionization detector were quantified with a Hewlett Packard 3392A integrator. Peak areas were measured relative to a known amount of n-tridecane internal standard and

factors were applied to convert peak areas to µg/bee for each compound. The secretions of the mandibular glands were analyzed on a Carlo Erba 2150 gas chromatograph fitted with a similar column to that used for the analysis of sting samples. Signals were quantitated using a Hewlett Packard 3390A integrator. Peak areas were measured relative to a known amount of n-nonane internal standard and factors were applied to convert peak areas to µg of 2-heptanone/bee. Identification of the components of the secretions was by comparison of retention times with those of authentic standards.

Behavioural assays. The laboratory test method of Collins and Rothenbuhler (1978) was used to assay the responses of variously aged bees to isolated intact stings, isolated intact mandibular glands and combined preparations of a sting and a pair of mandibular glands. Bees, that were 3, 4, 9, 15, 17, 20, 25 and 30 days old served separately both as donor age groups as well as recipient age groups. For each of the three sources of pheromones (stings alone, a pair of mandibular glands alone, stings and a pair of mandibular glands together) there was a total of 64 comparisons: 3 day old bees were donors in each of the three treatments to recipient groups of bees aged 3, 4, 9, 15, 17, 20, 25 and 30 days; 4 day old bees were donors to similar groups of recipients etc., such that 192 different comparisons were made, each of which was assayed between 20 and 25 times.

Recipient and donor bees for each test were collected from 6 field colonies and were randomly assigned to small cages two days prior to each analysis. For each test, three small cages each containing 25 bees of a particular age group were designated as recipient bees. Thus a small cage of 25 bees was the basis of all testing. Donors of a particular age group for each test were also collected and housed in a small cage. These bees were maintained in an incubator at 30 °C and fed pollen and sugar water. For each test, new recipients were collected so that no bee was ever used more than once as either a donor or a recipient.

All tests of the three treatments were conducted in an audiometry room at 30 °C. The order of presentation of the three treatments was randomized. A 30 min adjustment period was allowed after the recipient and donor bees were brought into the laboratory. In the first treatment, the responses of recipient bees to a donor sting (sting apparatus and associated glands) were investigated. The sting (sting and associated glands) was dissected free from the bee, placed onto a piece of cork and immediately presented to a recipient group of bees. The time to initiate a response and the duration of the response were measured in seconds. A response reaction was recorded when distinct wing flickering movements and an increased locomotion activity of all the recipient bees occurred (Collins and Rothenbuhler 1978). The time to initiate a response was taken from the time of the presentation of the treatment to the recipient bees until wing flickering and increased locomotion occurred. The duration was measured from the time a response was initiated until the bees returned to their former activities prior to the presentation of the stimulus. Fresh cork and test gland were used for each test. 20–25 donor stings were presented at random to any one of the three recipient groups. All tests were kept 15 min apart. This basic procedure was used in all of the treatments. In the second treatment, the responses of recipient bees to a pair of donor mandibular glands were examined. Similarly, in the third treatment, recipient responses to the presentation of a pair of mandibular glands and a sting together were measured. Thus, for each test, each of the three recipient groups of bees were randomly exposed to one of the three treatments, each treatment being administered 20–25 times.

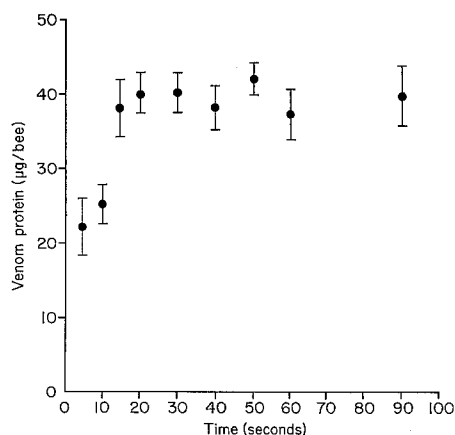


Fig. 1. Venom protein released from African worker honeybees of various ages as a function of stinging time. For each point, $n=55$, $\bar{x} \pm \text{SEM}$

Results

Venom proteins

Because the efficacy of stinging may be related both to the amount of venom secreted during a sting as well as to the rate of release, we measured venom release per unit time in a random sample of bees of various ages taken from an observation hive. This data (Fig. 1) demonstrates that venom was secreted within 20 s of the onset of stinging. There were no significant increases in either the amount or rate of secretion over an additional 80 s of stinging time. A sting in situ (rubber membrane) delivered the bulk of its venom load within 20 s.

Therefore, the amount of venom released during a fixed stinging interval of 30 s was measured in relation to the ages of worker bees. Bees of known ages precluded from foraging were assayed for venom protein production as a function of age (Fig. 2). Venom protein was just detectable in 3 day old bees (only some of which were capable of actually stinging the membrane). Thereafter the protein content of venom increased at about 1.5 µg/day until the bees were two weeks old. This was followed by a sharp increase in venom protein in bees between 15 and 17 days old to about 60 µg after which production remained constant, well into the foraging phase of the worker's life.

Alarm pheromones

All ten of the compounds associated with alarm behaviour in other races of honeybees were positively identified in African worker honeybees: isopentyl acetate, isopentyl alcohol, benzyl acetate, benzyl alcohol, 2-nonanol, n-hexyl acetate, n-decyl acetate, n-butyl acetate and n-octyl acetate from

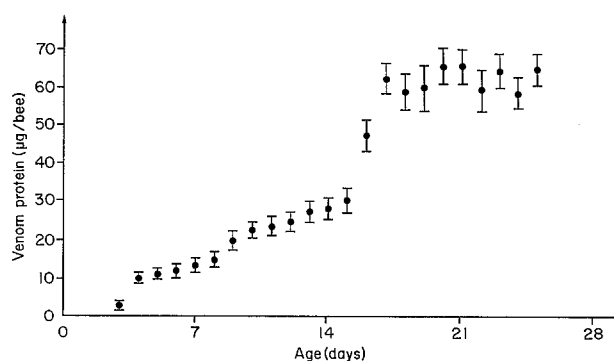


Fig. 2. Venom protein released during a 30 s stinging interval in relation to age in the African worker honeybee. For each point, $n=55$, $\bar{x} \pm \text{SEM}$

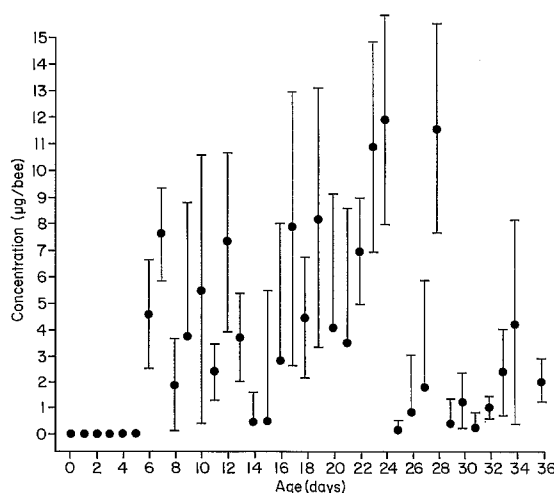


Fig. 3. Concentration of 2-heptanone in the mandibular glands of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm \text{SD}$

the sting apparatus and 2-heptanone from the mandibular glands. The relative concentrations of each of these compounds in African workers were determined for bees ranging in age from 1 to 36 days. The production of 2-heptanone was first detected in one week old bees (Fig. 3) and could be measured in all of the older bees. Much higher concentrations of 2-heptanone were found in bees aged 1 to 4 weeks than in 5 week old bees.

Four of the compounds isolated from the sting apparatus showed well developed concentration profiles that differed greatly with age. Of these, benzyl alcohol (Fig. 4) had the most age restricted distribution. This compound was virtually absent from 1 to 18 day old bees, was pronounced in 4 week old bees and disappeared from older bees. n-Hexyl acetate and isopentyl alcohol were detectable in bees of all ages assayed. In both cases (Figs. 5 and 6) the concentrations of the acetate and alcohol were relatively low over the first 16

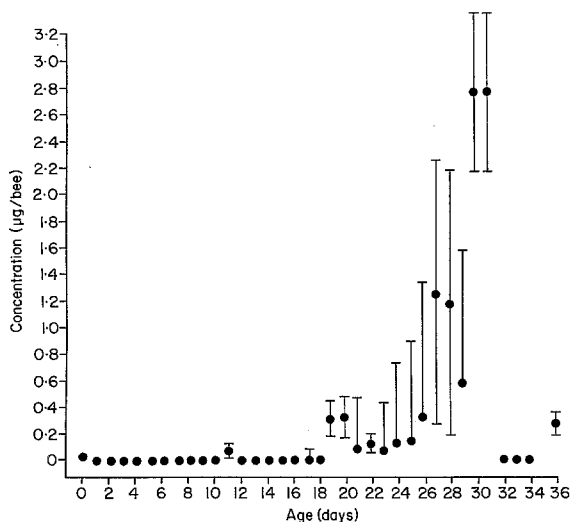


Fig. 4. Concentration of benzyl alcohol in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

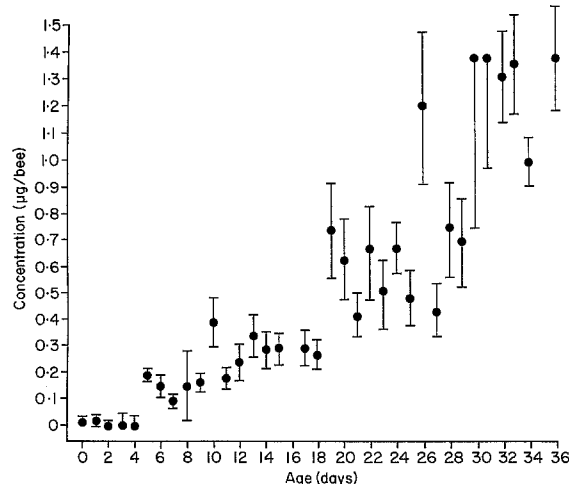


Fig. 6. Concentration of isopentyl alcohol in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

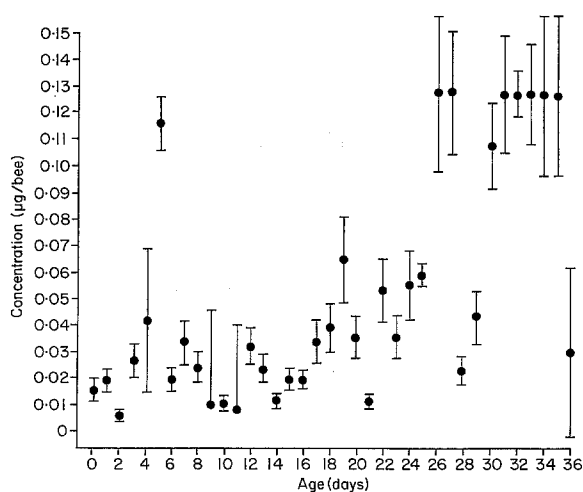


Fig. 5. Concentration of n-hexyl acetate in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

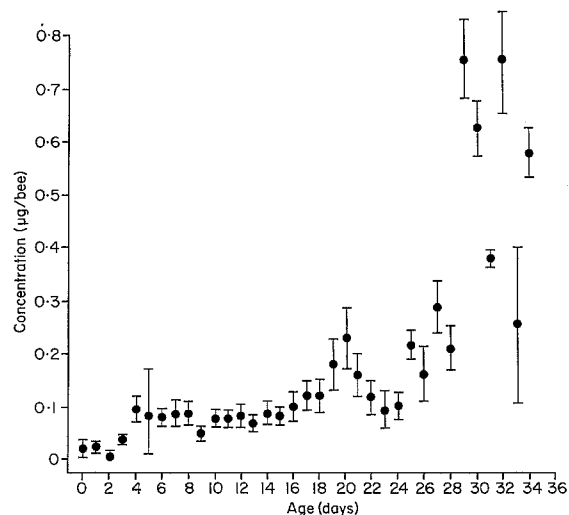


Fig. 7. Concentration of isopentyl acetate in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

to 17 days after which they more than doubled for the next ten days finally reaching peak concentrations. Isopentyl acetate was detectable in all bees (Fig. 7). There was an initial spurt of production of this acetate during the first week, followed by about two weeks of stasis and, finally, production was accelerated in bees more than 3 weeks old.

n-Butyl acetate, 2-nonanol and n-octyl acetate were present in bees of all ages (Figs. 8–10). The distribution of these compounds were not well correlated with age: however, n-butyl acetate and n-octyl acetate occurred in higher concentrations in the older groups of bees. The remaining two compounds, benzyl acetate and n-decyl acetate showed

similar concentration changes with age. The production of both these acetates was low during the first week after which the concentrations rose to reasonably firm levels over the next four weeks (Figs. 11 and 12).

Behavioural assays

A chi-square test of association was performed on the basic data obtained from the behavioural assays to test whether a positive response to stimulation in the donor-recipient interactions actually occurred. The sum total of all the recipient bees responding or not responding to a treatment were assayed and found to be significant ($\chi^2=44.95$,

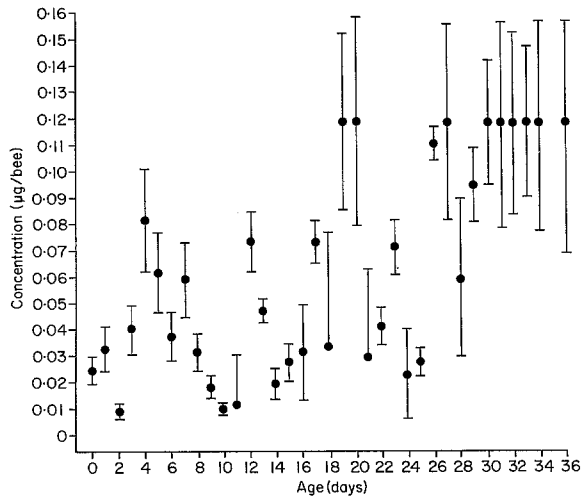


Fig. 8. Concentration of n-butyl acetate in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

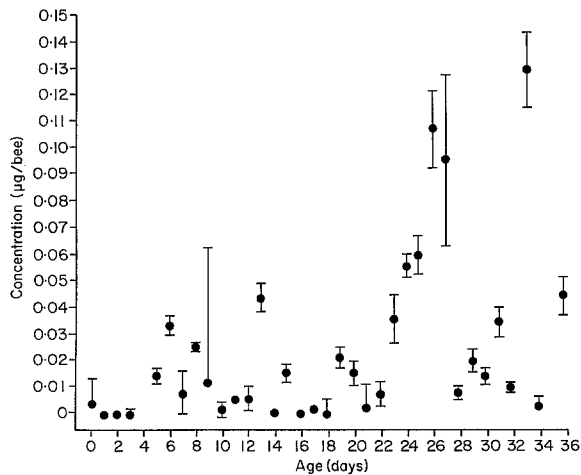


Fig. 9. Concentration of 2-nonanol in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

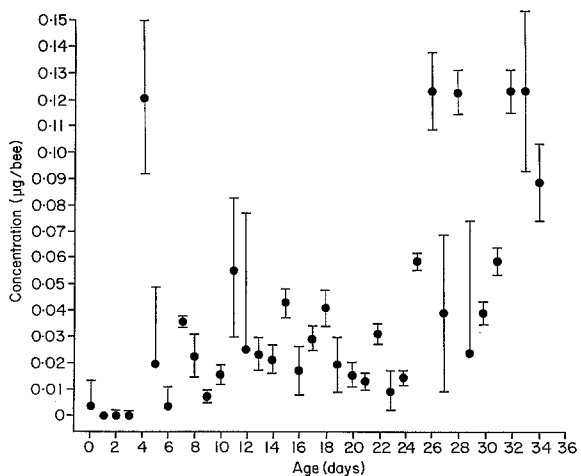


Fig. 10. Concentration of n-octyl acetate in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

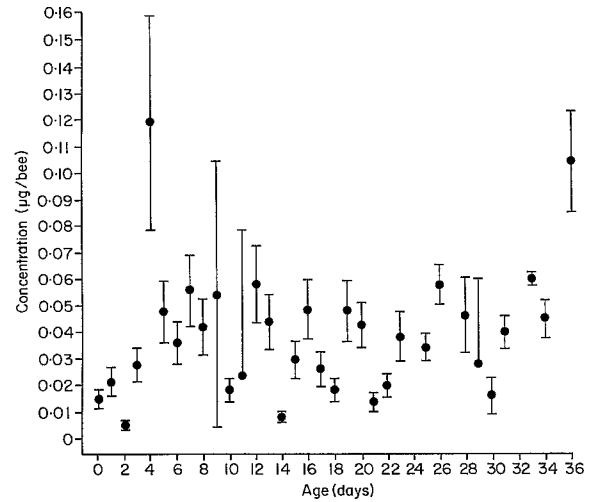


Fig. 11. Concentration of benzyl acetate in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

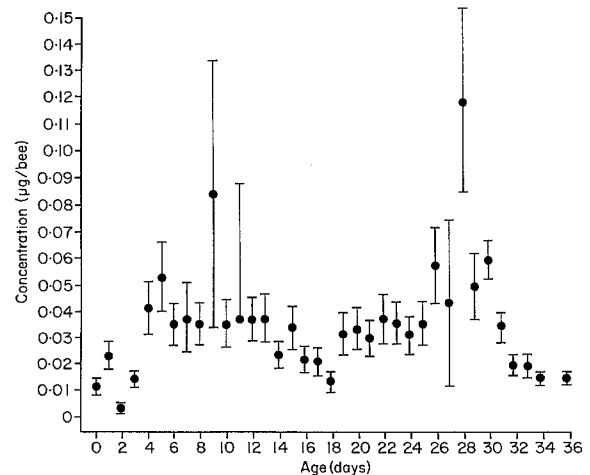


Fig. 12. Concentration of n-decyl acetate in the sting apparatus of African worker bees of different ages. For each point $n=5$, $\bar{x} \pm SD$

$df=2$, $P<0.05$). These responses are further explicated in Tables 1–3. Here, significant differences in the percentages of recipient bees responding to each of the three donor treatments is shown. 9–25 day old recipient bees responded most frequently to 17–25 day old stings (Table 1). 17–25 day old recipients responded most frequently to 15–25 day old pairs of mandibular glands (Table 2), while 15–25 day old recipients also responded readily to 15–25 day old donor stings and pairs of mandibular glands together (Table 3). These results indicate that 15–25 day old recipient bees responded most persistently to each of the treatments based on 15–25 day old bees. It is also interesting to note that the average percentage of recipient bees responding to treatments 1 and 3 are similar (43% and 42%, respectively), while fewer recipient bees

Table 1. The percentage of recipient bees of different ages responding to a particular sting (sting apparatus and associated musculature) from donor bees of different ages^a

Age (days) of sting donors	Recipient age (days)								
	3	4	9	15	17	20	25	30	$\bar{x}$
3	55	10	30	60	36	32	30	40	37
4	20	37	30	100	80	70	45	30	52
9	50	40	90	67	35	60	60	60	58
15	4	3	44	35	12	16	60	20	24
17	0	0	95	56	80	32	85	0	44
20	0	0	0	92	72	45	95	60	46
25	0	20	65	65	75	65	65	44	50
30	40	10	36	40	10	32	20	84	34

^a $\chi^2 = 758.5$, $df = 49$, $P < 0.005$

Each value given is the mean response of 25 separate assays

Table 2. The percentage of recipient bees of different ages responding to a particular pair of mandibular glands from donor bees of different ages^a

Age (days) of mandibular gland donors	Recipient age (days)								
	3	4	9	15	17	20	25	30	$\bar{x}$
3	5	16	43	24	8	16	15	30	20
4	20	37	50	46	30	45	20	20	34
9	40	10	70	27	60	56	20	44	41
15	4	10	24	60	64	20	50	15	31
17	0	0	10	48	85	16	55	0	27
20	0	15	0	80	60	60	80	52	43
25	5	10	60	60	55	65	50	32	42
30	20	10	24	25	15	16	36	64	26

^a $\chi^2 = 617.6$, $df = 49$, $P < 0.005$

Each value given is the mean response of 25 separate assays

Table 3. The percentage of recipient bees of different ages responding to a particular sting (sting gland and associated musculature) and a pair of mandibular glands from donor bees of different ages^a

Age (days) of sting & mandibular gland donors	Recipient ages (days)								
	3	4	9	15	17	20	25	30	$\bar{x}$
3	0	56	43	76	76	8	0	60	40
4	13	16	43	93	25	45	25	30	36
9	16	10	73	57	55	60	50	36	45
15	8	0	44	75	76	24	55	30	39
17	12	10	55	58	90	40	80	10	44
20	0	10	12	92	68	70	95	48	49
25	10	20	65	55	65	65	70	52	50
30	20	20	52	35	30	36	32	58	35

^a $\chi^2 = 515.0$, $df = 49$, $P < 0.005$

Each value given is the mean response of 25 separate assays

Table 4. The response times and duration of response of bees of different ages ($\bar{x} \pm SD$) to 3 day old donors for each of the three treatments^a

	Age of recipient bees (days)							
	3	4	9	15	17	20	25	30
Seconds to react								
S	7.3	2.3	13.1	6.1	8.4	13.6	10.5	8.0
$\pm$	5.3	2.3	10.7	7.1	5.5	10.8	8.6	12.3
$F=1.781$	df 7;75							
M	22.5	15.0	8.2	9.5	5.5	14.3	21.7	5.0
$\pm$	16.3	15.2	6.1	5.5	3.5	11.2	0.6	5.3
$F=2.517$	df 7;54							
B	0	4.9	3.8	6.9	2.1	2.5	0	10.3
$\pm$	—	4.9	4.2	5.3	1.8	0.7	—	11.9
$F=4.461$	df 7;87							
Duration of response (s)								
S	32.5	24.7	38.9	14.0	30.3	9.5	47.8	47.5
$\pm$	16.1	15.5	20.1	4.2	10.9	4.7	26.7	18.0
$F=4.554$	df 7;75							
M	37.5	40.2	34.7	4.0	11.5	18.8	23.7	36.7
$\pm$	16.3	51.6	17.1	—	7.8	23.8	21.1	36.7
$F=2.125$	df 7;51							
B	0	72.1	35.6	30.0	20.7	11.0	14.0	39.8
$\pm$	—	79.0	30.5	—	5.8	4.2	—	33.6
$F=3.992$	df 7;73							

^a Each value given is the mean number of 25 separate assays

One-way analysis of variance (square-root transformation) was used to analyse differences between recipient responses for the time to initiate a response and the duration of the response for each treatment. *S* sting treatment (sting gland and associated musculature). *M* pair of mandibular glands. *B* sting and a pair of mandibular glands. – indicates insufficient data to calculate SD

responded, on average, to isolated pairs of mandibular glands alone (33%).

The response times of recipient bees and the durations of the responses in each of the three treatments for each donor age category are presented in Tables 4–11. Recipient responses to all three treatments of 17 day old donors, for both the time to initiate a response and the duration of the response, differ significantly between the recipient groups (Table 8). This is the only donor age category where recipient responses differ according to age between all the treatments. For the time to initiate a response, 20–25 day old recipients responded the quickest to all three treatments. However, the older 25 day old recipients exhibited responses of longer duration to each of the treatments. 3, 4 and 30 day old recipients did not respond to donor treatments 1 and 2 (Table 8). These differential responses of the recipients to 17 day old donor bees also corresponds to the time of

Table 5. The response times and duration of response of bees of different ages ($\bar{x} \pm \text{SD}$) to 4 day old donors for each of the three treatments^a

	Age of recipient bees (days)							
	3	4	9	15	17	20	25	30
Seconds to react								
S	10.3	9.1	3.3	6.0	8.1	9.0	17.0	5.0
$\pm$	10.3	5.1	3.0	3.7	6.6	8.2	13.3	1.7
$F=1.204$	df 7;75							
M	19.0	8.0	10.6	12.2	16.2	7.7	10.6	16.0
$\pm$	8.6	2.2	10.4	6.6	9.9	10.5	5.7	5.8
$F=2.023$	df 7;62							
B	12.0	20.0	1.8	8.4	16.4	7.1	9.2	8.7
$\pm$	6.6	1.6	2.2	8.3	9.4	10.9	6.5	3.8
$F=5.489$	df 7;71							
Duration of response (s)								
S	39.2	21.7	36.9	23.0	41.8	33.8	26.4	18.0
$\pm$	19.9	11.9	23.4	7.8	25.2	31.7	17.1	5.3
$F=2.178$	df 7;88							
M	18.0	19.1	42.3	31.9	43.8	20.3	29.5	51.5
$\pm$	11.3	12.9	19.1	22.4	8.2	21.4	23.0	36.1
$F=1.217$	df 7;55							
B	21.5	14.4	45.6	26.7	21.2	23.0	40.8	12.3
$\pm$	23.5	13.2	29.7	13.9	10.0	24.9	22.5	4.7
$F=3.297$	df 7;70							

^a Explanations and symbols see Table 4

maximum venom protein production and the presence of benzyl alcohol in the sting apparatus. Isopentyl acetate, isopentyl alcohol, and n-hexyl acetate concentrations also increase at this donor age.

Recipient responses to 20 day old donors showed differential reactions for the time to initiate a response in treatment 1 and in all of the treatments for the duration of the response (Table 9). 15 day old recipients responded the fastest to donor (20 day old) stings. However, 25 day old recipients responded the longest to all three treatments. 15–17 day old recipients also showed rather long responses to the third treatment. 3 day old bees did not respond to any of the treatments while 9 day old bees only responded to treatment 3. Once again, the differential response of the recipients to 20 day old donor treatments coincided with maximum venom protein concentration and elevated levels of the four sting alarm pheromones, benzyl alcohol, isopentyl acetate, isopentyl alcohol and n-hexyl acetate.

Nine and 15 day old donors elicited differential responses for the time to initiate a response in treatments 1 and 2 and for the duration of the

Table 6. The response times and duration of response of bees of different ages ($\bar{x} \pm \text{SD}$) to 9 day old donors for each of the three treatments^a

	Age of recipient bees (days)							
	3	4	9	15	17	20	25	30
Seconds to react								
S	3.3	6.5	2.0	11.4	9.9	6.7	10.0	6.2
$\pm$	4.1	6.7	0.5	6.8	5.7	9.7	11.5	5.3
$F=5.5$	df 7;117							
M	10.6	18.3	4.5	15.0	11.9	4.3	3.3	8.2
$\pm$	5.7	15.5	4.5	9.5	9.2	4.2	4.5	5.9
$F=4.328$	df 7;84							
B	1.8	4.7	3.4	8.0	8.5	6.1	4.7	9.0
$\pm$	1.6	4.0	3.6	9.3	7.0	6.7	4.2	4.4
$F=1.777$	df 7;90							
Duration of response (s)								
S	41.3	31.9	26.6	39.2	18.9	29.9	31.7	49.0
$\pm$	17.8	19.1	22.8	7.6	23.9	13.0	19.3	13.2
$F=2.295$	df 7;123							
M	32.8	42.0	15.3	35.1	21.8	40.7	21.8	50.5
$\pm$	19.7	33.0	6.1	19.3	10.3	34.8	9.7	33.6
$F=3.503$	df 7;84							
B	27.4	16.3	26.9	32.5	14.6	34.3	26.9	40.3
$\pm$	24.3	8.0	12.4	19.6	7.7	24.3	16.3	15.2
$F=3.703$	df 7;135							

^a Explanations and symbols see Table 4

response in treatment 3 (Tables 6 and 7). Nine day old recipients responded the fastest to 9 day old donor stings, while 25 day old recipients responded the fastest to 9 day old pairs of mandibular glands. However, 17 and 25 day old recipients responded the fastest to 15 day old donors for treatments 1 and 2. Nine day old recipients showed the longest duration of response to 15 day donors for treatment 3, while 30 day old recipients showed the longest response to 9 day old donors for the same treatment.

Seventeen day old recipient bees responded the fastest to 25 day old donor stings (Table 10). Three day old recipients did not respond to donor sting treatments for the time to initiate a response. Once again, the 25 day old recipients responded the longest to the 25 day donor treatments 1 and 3. Tables 4, 5 and 11 show the responses of recipient bees to donors aged 3, 4 and 30 days. Many of the recipient responses did not differ between the age categories for each donor age treatment. This suggests that these recipients all respond in similar ways to these donor age groups. It is therefore unlikely that these recipient bees respond to these donor age categories in colony defence.

Table 7. The response times and duration of response of bees of different ages ($\bar{x} \pm \text{SD}$) to 15 day old donors for each of the three treatments^a

	Age of recipients (days)							
	3	4	9	15	17	20	25	30
Seconds to react								
S	14.0	4.0	4.0	13.6	3.6	10.8	3.5	13.0
$\pm$	—	—	5.9	4.5	3.9	13.3	3.2	4.3
$F=6.283$	df 7;57							
M	31.0	11.0	14.2	16.8	6.1	14.2	6.2	1.3
$\pm$	—	3.5	11.1	10.4	9.4	6.5	9.8	0.6
$F=4.376$	df 7;54							
B	19.0	0	6.9	9.4	3.3	8.7	8.3	6.8
$\pm$	19.1	—	7.8	6.4	4.3	3.1	8.2	8.3
$F=3.564$	df 7;71							
Duration of response (s)								
S	28.0	12.0	19.6	24.7	23.3	26.0	33.9	13.0
$\pm$	13.3	—	6.7	15.4	15.6	13.6	24.4	5.7
$F=1.55$	df 7;57							
M	22.8	8.7	20.2	27.6	22.3	23.6	34.5	19.0
$\pm$	27.9	7.4	22.9	14.0	15.2	29.0	29.5	3.6
$F=2.5$	df 7;53							
B	23.8	0	54.4	46.6	24.5	9.2	32.2	19.8
$\pm$	16.7	—	49.2	21.1	13.0	2.6	29.9	10.2
$F=6.563$	df 7;68							

^a Explanations and symbols see Table 4

Once again, recipient bees responded more quickly and longer to donor stings than to donor pairs of mandibular glands. Preparations of a sting and a pair of mandibular glands together were no more efficacious than were isolated sting preparations. Older bees also evoked responses more quickly than did younger bees. These results show that 25 day old bees responded most vigorously to 17 and 20 day old donor treatments 1 and 3.

Discussion

While great strides in the analysis of colony defence in honeybees have been made in the last two decades, it is still necessary to fully define the relationship of venom production to defence, to describe and fully evaluate the importance of numerous components of pheromones and to integrate this information in terms of observable behaviour. When a colony of honeybees is disturbed, alarmed guard bees open their sting chambers, expose their stings and release the characteristic fruity aroma long associated with colony defence. The excited bees fan vigorously, distributing the scent, and run back into the nest from which scores of newly rec-

Table 8. The response times and duration of response of bees of different ages ($\bar{x} \pm \text{SD}$) to 17 day old donors for each of the three treatments^a

	Age of recipients (days)							
	3	4	9	15	17	20	25	30
Seconds to react								
S	0	0	11.4	10.1	12.9	9.1	5.5	0
$\pm$	—	—	8.5	7.1	7.7	7.3	4.5	—
$F=12.239$	df 7;82							
M	0	0	14.5	14.1	12.7	4.3	6.8	0
$\pm$	—	—	10.8	8.2	11.2	5.9	7.0	—
$F=6.88$	df 7;44							
B	4.7	4.5	10.4	13.9	10.9	6.8	5.4	16.5
$\pm$	1.2	11.3	4.7	7.8	6.3	5.5	7.7	17.7
$F=7.338$	df 7;81							
Duration of response (s)								
S	0	0	29.4	31.3	27.6	13.0	45.7	0
$\pm$	—	—	13.9	25.9	14.1	6.8	23.7	—
$F=19.98$	df 7;66							
M	0	0	22.5	28.4	23.9	17.8	33.6	0
$\pm$	—	—	16.3	18.3	8.5	16.4	21.3	—
$F=10.875$	df 7;45							
B	15.0	20.0	16.2	20.5	23.5	35.6	37.5	40.7
$\pm$	7.1	10.8	12.3	8.5	14.4	18.9	24.7	18.2
$F=2.189$	df 7;75							

^a Explanations and symbols see Table 4

ruited bees are likely to emerge (Free 1961; Free and Simpson 1968). It is apparently much the same in all races of *A. mellifera*; however, the African honeybee, *Apis mellifera scutellata*, is more readily recruited and more persistent in defence than bees of European origin (Stort 1974; Collins et al. 1982).

Inasmuch as colony defence has been associated with response to two 'active principles', isopentyl acetate and 2-heptanone, the age groups chosen for behavioural assays in our experiments were selected on the basis of age related changes in these two chemicals (Boch and Shearer 1966, 1967; Crewe and Hastings 1976) as well as those of venom protein (Fig. 2). But, because the behavioural response is the ultimate test of a colony's reaction to any putative stimulus, results of these assays are used in our evaluation of the relative importance of the changes in the profiles of both pheromones and venom protein that occur with age.

The amount of venom protein secreted by a random sample of African honeybees (Fig. 1) is comparable to that obtained from bees of European origin, but ontogenetic data for the latter is

Table 9. The response times and duration of response of bees of different ages ($\bar{x} \pm \text{SD}$) to 20 day old donors for each of the three treatments^a

	Age of recipients (days)							
	3	4	9	15	17	20	25	30
Seconds to react								
S	0	0	0	3.4	6.8	3.7	3.7	6.0
$\pm$	—	—	—	3.8	7.0	2.9	4.5	7.0
$F=4.765$	df 7;83							
M	0	10.0	0	3.5	5.8	7.6	2.4	6.2
$\pm$	—	10.1	—	2.8	6.2	10.6	1.8	6.9
$F=2.267$	df 7;77							
B	0	5.0	8.3	4.0	3.9	9.4	2.5	5.3
$\pm$	—	5.7	7.8	2.9	3.2	8.6	3.4	7.7
$F=2.375$	df 7;89							
Duration of response (s)								
S	0	0	0	29.0	17.6	33.8	40.3	32.1
$\pm$	—	—	—	17.0	16.4	20.8	27.3	22.5
$F=11.304$	df 7;84							
M	0	21.3	0	28.7	29.5	44.0	46.6	32.1
$\pm$	—	13.6	—	19.9	23.9	22.9	25.7	17.4
$F=8.594$	df 7;78							
B	0	10.5	17.3	42.7	41.6	24.6	40.8	38.8
$\pm$	—	2.1	9.1	26.8	29.6	22.7	25.7	17.4
$F=6.957$	df 7;87							

^a Explanations and symbols see Table 4

lacking. Venom protein was first detected in trace amounts in three day old African workers (Fig. 2). Indeed, none of the hundreds of individuals of younger bees and about half of the three day old bees were actually capable of stinging through the rubber membrane or human fingers. Young bees were also lacking in detectable amounts of the ten alarm pheromone components assayed (Figs. 3–12). In the behavioural assays, these young bees proved to be least responsive to older donor treatments (Tables 8–10). Moreover, it has recently been shown that the olfactory system of a young bee is undeveloped (Masson and Arnold 1984) suggesting an explanation for the lack of responsiveness in young bees. Thus, the potential contribution of young bees (from eclosion to 4 days old) to defence behaviour is probably very small indeed.

Towards the end of the first week of an adult worker's life important changes in pheromone and venom protein synthesis began to occur. There was a steady increase in venom protein through the fifteenth day and all of the pheromone components were measurable except benzyl alcohol (Fig. 4). It was at this time that a greater percentage of these bees began to respond to each of the three treat-

Table 10. The response times and duration of response of bees of different ages ($\bar{x} \pm \text{SD}$) to 25 day old donors for each of the three treatments^a

	Age of recipients (days)							
	3	4	9	15	17	20	25	30
Seconds to react								
S	0	14.0	7.8	5.2	1.9	4.3	2.6	9.5
$\pm$	—	6.8	6.8	6.4	1.7	4.9	5.0	6.3
$F=5.479$	df 7;80							
M	17.0	9.0	8.9	8.3	4.3	5.2	3.8	11.3
$\pm$	—	—	7.5	8.3	2.0	3.6	2.8	6.4
$F=2.617$	df 7;68							
B	19.5	10.8	10.1	4.9	2.2	2.2	4.6	10.8
$\pm$	9.2	4.6	8.4	4.4	2.1	1.2	7.9	7.1
$F=7.656$	df 7;65							
Duration of response (s)								
S	0	16.5	26.8	26.8	47.8	26.6	51.6	18.9
$\pm$	—	4.4	21.3	9.2	29.6	12.7	36.1	12.6
$F=5.84$	df 7;81							
M	8.0	7.5	33.3	36.0	46.8	29.6	45.0	21.9
$\pm$	—	2.1	19.7	24.4	26.5	26.1	26.3	12.2
$F=3.215$	df 7;68							
B	9.0	16.0	24.4	26.0	32.9	26.5	53.3	18.0
$\pm$	1.4	13.0	16.5	11.4	21.5	11.6	43.5	9.1
$F=4.871$	df 7;82							

^a Explanations and symbols see Table 4

ments (Tables 1–3). Bees aged 15–25 days responded most readily to 15–25 day old donor treatments. However, the results from Tables 4–11 show that the older bees (25 day olds) responded most strongly to 17–20 day old donor treatments (and in particular to treatments 1 and 3).

The interpretation of actual recruitment and responsiveness to a panoply of alarm signals is difficult. For example, while assays of isolated pairs of mandibular glands showed them to be significantly less active than stings alone, they could still evoke responses as previously noted by others (Boch and Shearer 1966; Collins and Blum 1982). A substantial production of 2-heptanone occurred during the third week of worker life (Fig. 3), a result similar to those of Crewe and Hastings (1976) and Boch and Shearer (1966, 1967). The concentration of isopentyl acetate (Fig. 7) was notably higher in the 15 to 22 day old group than in younger bees and then increased in bees aged 25 days and older. The ranges of concentrations of both isopentyl acetate and 2-heptanone are close to those found in other assays (Boch and Shearer 1966, 1967; Crewe and Hastings 1976). The age-relatedness of concentration of late summer bees

Table 11. The response times and duration of response of bees of different ages ($\bar{x} \pm \text{SD}$) to 30 day old donors for each of the three treatments^a

	Age of recipients (days)							
	3	4	9	15	17	20	25	30
Seconds to react								
S	10.8	9.0	3.6	4.3	12.0	3.1	8.4	3.0
$\pm$	9.2	—	2.5	3.2	7.1	2.6	6.8	3.2
$F=4.267$	df 7;57							
M	11.0	31.0	13.2	9.4	4.3	5.5	9.2	5.0
$\pm$	5.7	—	6.5	8.9	3.1	2.1	7.2	4.8
$F=3.754$	df 7;65							
B	15.0	6.0	6.2	4.7	7.0	2.2	9.8	7.0
$\pm$	2.8	7.1	7.2	6.4	6.6	1.9	7.2	9.5
$F=1.11$	df 7;65							
Duration of response (s)								
S	32.0	5.0	33.9	43.6	24.0	35.5	21.0	23.1
$\pm$	17.3	—	15.9	38.5	24.5	15.1	12.0	11.4
$F=1.848$	df 7;57							
M	16.5	6.0	48.0	25.0	16.7	15.0	29.4	29.8
$\pm$	6.4	—	32.3	12.4	5.7	8.3	21.1	11.4
$F=2.390$	df 7;45							
B	12.5	31.5	31.8	29.1	28.5	24.7	26.0	34.9
$\pm$	7.8	6.4	24.3	16.3	20.9	8.9	13.0	17.7
$F=1.137$	df 7;59							

^a Explanations and symbols see Table 4

in our assays are closer to the late summer results of Boch and Shearer (1966, 1967) than to those early summer results of Crewe and Hastings (1976).

The trends in the relationships between the production of chemical signals and behavioural responsiveness to them may be further traced. The concentrations of n-hexyl acetate, isopentyl acetate and isopentyl alcohol in the 15 to 25 day old groups were about twice those of younger bees (Figs. 5–7) but even higher in older bees. The production of benzyl alcohol (Fig. 4), the least active compound in the assays of Collins and Blum (1982), closely coincided with the peak ages of excitability and responsiveness. Yet, the possible significance of this compound is unknown at present. The relative concentrations of n-butyl acetate, n-octyl acetate, n-decyl acetate and benzyl acetate (Figs. 8, 10–12) are not obviously correlated with recruitment and responsiveness. The higher concentrations of the first two compounds in bees more than 25 days old are difficult to interpret in the absence of assays of very old bees.

In individual assays of all nine of the compounds identified from the sting gland, Collins and

Blum (1982) found that four of them – isopentyl alcohol, benzyl alcohol, n-octyl acetate, n-decyl acetate – made little contribution to recruitment for defence. In our data, the two acetates are ontogenetically unremarkable (Figs. 10 and 12), but isopentyl alcohol (Fig. 6) and benzyl alcohol (Fig. 4) in particular had well defined ontogenetic characteristics. While the latter two compounds presented alone, may not make a direct contribution to the excitement to alarm they may well have some unrealized importance.

The age groups of test donors and recipients used in our experiments, as well as the methodology, preclude an assessment of the significance of any particular compound on its own, but the ontogenetic observations at least point to areas worthy of further investigation. For example, Collins and Blum (1982) found 2-nonanol to be highly effective in alerting bees, yet our data (Fig. 9) fail to indicate any relationship between the ontogenetic characteristics of this alcohol and the behavioural responses of the bees. Similarly, while the efficacy of 2-heptanone in alarm was well established in other experiments (Boch and Shearer 1966, 1967; Collins and Blum 1982), and also elicited responses in our own experiments (tests of isolated mandibular glands, Tables 2 and 12), the very high degree of variation in its production with respect to age (Fig. 3) makes its contribution to a smoothly age regulated defence of the colony questionable (also cf. Free 1987). Similarly, n-butyl acetate (Fig. 8) and n-octyl acetate (Fig. 10) are not well correlated with that age group of bees most responsive in the context of colony defence.

It would appear that defence in the African honeybee involves the temporal coincidence of maximum venom protein and the ability of bees to both elicit response reactions as well as respond to chemical signals. These two aspects of defence are well correlated for bees of 15 to 25 days old and are very supportive of defence being an element of the division of labour. Defensive behaviour goes hand-in-hand with the chemical weaponry. The evidence of the present study coupled with the data of Boch and Shearer (1966, 1967), Crewe and Hastings (1976) and Collins and Blum (1982) indicate that successful recruitment in these honeybees is not related to the maximum concentrations of any of the ten defensive pheromones which have been studied, but can be correlated with certain minimal titres presumably necessary to constitute a threshold response.

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