False Queens: A Consequence of Mandibular Gland Signals in Worker Honeybees

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Observations on queenless honeybees have indicated that it is possible for workers to become egg layers [1–7]. What is even more curious is that some of these laying workers are capable of becoming what Sakagami has called "false queens" [3, 5]. The criteria for this designation being dependent on their ability to elicit retinue behaviour and in being able to inhibit emergency queen rearing in other workers should female larvae be available. Laying workers and false queens are both able to inhibit to some extent ovarial development in other workers.

These behavioural and physiological effects can be understood in terms of the production of chemical signals a false queen emits which allow her to mimic queen signals [6]. The queen mandibular gland signals, within the hive, are involved in eliciting retinue behaviour and in the inhibition of both emergency queen rearing and ovarial activation in the workers [7]. The chemical constituents of this gland have been extensively investigated. The major component of the queen mandibular gland has been identified as (*E*)-9-oxo-2-decenoic acid (9-ODA) [8, 9], the so-called queen substance. The worker mandibular gland produces (*E*)-10-hydroxy-2-decenoic acid (10-HDA), which is also a component of royal and worker jellies [10] and 2-heptanone which is thought to be an alarm substance [11, 12]. Recently a number of other fatty acid constituents have been identified in royal jelly and the mandibular glands of workers and queens [13]. Thus worker mandibular gland contents are said [14, 15] to differ markedly from those of the queen and hence the signals workers may be capable of releasing should be very different from those of the queen.

The distinction between queen and worker signals has been blurred by the discovery that *Apis mellifera capensis* workers can synthesize the major component of the queen mandibular gland secretion, 9-ODA, in their mandibular glands [16, 17]. Workers of *A. capensis* are capable of becoming laying workers very readily [1] and these workers, when placed in queenless groups of other races [16], very rapidly establish themselves as false queens. That these laying workers are capable of mimicking queen mandibular gland secretions and thus could produce queen-like chemical signals, destroyed the distinction [14, 18] that had previously been drawn between queen and worker mandibular gland products. As with ovarial development, the effects of caste determination on the biosynthetic capabilities of the mandibular gland are not irrevocable.

The fact that *A. capensis* workers (amongst other pecularities) can become laying workers and false queens so readily, suggested that they occupied an intermediate position between *A. mellifera* workers and *A. mellifera* queens in terms of their ability to produce queen-like signals. However, the occasional appearance of false queens among *A. mellifera* laying workers [4, 7] was the spur which made us reinvestigate these mandibular gland signals.

The mandibular glands of the series of honeybees (Table 1) were investigated by removing the pair of mandibular glands from each individual and analysing their contents gas-chromatographically (Fig. 1) [19, 20]. The bees used in these experiments were reared and maintained according to methods described in [5].

The *A. mellifera* workers in group A (Table 1), as has been reported previously [14, 15], have secretions dominated by 10-HDA. This fatty acid is said to be "characteristic" of workers [18] yet is present in all the individuals examined. The two egg-laying individuals produced a greater range of acids (10-HDA, 10-HDAA, 9-HDA) than workers with underdeveloped ovaries with one exception. The exceptional individual with underdeveloped ovaries also produced a range of acids similar to that of the laying workers. The enhanced production of acids in this individual suggests that the production of appropriate mandibular gland signals may precede ovarial development.

The secretions of the *A. mellifera* laying

Table 1. The occurrence of mandibular gland substances in workers and queens of *Apis mellifera mellifera* and workers of *A. m. capensis* in relation to the activation of the ovary. Groups are as follows: A) two egg-laying and 15 other workers (chosen at random) from an isolated group of 50 *A. mellifera* workers; B) laying workers removed from a colony of queenless *A. mellifera* workers in a two-frame observation hive; C) individual *A. capensis* workers from groups of either 5 *A. capensis* workers (*cap/cap*) or one *A. capensis* and 4 *A. mellifera* workers (*cap/mell*); D) *A. mellifera* queens of different ages. Abbreviations for the compounds present are as follows: 10-HDA = (*E*)-10-hydroxy-2-decenoic acid; 10-HDAA = 10-hydroxydecanoic acid; 9-HDA = (*E*)-9-hydroxy-2-decenoic acid; MHPE = 2-methoxy-4-hydroxy phenylethanol; 9-ODA = (*E*)-9-oxo-2-decenoic acid; 8-HOA = 8-hydroxyoctanoic acid; 4-HOB = methyl p-hydroxybenzoate (the identity of MHPE requires confirmation)

	Group	Total acids [µg/head]	Component present [%]						
			10-HDA	10-HDAA	9-HDA	MHPE	9-ODA	8-HOA	4-HOB
A	4 individuals	1.5	100.0						
	10 individuals	3.0	87.0	13.0					
	1 individual	4.3	78.4	15.7	5.9				
	2 egg layers	4.5	81.4	13.6	5.1				
B	3 laying workers	5.4	65.2	34.8					
	7 laying workers	6.0	80.3	12.2	7.5				
	1 laying worker	22.5	52.5	7.8	12.0		18.3	9.4	
C	17 *cap/cap*	61.5	42.4	3.0	6.8		33.9	14.0	
	3 *cap/mell*	22.4	5.5	0.5	7.7		76.2	10.0	
D	7 1-day queens	136.7	61.8	1.8	7.0		26.5	2.3	0.6
	5 mated, laying queens	197.2	12.1	7.9	32.2	2.4	36.1	6.9	2.3

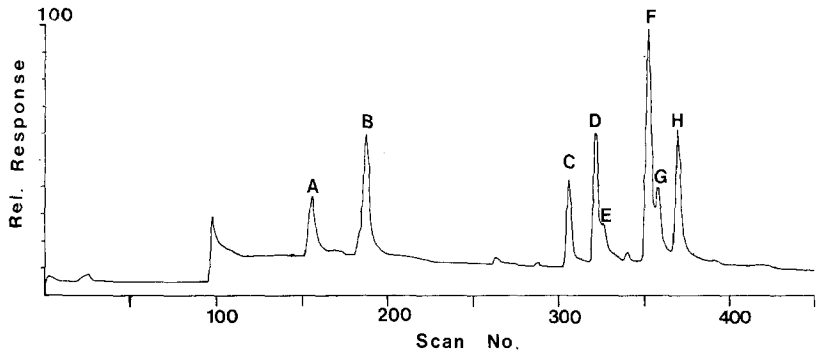

Fig. 1. A chromatogram of the mandibular gland extract of a laying *A. mellifera* queen run on a 2-m glass column packed with 5% OV-101. The acids were silylated according to [20] with BSTFA. The identity of the peaks is as follows: *A* dodecane (internal standard); *B* octanoic acid (internal standard); *C* 8-hydroxyoctanoic acid; *D* (*E*)-9-oxo-2-decenoic acid; *E* 2-methoxy-4-hydroxy phenylethanol (?); *F* (*E*)-9-hydroxy-2-decenoic acid; *G* 10-hydroxydecanoic acid; *H* (*E*)-10-hydroxy-2-decenoic acid; and peak at Scan No. 267: methyl p-hydroxybenzoate. Identity of compound *E* requires confirmation

workers which were analysed as group B indicate that a range of fatty acids are present although 10-HDA is quite clearly the dominant product. One of these laying workers provided us with a startling exception to the general pattern of fatty acid production in *A. mellifera* worker mandibular glands in that significant quantities of 9-ODA, a compound thought to be produced exclusively by queens and *A. capensis* laying workers, were present. However, even though capable of producing 9-ODA, this individual's secretion was still dominated by 10-HDA and was worker-like in this respect.

Although *A. capensis* laying workers are capable of producing 9-ODA [16, 17], the unique biosynthetic capabilities of their mandibular glands are made clear by the results obtained from the individuals in group C in Table 1. Their mandibular glands produce almost the full range of components found in queen mandibular gland extracts, the secretions are not completely dominated by 10-HDA and the proportion of 9-ODA present is high. Where *A. capensis* workers are kept in groups with other *A. capensis* workers, the degree of differentiation among the individuals was not great, i.e., ovarial development was retarded by comparison with the *A. capensis* workers placed in groups of *A. mellifera* workers. Thus the amount of 9-ODA was roughly equivalent to the amount of 10-HDA and the level of production (61.5 μg/head) was relatively high for workers. The *A. capensis* in groups of *A. mellifera* had well developed, activated ovaries and a conspicuous court. Their mandibular gland secretions were overwhelmingly queen-like in that 9-ODA predominated as it does in queens.

Comparison of the results obtained from the workers, with those obtained from *A. mellifera* queens (group D), indicates clearly that the queen's level of total fatty acid production was significantly higher than that of the workers even for one-day-old queens. Furthermore, their secretions may have a greater diversity of components. The startling aspect of these results is that 10-HDA predominates in the secretion of the young queens and in this sense they were producing a worker-like secretion. Mated laying queens produce a secretion in which 9-ODA predominates, 9-HDA is present in significant amounts and 10-HDA is a relatively minor component of the mixture.

Clearly the distinctions between mandibular gland secretions of queens and workers is rather more subtle than simply a distinction between the ability to biosynthesize 10-HDA and 9-ODA. The biosynthetic capabilities of the two castes are apparently similar, with the particular mixture which is produced being dependent on: a) caste differentiation in the sense that queens produce a range and quantity of material which is greater than that of the workers; b) social position of the workers in queenless groups that can result in the dominant individuals [21] becoming false queens and producing 9-ODA in their mandibular glands; c) the production of 9-ODA in the mandibular glands of the workers preceding and not synchronized directly with ovarial development. The fact that the various components of the secretions are found in both workers and queens implies that the signal or pheromone is produced by a characteristic blend of components.

The *A. mellifera* laying worker results resolve one of the puzzling features of earlier behavioural observations [5, 6] in which it had been noted that functional laying workers could be divided into those which acted as false queens and those that were simply egg layers. In group B, the one that produced 9-ODA indicated that the mandibular gland composition of laying workers is idiosyncratic with several grades of mimicry, making the division between simple egg layers and false queens more understandable.

The mandibular gland pheromone of the honeybee is thus not completely defined by the process of caste determination, rather as Sakagami [4] first indicated: "...certain important elements of such a stable differentiation are yet modifiable even at the adult stage". In the case of the *A. mellifera* laying workers, modification in the adult stage was rare, while in *A. capensis* it occurred readily, especially when an *A. capensis* worker was placed in a group of workers of another race. Surprisingly, modification in the adult stage even occurred in the queens where 9-ODA production went from 25% of the total mixture to 36% while 10-HDA declined from 62 to 12%.

These variations in the mandibular gland secretions may represent the principal social signals which govern the relationships between castes within a colony. Analysis of these systems may have important consequences for an understanding of the evolution of sociality in these organisms [22].

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Spike Count and Response Latency

Two Basic Parameters Encoding Sound Direction in the CNS of Insects

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For animals with small interaural disparities and simple auditory systems it is difficult to imagine that interaural time differences—for insects about 10 µs or even less—can be used for sound localization. Temporal discrimination in this magnitude has been found only in highly developed auditory systems, e.g., in man and bats. However, based on the suggestions of Autrum et al. [1], Mörchen et al. [2] verified on single receptor fibers of the locust that spike count is inversely correlated with response latency and, more interestingly, that both encode sound direction. Therefore, bilateral spike count differences and bilateral time differences (in the order of 6 to 7 ms) are obviously important parameters for detecting sound direction.

Single fiber activity was recorded with micropipettes (15–20 MΩ) from interneurones in the three metathoracic ganglia of *Locusta migratoria* using different sound directions in the horizontal plane (for details of preparation see [3]). Spike count and response latency were averaged from 10 repeated sound stimuli (25 kHz, 22 ms duration, rise- and fall-time 1 ms).

For comparison, the response of a typical auditory receptor fiber is shown in Fig. 1 a as a function of sound direction. Moving the sound source from the ipsi- to the contralateral side, spike count decreases by about 3 spikes/stimulus whereas response latency increases by about 5 ms. The ipsilateral tympanal organ is thus excited more strongly and consequently earlier than the contralateral one at lateral sound stimulation. (Remarkably, the "physiological" time difference exceeds the "physical" one by a factor of 10^2–10^3.)

Fig. 1 b shows the directional response of an auditory interneurone. Moving the sound source from the ipsi- to the contralateral side of the preparation again both parameters change significantly: spike count decreases by about 6 spikes/stimulus from 7.1 (S.D. = 1.47) to 1.4 (S.D. = 0.53) spikes/stimulus whereas response latency increases by nearly 20 ms from 20.8 (S.D. = 0.70) to 40.2 (S.D. = 5.38) ms. Thus, on the higher neuronal level of the locust's auditory system, the same phenomena as on the preceding level of the receptor fibers can be observed; however, the degree of variance is much stronger. This is especially true for the response latency (for similar observations in the cricket see [4]). This neuronal response characteristic was found in 70% of all directionally sensitive interneurones.

In the remaining interneurones, however, only one of the two parameters varies with sound direction. In Fig. 1 c a typical example is given: in this neurone the spike count remains more or less independent of sound direction, whereas response latency again increases (here by about 12 ms) when turning the sound source from the ipsi- to the contralateral side. Other neurones operate in quite the opposite way (not shown here). They encode the sound direction only by means of spike count, whereas response latency was found to be directionally independent.

These results record that on the level of the insects' CNS there exist three different modes of encoding sound direction. One portion of the directionally sensitive interneurones simply shows the response characteristics of the receptor fibers, but much more distinctly (namely by reinforcing both peripheral parameters). The other

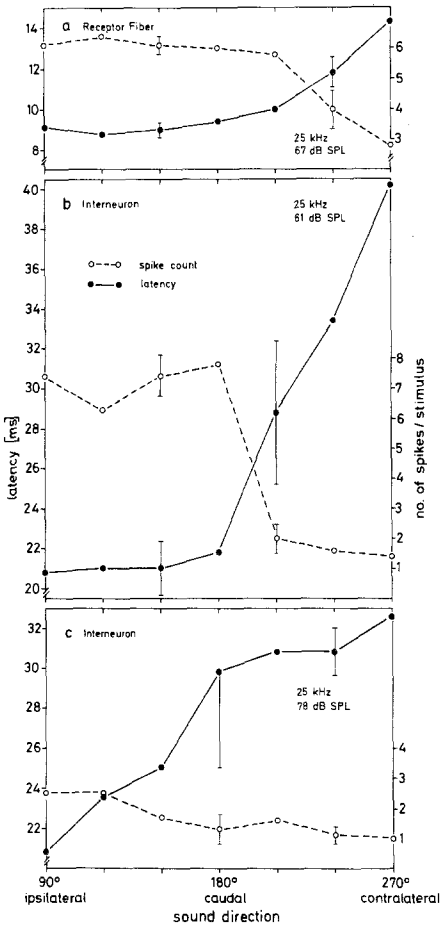

Fig. 1. Spike count and response latency as the function of sound direction of a receptor fiber (a) and two interneurones (b, c). Solid lines represent response latency, dashed lines spike count. Response latency was measured from the arrival of the sound at the ear to the arrival of the first spike at the recording site