Journal of Apicultural Research 34(1): 31–37 (1995)

Evidence for a queen-produced egg-marking pheromone and its use in worker policing in the honey bee

FRANCIS L W RATNIEKS[1]

Division of Entomology, University of California, Berkeley, CA 94720, USA

(Received 23 July 1993, accepted subject to revision 9 September 1993, accepted for publication 22 February 1995)

SUMMARY

Worker-laid and queen-laid male eggs were transferred into combs of empty drone cells in four honey bee (*Apis mellifera ligustica*) colonies. Worker-laid eggs treated with an ethanol extract of queen Dufour's gland were removed by workers (worker policing) at a significantly lower rate than either untreated or ethanol-treated worker-laid eggs, but this effect was less when a 1:10 dilution was used and it disappeared at a 1:100 dilution. Worker-laid eggs that had been touched to an area of a queen at the base of the sting and between the sting sheaths ('sting-wipe' treatment) were also removed at a significantly lower rate than untreated worker-laid control eggs. In all trials, the removal rate of worker-laid eggs exceeded that of queen-laid eggs. Queen-laid eggs treated with the polar solvents methanol and ethanol were removed more rapidly than those treated with the less-polar hexane and methylene chloride, but it was not possible to determine if this was because methanol and ethanol were more effective at removing a possible pheromone or because they caused more damage to the eggs. The results support a hypothesis that recognition of worker-laid eggs during worker policing is via a queen-produced egg-marking pheromone. Possible sources of pheromonal material besides the Dufour's gland are discussed.

Keywords: worker policing, laying worker honey bees, queen honey bees, *Apis mellifera*, eggs, Dufour's gland, pheromones, sting apparatus

[1]Present address: Department of Entomology, University of California, Riverside, CA 92521, USA

INTRODUCTION

Honey bee (*Apis mellifera*) workers are not sterile. Although workers cannot mate they possess ovaries and can lay male eggs (males in the Hymenoptera are haploid). Reproductive honey bee workers have considerable fecundity, with laying workers in queenless colonies each producing *c.* 19–32 eggs per day (Perepelova, 1928, cited in Ribbands, 1953). However, in queenright colonies worker reproduction is rare. In honey bees of European races only about one worker in ten thousand has fully developed ovaries (Ratnieks, 1993), and only one adult drone in a thousand is a worker's son (Visscher, 1989). The low production of males by workers is, at first, surprising. This is because a worker could increase her inclusive fitness by producing males, because she is more related to her sons (0.5) than to brothers (queen's sons, 0.25). Low male production by workers is thought to be due to 'worker policing', the mutual prevention of reproduction by workers (Ratnieks, 1988). Worker policing is selectively favoured because honey bee workers are more related to brothers (0.25) than to the sons of other workers (*c.* 0.15) because queens are inseminated by several males. Specifically, worker bees remove worker-laid eggs from cells but leave queen-laid eggs (Ratnieks & Visscher, 1989). Worker policing is probably important in reducing male production by workers at two time scales. Within the life of a single colony, worker policing can prevent the relatively few egg-laying workers from producing more than a few adult males. Over many generations, effective worker policing has probably resulted in selection against attempted reproduction by most workers. In addition to worker policing and self-restraint, lack of male production by workers may also be caused by queen policing (actions by the queen, such as dominance behaviour or egg-eating, which directly reduce worker reproduction). Queen policing is well-known in species which form small colonies such as *Polistes* wasps, halictid bees, some ants, and naked mole rats (Ratnieks & Reeve, 1992). However, it seems unlikely that queen policing could be effective in species, such as the honey bee, with large colonies in which there are tens of thousands of both workers to control and cells that could contain worker-laid eggs (Ratnieks & Reeve, 1992).

Discrimination of eggs necessitates a source of information by which police workers can recognize whether an egg is queen-laid or worker-laid. One possible source for this information would be a 'queen-produced egg-marking pheromone' (Ratnieks, 1988; Ratnieks & Visscher, 1989). If the eggs laid by the queen were chemically distinct from those laid by workers, then workers could use this difference in egg recognition and discrimination. Importantly, in the honey bee such a pheromone would be favoured by natural selection (Lloyd, 1983; Seeley, 1989) because both the producer (queen) and receiver (police workers) would benefit via higher inclusive fitness. The queen would benefit because the pheromone would help workers rear her sons (relatedness 0.5) over her grandsons (relatedness 0.25). Police workers would benefit because it would help them rear brothers (relatedness 0.25) over nephews (relatedness 0.175–0.1375 assuming queens effectively mate with 5–20 males).

The hypothesized queen-produced egg-marking pheromone, if it exists, would most likely be produced in the queen reproductive system or one of its associated glands and structures, and be applied to eggs either during formation or oviposition. Currently, there are three glands known in this region (Dufour's, sting gland, and Koschevnikov (Free, 1987)). In addition, structures such as the median oviduct, which is lined with secretory gland cells (Camargo & Mello, 1970), have the potential to act as a source of pheromone. This study presents evidence for the existence of a queen-produced egg-marking pheromone by showing that worker-laid male eggs are removed from drone cells more slowly after they have been treated with ethanol extract of queen Dufour's gland, or material originating in the Dufour's gland that accumulates between the sting sheaths of queens.

MATERIALS AND METHODS

Pheromonal effect was looked for by comparing the removal rates of eggs from drone cells of control queen-laid and worker-laid eggs versus worker-laid eggs treated with possible pheromone extracts. All colonies and queens were Italian bees, *Apis mellifera ligustica*. Mated queens were purchased from various commercial queen breeders in the USA. The experiment was carried out in Berkeley, California, from July to September; a time when drone rearing occurs.

I transferred male eggs, 0–1 days old, into combs of drone cells (i.e. the larger hexagonal cells used for rearing males) which were then placed into one of four queenright discriminator colonies, and determined the numbers remaining after 1, 2, 3, 4, and 20 ± 2 h. After 20 h the frame of drone cells was permanently removed from the discriminator colony. Any remaining eggs were removed using forceps so that the frame could be reused. (The presence of a queen-laid egg in a cell does not decrease the probability that a subsequent worker-laid egg in that cell will be removed (Ratnieks & Visscher, 1989).) The same four discriminator colonies were used throughout the experiment. Discriminator colonies were strong in population, having an estimated 20 000–40 000 workers, and were housed in two 10-frame Langstroth hive bodies (volume *c.* 90 litres). The queen was confined to the lower hive body by using a queen excluder. The experimental frames of drone comb containing the transferred eggs were placed above the queen excluder between two frames containing worker brood of all ages.

Queen-laid male eggs were obtained from queenright colonies, in which a single egg-laying queen laid the eggs. Worker-laid male eggs were obtained from queenless colonies with laying workers. Several source colonies of both types were used during the experiment. Egg source colonies were unrelated to the discriminator colonies. Methods for obtaining and transferring eggs were as previously described (Ratnieks & Visscher, 1989), with the following minor modifications:

- Egg-source queens were not caged on frames of drone cells to obtain male eggs, but were given an empty frame of drone cells in the brood area of their colony.
- After removal from the cell in which they were laid, eggs were temporarily positioned in rows on glass slides for 0.5–2 h to facilitate handling and treatment and were then transferred to experimental frames of drone cells.

The room used for handling eggs was humidified to prevent desiccation.

Experiment 1

This experiment compared the removal rates of worker-laid male eggs treated with ethanol extract of the queen Dufour's gland with two controls: untreated and ethanol-treated worker-laid eggs. In addition, queen-laid male eggs were also transferred enabling comparison between treatment eggs and normal queen-laid eggs. Dufour's gland extract was produced by crushing a single gland, removed from a mated egg-laying queen, in 3 µl of ethanol contained in a capillary tube. This was sufficient to treat up to *c.* 150 eggs. Eggs were treated by inserting the capillary over the top half of an egg for 2–4 s while the egg was positioned on a glass slide. Fresh gland extract was made on each trial day. For each day's trial, all four discriminator colonies received eggs treated with solvent extract from the same gland-source queen.

Experiment 2

The second experiment followed the same protocol as Experiment 1 but compared the removal rates of worker-laid eggs treated with 10-fold and 100-fold dilutions of the gland extract as well as the undiluted extract. The purpose of this experiment was to look for dose-dependent effects of the gland extract.

Experiment 3

The Dufour's gland opens at the base of the sting (Lello, 1976). When holding CO_2-anaesthetized queens in a queen insemination apparatus (Laidlaw, 1977) I observed that a white waxy material, closely resembling the contents of the queen Dufour's gland, accumulated along the sting shaft and between the sting sheaths (Snodgrass, 1956). This was particularly evident in queens previously confined for a few days with attendant workers in a mailing cage. Experiment 3 compared the removal rates of worker-laid male eggs touched to this area of a queen, referred to as the 'sting-wipe' treatment, with untreated worker-laid male eggs as controls. During this procedure queens were held under CO_2 anaesthesia in an insemination device. From 5 to 15 eggs were wiped on each queen. Queens used were mated and had previously been held in a mailing cage for 1–5 days with 5–10 worker bees to look after them.

Experiment 4

The removal rates of control queen-laid eggs were compared with those of queen-laid male eggs that had been treated with methanol, ethanol, methylene chloride, and hexane, using the capillary method described above. The purpose of this experiment was to gather data on the effect of various solvents on the acceptance of queen-laid eggs.

All trials used 20 eggs for each treatment and control, except for Experiment 3 which used 10 eggs for the sting-wipe treatment but 20 for the controls. Replication was carried out by using four different discriminator colonies, each used in the following experiments for the following number of trials: Experiment 1, 10 trials per discriminator colony (= 40 trials in total); Experiment 2, three trials per discriminator colony (= 12 in total); Experiment 3, 10 trials per discriminator colony (= 40 in total); Experiment 4, three trials per discriminator colony (= 12 in total). For each day's trials all four discriminator colonies were used, and all eggs used were from a single queenright colony and a single laying worker source colony.

The experimental design permits paired comparisons of the number of treatment eggs remaining in each discriminator colony with the number of control eggs. The paired design occurs because controls were run in every trial. Significance levels are based on one-tailed sign tests of the sign of the difference in the number of eggs remaining (Experiments 1, 2, 4), or the proportion remaining when the initial numbers of control and treatment eggs were unequal (Experiment 3), under the experimental hypothesis that worker-laid treatment eggs are removed more slowly than worker-laid control eggs (Experiments 1, 3), or that eggs treated with a more concentrated gland extract are removed more slowly than those with a more dilute extract (Experiment 2).

RESULTS

The results of all four experiments are presented in figure 1, which shows the percentage of eggs remaining 1, 2, 3, 4, and 20 hours as a function of time after the experimental frames of drone cells were put into

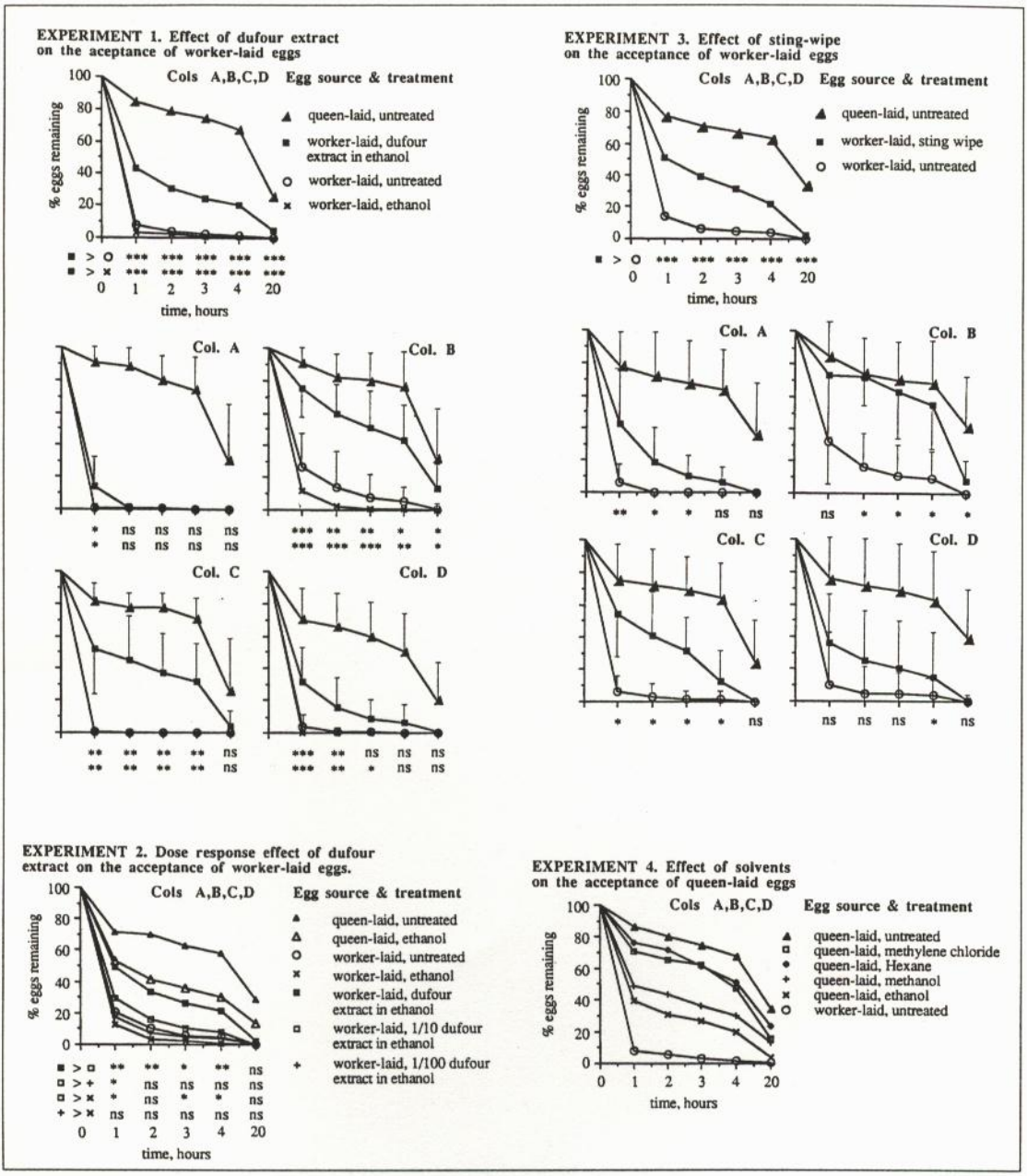

FIG. 1. The rates at which treatment and control worker-laid male eggs were removed from drone cells in four discriminator colonies (A, B, C, D) in four experiments (1, 2, 3, 4).

The error bars show the standard deviation. The significance levels are represented: $P > 0.05$, ns; $0.05 > P > 0.01$, *; $0.01 > P > 0.001$, **; $P < 0.001$, ***. The hypotheses being tested are given under the x-axis of the first figure in each experiment (Experiments 1, 2, 3). In Experiments 1 and 3 the experimental hypotheses are that eggs treated with ethanol extract of queen Dufour's gland (Experiment 1) or by touching to the sting area of a queen (Experiment 3) are removed more slowly than control worker-laid eggs. In Experiment 1 there are two controls, untreated worker-laid eggs and ethanol-treated worker-laid eggs. In Experiment 2 the experimental hypotheses are that lower levels of gland extract (1/10 or 1/100 dilution) are less effective in reducing the removal rate. Replication is 10 trials per discriminator colony (Experiments 1, 3) and 3 trials per discriminator colony (Exeriments 2, 4). In all experiments queen-laid eggs were removed significantly more slowly than worker-laid treatment eggs ($P < 0.001$, one-tailed sign test).

the discriminator colonies. The axes on all figures are the same. Error bars, where given, show the standard deviation. Significance levels are presented along the horizontal axis of each figure. The hypotheses being tested are indicated by the symbols beside the significance levels. Combined data from all four discriminator colonies are presented for all experiments. In Experiments 1 and 3, the large number of trials (10) per discriminator colony makes it worthwhile also to present the results and significance levels for each discriminator colony.

Experiment 1
Ethanol extract of Dufour's gland

Worker-laid eggs treated with Dufour's gland extract in ethanol were removed at a significantly lower rate than either untreated or ethanol-treated worker-laid control eggs. Comparison of the two controls, ethanol-treated worker-laid eggs and untreated worker-laid eggs, shows that ethanol alone did not lower the removal rate. The combined data from all four discriminator colonies (Experiment 1, colonies A, B, C, D) show highly significant differences in the removal rates of treated worker-laid eggs and the two controls ($P < 0.001$) for the 1, 2, 3, 4 and 20 h observations. Data from individual discriminator colonies also show significant differences in the removal of treatment and control eggs, for at least the first hour's observation (colony A) or for up to 20 hours (colony B). The four discriminator colonies also show differences in discrimination rates. In particular, in colony B worker-laid eggs were removed more slowly than in other colonies and in colony A more rapidly. However, even in colony B removal of worker-laid control eggs was complete after 20 h, indicating that few if any worker-laid eggs would survive the three-day egg stage in the honey bee.

Experiment 2
Dilutions of Dufour's gland extract

Serial dilutions of the Dufour's gland extract had a declining effect on reducing egg-removal rate. Worker-laid male eggs treated with Dufour's gland extract were removed more slowly than worker-laid eggs treated with the 1/10 dilution (at the 1, 2, 3, 4 h observations). Worker-laid male eggs treated with 1/10 dilution were removed more slowly than eggs treated with the 1/100 dilution (at the 1 h observation) and also more slowly than control worker-laid eggs treated with ethanol alone (at the 1, 3, 4 h observations). The removal rates of eggs treated with the 1/100 gland extract were not different from controls treated with ethanol alone.

Experiment 3
'Sting wipe' treatment

Eggs treated by contact with the queen sting area, ('sting-wipe'), were removed at a significantly lower rate than untreated worker-laid control eggs. This difference is highly significant ($P < 0.001$) for all five observation times when data from all 40 trials are pooled. Within each discriminator colony, the removal rate of the treated eggs was significantly lower than that of worker-laid controls for at least one observation time. As in Experiment 1, control worker-laid eggs were removed more slowly in colony B than in the other colonies and in colony A more rapidly, although not as rapidly as in Experiment 1.

Experiment 4
Effect of solvents

In this experiment queen-laid eggs treated with the polar solvents methanol and ethanol were removed more rapidly than those treated with the less-polar solvents hexane and methylene chloride. It was not possible to determine if differences in removal were caused by methanol and ethanol being more effective at removing possible pheromone from queen-laid eggs, or by methanol and ethanol causing more damage to the eggs.

In all four discriminator colonies and in experiments 1–3, queen-laid male eggs were removed significantly less rapidly than any control or treatment worker-laid eggs. These differences were highly significant in all experiments at all five time intervals ($P < 0.001$, one-tailed paired sign test). This suggests that the queen treatments used to increase the acceptance of worker-laid eggs did not make worker-laid eggs as acceptable as normal queen-laid eggs. Possible reasons for this are given in the discussion.

In all four experiments approximately 60% of the queen-laid eggs were removed after 20 h, which was similar to previous results (Ratnieks & Visscher, 1989). This is greater than the removal rates of queen-laid male eggs that are left undisturbed in the cell they are laid in (Ratnieks, unpublished data). The greater removal of queen-laid eggs in these experiments probably had two main causes. First, eggs were removed from the cells they were originally laid in and then relocated by the experimenter, which probably resulted in some eggs being damaged or not being well fixed into their new cells. (Honey bee eggs have a tacky coating which causes them to come unstuck or stuck with light pressure.) Second, the frame of drone cells into which eggs were transferred contained no other eggs or brood, possibly resulting in the transferred eggs being less acceptable because some worker bees perceived this frame as not being in the brood area. (In a normal colony, eggs are normally laid in combs containing brood.) To minimize this problem the experimental frame was sandwiched between two frames containing brood. The reduction

of egg acceptability by these possible causes does not invalidate the experimental method, because control and experimental worker-laid eggs were treated equally in all respects other than treatment and would have suffered any disadvantage equally.

DISCUSSION

The results from Experiments 1 and 2 show that Dufour's gland material from the queen can lower the removal rate of worker-laid eggs. Importantly, the Dufour's gland extract has a declining effect on egg acceptance as it is diluted (Experiment 2). This, together with the evidence that worker-laid eggs wiped between the sting sheaths of a queen (Experiment 3) and worker-laid eggs wiped against a queen-laid male egg (Ratnieks, 1992) are removed more slowly, supports the hypothesis (Ratnieks, 1988; Ratnieks & Visscher, 1989) that honey bee queens produce an egg-marking pheromone that is used in discrimination against worker-laid eggs by police workers.

In Experiments 1, 2 and 3 the removal rates of worker-laid eggs were decreased by queen treatments, but not down to the level of queen-laid male eggs. In all cases queen-laid eggs were removed significantly more slowly than treated worker-laid eggs. There are a number of possible reasons for this difference:

- The experimental methods used to transfer pheromone are unlikely to have been as effective as those in nature. In Experiment 3 only about 1/4 of the egg surface came into contact with the queen sting base. Furthermore, to avoid damaging the egg the contact made was very light in pressure, which may have reduced transfer of material. In Experiments 1 and 2 only the upper half of the egg was treated with solvent extract.
- The additional handling or solvent used on the treatment eggs may have resulted in some damage to eggs; Experiment 4 shows that solvents have detrimental effects on the acceptance of queen-laid eggs, which may result from damage in addition to the removal of pheromone.
- There may be physical or other differences between worker-laid and queen-laid eggs in addition to any chemical differences.
- Pheromonal components from other glands, or any cuticular surface or structure, in the tip of a queen's abdomen may contribute to egg recognition.

Pheromonal components additional to those of the Dufour's gland must be considered a strong possibility given that there are at least four known gland sources (median oviduct, sting gland, Dufour's gland, Koschevnikov gland) in the queen reproductive system. Furthermore, honey bees set several precedents for redundancy in pheromonal signalling systems. Queen attractiveness to workers is mediated by material from the mandibular, tergal and Koschevnikov glands (Velthuis, 1985; Free, 1987; Slessor *et al.*, 1988; Cassier & Lensky, 1992). Alarm pheromones are produced in the mandibular and Koschevnikov glands of workers (Grandperrin & Cassier, 1983; Duffield *et al.*, 1984; Free, 1987). A further question is the chemical nature of the honey bee egg-marking pheromone. Experiment 4 (fig. 1), which shows that queen-laid eggs treated with the polar solvents ethanol and methanol are removed more rapidly than queen-laid eggs treated with the less polar hexane and methylene chloride, suggests that the pheromone may be polar in nature.

Experiments 1 and 2, and less directly Experiment 3, suggest that the Dufour's gland may be an important pheromone source. The function and chemistry of the Dufour's gland in the highly social Apidae (Hefetz, 1987), including the honey bee (Free, 1987), is not well known. However, Dufour's gland function and chemistry have been widely studied in other bees (Duffield *et al.*, 1984; Hefetz, 1987) and other Hymenoptera. In certain cases it is well established that the Dufour's gland plays a role in communication. For bees in general, Hefetz (1987) proposes an evolutionary scenario in which the Dufour's gland product is used primitively in cell construction by solitary bees (many ground-nesting bees use it to line their cells (Duffield *et al.*, 1984; Hefetz, 1987)). The gland product then becomes a means for chemical communication, initially for bees to recognize their own nest but subsequently for social communication. For example, in the carpenter bee *Xylocopa virginiana*, the Dufour's gland product is used to mark flowers and serves as a short-term deterrent to conspecific foragers (Frankie & Vinson, 1977). Interestingly, in other Hymenoptera the Dufour's gland also has a role in communication related to oviposition. The Dufour's gland product acts as a deterrent to superparasitism in the insect parasitoid *Campoletis perdistinctus* (Guillot & Vinson, 1972), and in the social wasp *Polistes fuscatus* (Downing, 1991) it permits dominant females to distinguish their own eggs from the eggs of subordinates.

In the queen honey bee, the Dufour's gland is well placed to serve as a source of egg-marking pheromone. The gland opening is 1–2 mm dorsal and distal to the opening of the median oviduct (personal observations) so the gland product could spread to the tissue surrounding the opening of the median oviduct; eggs could pick up the secretion from here or by brushing against the sting sheaths and sting as they are laid.

To be effective in preventing worker reproduction worker policing must have a low cost to the colony (Ratnieks & Reeve, 1992). In the honey bee the cost of policing is likely to be low because:

- Workers can readily recognize the maternity of eggs as either queen-laid or worker-laid (Ratnieks & Visscher, 1989), thereby reducing the cost of

recognition errors during policing which result in removal of queen-laid eggs or non-removal of worker-laid eggs (Reeve, 1989).

- Eggs are held in open cells, thereby greatly reducing the time cost of checking an egg (Ratnieks & Reeve, 1992).

By providing a mechanism facilitating recognition, an egg-marking pheromone may be one of the major factors that has permitted the evolution of worker policing in the honey bee.

ACKNOWLEDGEMENTS

This research was funded by NSF grant BNS-9009197. Juliet Furneaux provided technical assistance.

REFERENCES

The numbers given at the end of references denote entries in *Apicultural Abstracts*.

CAMARGO, J M F DE; MELLO, M L S (1970) Anatomy and histology of the reproductive tract, spermatheca, spermathecal duct and glands of *Apis mellifica* queens (Hymenoptera: Apidae). *Apidologie* 1(4): 351–373. 336/73

CASSIER, P; LENSKY, Y (1992) Structure et rôle social de quelques glandes exocrines a sécrétion phéromonale chez l'abeille domestique, *Apis mellifera* L. (Hymenoptera: Apidae). I. Glandes tarsales, glandes tergales et glandes de Koschewnikow. *Année Biologique* 31: 61–78.

DOWNING, H A (1991) A role of the Dufour's gland in the dominance interactions of the paper wasp, *Polistes fuscatus* (Hymenoptera: Vespidae). *Journal of Insect Behavior* 4: 557–565.

DUFFIELD, R M; WHEELER, J W; EICKWORT, G C (1984) Sociochemicals of bees. *In* Bell, W J; Cardé, R T (eds) *Chemical ecology of insects*. Sinauer Associates; Sunderland, USA; pp 387–428. 1106L/85

FRANKIE, G W; VINSON, S B (1977) Scent marking of passion flowers in Texas by females of *Xylocopa virginica texana* (Hymenoptera: Anthophoridae). *Journal of the Kansas Entomological Society* 50(4): 613–625. 95/79

FREE, J B (1987) *The pheromones of social bees*. Chapman and Hall; London, UK; 218 pp. 540/88

GRANDPERRIN, D; CASSIER, P (1983) Anatomy and ultrastructure of the Koschewnikow's gland of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae). *International Journal of Insect Morphology and Embryology* 12(1): 25–42. 861/85

GUILLOT, S B; VIINSON, S B (1972) Sources of substances which elicit a behavioural response from the insect parasitoid, *Campoletis perdistinctus*. *Nature* 235: 169–170.

HEFETZ, A (1987) The role of Dufour's gland secretions in bees. *Physiological Entomology* 12(3): 243–253. 14/88

LAIDLAW, H H (1977) *Instrumental insemination of honey bee queens*. Dadant & sons; Hamilton, USA; 144 pp. 924/78

LELLO, E DE (1976) Adnexal glands of the sting apparatus in bees: anatomy and histology, V (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society* 49(1): 85–99. 371/78

LLOYD, J E (1983) Bioluminescence and communication in insects. *Annual Review of Entomology* 28: 131–160.

PEREPOLOVA, L I (1928) Biology of laying workers II, III. *Opitnaya Paseka*; 6–10, 59–61 (In Russian, cited in Ribbands, 1953).

RATNIEKS, F L W (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *American Naturalist* 132(2): 217–236. 366/90

RATNIEKS, F L W (1992) Evidence for an egg marking pheromone in the honey bee. *American Bee Journal* 132(12): 813. 154/94

RATNIEKS, F L W (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. *Behavioral Ecology and Sociobiology* 32:191–198.

RATNIEKS, F L W; VISSCHER, P K (1989) Worker policing in the honeybee. *Nature* 342: 796–797. 905/91

RATNIEKS, F L W; REEVE, H K (1992) Conflict in single-queen Hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *Journal of Theoretical Biology* 158: 33–65.

REEVE, H K (1989) The evolution of conspecific acceptance thresholds. *American Naturalist* 133: 407–435.

RIBBANDS, C R (1953) *The behaviour and social life of honeybees*. Bee Research Association; London, UK; 252 pp. 8/54

SEELEY, T D (1989) The honey bee colony as a superorganism. *American Scientist* 77(6): 546–553. 510/92

SLESSOR, K N; KAMINSKI, L-A; KING, G G S; BORDEN, J H; WINSTON, M L (1988) Semiochemical basis of the retinue response to honey bee queens. *Nature* 332(6162): 354–356. 149/90

SNODGRASS, R E (1956) *Anatomy of the honey bee*. Cornell University Press; Ithaca, USA; 334 pp. 90/57

VELTHIUS, H H W (1985) The honeybee queen and social organization of her colony. *In* Hölldobler, B; Lindauer, M (eds) *Experimental behavioral ecology and sociobiology*. Gustav Fischer Verlag; Stuttgart, German Federal Republic; pp 343–357. 1219/87

VISSCHER, P K (1989) A quantitative study of worker reproduction in honey bee colonies. *Behavioral Ecology and Sociobiology* 25: 247–254. 169/91