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FORUM

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Evolution of worker sterility in honey bees: egg-laying workers express queen-like secretion in Dufour's gland

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Unlike many social insects in which overt conflict over male production may exist between queens and workers, the queen honey bee seems to have almost absolute dominance over male production (Page and Erickson 1988; Visscher 1989, 1996). The evolutionary explanation for this successful domination is that multiple mating by the queen creates a new genetic asymmetry between workers and males in the colony. Consequently, it becomes more profitable to rear brothers rather than nephews, providing a driving force for the evolution of selective elimination of worker-born eggs by other workers in the colony (Woyciechowski and Lomnicki 1987), a phenomenon later termed "worker policing" (Ratnieks 1988). In a later study it was further shown that egg discrimination is pheromonally mediated and that the pheromone source is the queen's Dufour's gland (Ratnieks 1995). Recently, Oldroyd and Ratnieks (2000) reported that anarchistic workers evade policing by laying eggs that are more acceptable than those of normal workers, presumably because they counterfeit the queen-produced egg-marking pheromone. In support of their hypothesis, they cite Katzav-Gozansky et al. (1997a) as follows:

"This idea is consisted with data of Katzav-Gozansky et al. (1997a, b) who showed that the chemical composition of the Dufour's gland differs between queens and normal laying workers. We hypothesize that unlike the normal workers studied by Katzav-Gozansky et al. (1997a, b), anarchistic workers have Dufour's glands that secrete chemicals similar to those from the queen gland. This probably results from a mutation that allows anarchistic to become more queen-like than normal workers with respect to their Dufour's gland secretions".

While we cannot comment on the nature of Dufour's gland secretion of anarchistic bees, we would like to emphasize that our results concerning the caste specificity of Dufour's gland secretion in normal honey bees were misinterpreted by these authors.

A queen's Dufour's gland is much more developed than that of queenright (QR) workers, containing an average of 20.5±3.0 µg/gland secretion, as compared to an average of 1.15±0.23 µg/gland for workers. The queen secretion is typified by saturated and unsaturated hydrocarbons (30–40%) accompanied by long-chain, saturated and unsaturated esters. The gland of QR workers, however, possesses only odd long-chain alkanes and completely lacks the esters. However, when workers are orphaned from their queen, some develop ovaries and initiate egg laying, and concomitantly their Dufour's gland expands and contains more secretion (3.44±0.88 µg/gland), fortified by the queen-characteristic esters. Queenless (QL) foragers which do not have developed ovaries do not possess the queen-like esters in their Dufour's gland (Katzav-Gozansky et al. 1997a).

This chemical plasticity was further studied by following the biosynthesis of the glandular constituents (using [1-^{14}C] sodium acetate as a precursor) both in vivo and in vitro, in queens and workers under various social conditions. The composition of the newly synthesized secretion in vivo was as predicted from the glandular chemistry. In queens, there was a considerable incorporation of acetate into both hydrocarbons and esters (Katzav-Gozansky et al. 1997b), whereas the gland of QR workers incorporated acetate only into hydrocarbons. As predicted, the gland of QL egg-laying workers, but not that of QL foragers, incorporated acetates into both hydrocarbons and esters in a queen-like manner. The results of the incubations in vitro were quite different (Katzav-Gozansky et al. 2000). None of the glands possessed newly synthesized hydrocarbons. It would thus seem that these are synthesized elsewhere in the body and are then sequestered by the gland. Queens and

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QL egg-laying workers produced large amounts of radiolabelled esters, as predicted from their social status. Unlike the in vivo situation, glands of QR workers synthesized esters in vitro. A time-dependent study has shown that the alcohol precursors accumulate first, and then their amounts decline with the concomitant accumulation of the respective esters. This time lag in ester biosynthesis seems to be necessary for the accumulation of precursors and/or the induction of the appropriate enzymes.

These data cast some doubt on the role of the secretion as an egg-discriminator pheromone. The features that support the hypothesis that Dufour's gland is the source of an egg-discriminating pheromone are: (1) this abdominal gland opens into the dorsal vaginal wall (Billen 1987) and its secretion therefore may be controllably applied onto the egg before deposition; (2) the glandular secretion is caste-specific, and thus the specific esters can serve as a queen-specific signal that is deposited on the queen-born eggs (haploid or diploid) and protects them from policing. Other features regarding the selective expression of Dufour's gland, however, seem to negate its role as the egg-discrimination pheromone. Although the queen-specific esters were not detected in QR workers, under QL conditions egg-laying workers produce them. While ester production indicates that QL egg-laying workers have regained the ability to biosynthesize them, it does not necessarily suggest that potential egg-laying workers under QR condition also produce them. The studies of the biosynthesis in vitro, however, lend credence to the suggestion that at least some of the QR workers within the hive are able to mimic the queen's Dufour's secretion. If they are able to protect their eggs from policing, these workers can thus compete with the queen over male production. Why then is the number of worker-born males in natural colonies so low? If this small percentage is due to effective worker policing, then it can be concluded that it is not Dufour's gland secretion that serves as the egg-discrimination pheromone. Furthermore, the ability of QR workers to synthesize the queen-like esters in vitro indicates that the biosynthetic pathways do exist in workers, but are under queen suppression. Therefore, if mutations have arisen in anarchistic bees, it may have been in their response to the queen suppression rather than a Dufour's gland expression. Considering the fact that under QR conditions, Dufour's gland secretion is caste-specific, we suggest that rather than constituting an egg-marking pheromone, it may instead constitute a component in the complex queen signal.

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