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Ant Queens Deposit Pheromones and Antimicrobial Agents on Eggs

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Queen ants control the activities of workers by producing releaser pheromones that directly act on workers by eliciting attraction and queen-tending activities [1]. In addition, queens can produce primer pheromones which indirectly affect the behavior of workers toward developing sexual forms or inhibit ovariole development in female alates or workers within the colony. These activities are usually related to the queen's fecundity. We report for the first time that the sting apparatus is intimately involved in the egg-laying process and that related glandular products are deposited on eggs as they are laid. This unique behavior provides a direct link between pheromone distribution through the handling of eggs by workers and the queen's egg-laying rate (fecundity). Thus, whether queen and/or worker control of reproduction is involved, this behavior provides a way in which a queen's fitness can be readily expressed by the queen and assessed by workers. The process also is beneficial for the eggs laid, since worker attractants and antimicrobial agents have been demonstrated to be deposited on the eggs.

The reproductive system [2] and sting apparatus [3] of the fire ant, *Solenopsis invicta*, have been fully described. However, the behavioral events associated with egg deposition have not been documented until now. A typical oviposition event for *S. invicta* queens occurs as follows: the vulva opens as the sting fully extends; a series of contractions force an egg to the vulva's outer surface; the vulva closes and forces the egg to the sting base, whereupon the sting is retracted across the egg (Fig. 1). A comprehensive analysis of this behavior will be published elsewhere. Independent musculature where the Dufour's and poison gland lumens enter the sting apparatus allows the queen to control the flow of exocrine products. This is true for *S. invicta* and other species in the Formicidae [4, 5]. Since the sting is composed of two lancets that slide along the sting shaft, exocrine secretions from the poison and Dufour's glands are dispensed along the entire sting shaft rather than just at the sting tip [3]. Consequently, an egg can be inoculated with exocrine gland products if it contacts any part of the sting. We tested the hypothesis that the physical behavior described above is coupled with deposition of exocrine gland products on the eggs.

The chemistry and function of the Dufour's and poison gland in *S. invicta*

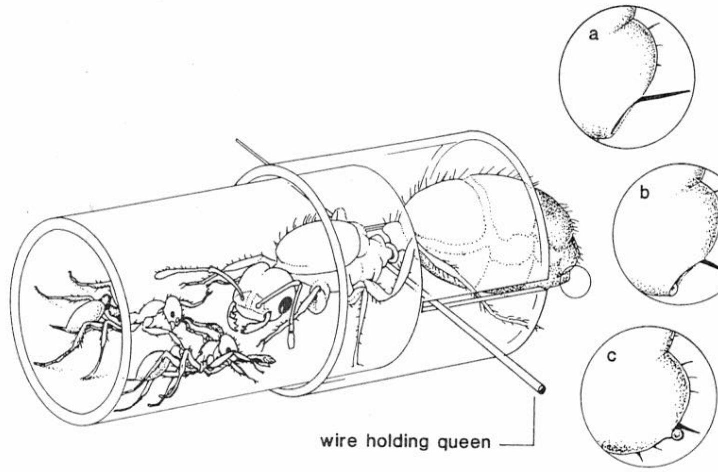

Fig. 1. Diagram of the apparatus used to collect eggs directly from fire ant queens and an expanded view of the sting's involvement in the egg-laying sequence: *a* vulva opens with full extension of the sting; *b* with a series of contraction-like movements an egg is forced out of the vulva with the sting still extended; *c* simultaneously, the vulva closes, pushing the egg to the base of the sting, and the sting retracts across the egg

workers have been thoroughly investigated in terms of recruitment and defense. Worker poison glands produce a series of *trans*-2-methyl-6-alkyl or alkenyl piperidine alkaloids [6], which can be distinguished chromatographically from the main product of the queen poison gland, *cis*-2-methyl-6-undecyl piperidine (Fig. 2). Workers are highly aggressive and use the physiologically active alkaloids in many ways, i.e., prey capture, defense, and nest hygiene [7]. Queens are not aggressive, yet they have a well-developed poison gland and reservoir containing piperidine alkaloids at levels equivalent to that of workers. Although the function of the queen alkaloids is unknown, the queen poison gland also produces a pheromone that attracts workers [8]. The main queen alkaloid (ca. 10 µg/queen) is present in the poison sac in concentrations about 2500 times that of the attractant pheromone (ca. 4 ng/queen) and is readily detected by gas chromatography.

To determine whether exocrine gland products are applied by the queen to eggs via her sting we used an apparatus that allowed the immediate removal of eggs from ovipositing queens. A piece of Tygon® hose was placed over a glass tube such that only half the hose covered the tube. Two slots were cut in the overhanging hose (see Fig. 1). The queen to be tested, along with a few workers found near her, was placed in a freezer for 1.5 min, after which a fine copper wire belt was tied around the queen's petiole. The two ends of the belt were inserted into the slots of the Tygon® hose, such that the queen's head and thorax were inside the glass tube and the end of her abdomen extended just beyond the hose. The apparatus was placed in a Petri dish (5 cm dia.; inner side painted with Fluon® and bottom covered with moist filter paper) along with a few workers. The Petri dish and the queen's abdomen were positioned under a dissecting microscope so that the egg-laying process could be observed. The queen's abdomen was suspended in air. Eggs were collected as laid, using bristles from a camel's hair brush, counted and immediately placed in hexane (100 µl) for subsequent gas chromatographic analysis. Between 200 and 300 eggs were collected from each queen within a 4-h period. At the end of each collection, *n*-pentacosane (1 µl of a 0.1% hexane solution) was added to the hexane/eggs as an internal standard. The assay was replicated six times. All *S. invicta* queens were from colonies collected in North Central Florida. All colonies were maintained in the laboratory for several months prior to use.

The amount of queen-specific alkaloid deposited in the glass insert was quantified by gas chromatography (GC) carried out and analyzed as previously described [9] with a temperature program of 150–285 °C at 5 °C/min. A combination of GC and GC/mass spectroscopy was used to identify *cis*-2-methyl-6-undecyl piperidine (1.78 ± 0.38 ng/egg; $N = 6$), the queen-specific alkaloid (Fig. 2). Mass spectra were obtained on a Hewlett Packard 5988A GC-MS (Hewlett-Packard, Palo Alto, CA), operated in EI mode (70 eV), equipped with an HP 9000/300 Chemstation and interfaced with an HP 5890 GC operated in the splitless mode. The GC column and operating conditions were the same as described for the GC analysis. Standard *S. invicta* queen and worker venom alkaloids were obtained from dissected poison sacs. Similar quantitative results were obtained with a second experimental design (3.92 ± 0.85 ng/egg; $N = 21$) where a monogyne *S. invicta* queen, five workers located near her, and five 4th instar larvae and/or prepupae were removed from the colony. The queen was weighed and placed into a 100-µl glass insert contained in a 2-ml vial along with the workers and brood. Observations of the queen were made under a dissection microscope for the next 2 h. At the end of this observa-

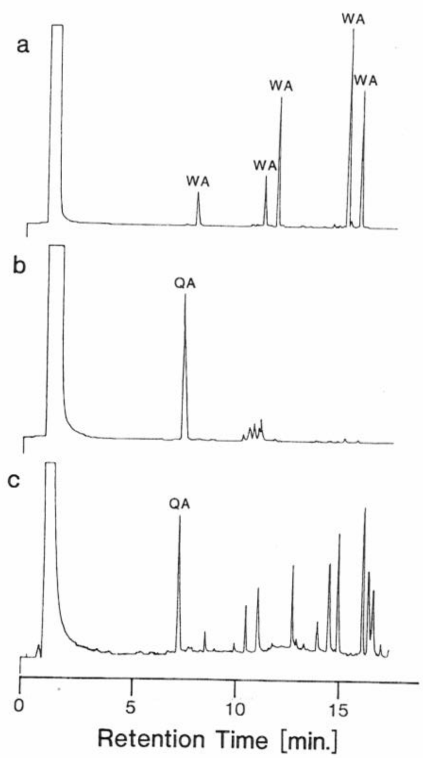

Fig. 2. Comparison of venom alkaloid gas chromatograph profiles: a) worker, b) queen, c) hexane rinse of eggs. *QA* queen-specific piperidine alkaloid; *WA* worker-specific alkaloids. Chromatograms (a) and (b) are from worker and queen poison sac extracts, respectively, and are very concentrated compared to chromatogram (c)

tion period, the workers and brood were discarded and the queen returned to its colony. Hexane followed by an internal standard (*n*-pentacosane) was added to the glass insert containing the eggs laid. Detection of the predominant and easily analyzed alkaloid infers that attractant pheromone and alkaloids are applied to the eggs, because they can not be released from the poison sac independently. No attempt was made to demonstrate whether or not Dufour's gland products (recruitment pheromones) [10] were also deposited on the eggs, due to the lack of a suitable chemical marker.

Application of poison sac contents onto eggs has two clear functions. First, the alkaloids have potent fungicidal and antibacterial activity [11]. The quantity deposited on an egg is enough to inhibit germination of entomopathogenic fungi [12], thus giving the eggs protection in their subterranean habitat. Secondly, poison-gland-derived queen pheromones attract workers to the eggs and queen, thereby insuring prompt care. Worker attraction to the posterior portion of the queen's abdomen is obvious, and laboratory bioassays showed positive worker response to queen pheromone concentrations of less than 0.01 queen poison sac equivalents [13]. These observations and data suggest that, at least for this species of stinging Hymenoptera (aculeata), the queen has retained a reproductive function for its sting and associated glands, similar to that found with non-stinging Hymenoptera [14].

Deposition of pheromones on eggs through the egg-laying behavior described here may be a primary means of information exchange between queen and workers. Whether derived from the Dufour's gland or poison gland, the queen's sting apparatus provides an ideal conduit for the dissemination of physiologically active compounds to other colony members. In addition, the release of worker attractants by the queen may be necessary for the effective distribution of other pheromones produced by the queen [15]. The implications of this work are far ranging and may involve regulation of queen number, control of dealation and oogenesis in virgin queens, and control over the production of sexuals [16]. These regulatory effects are dependent on the distribution of queen pheromones and are linked to a queen's fecundity. For *S. invicta*, release of the queen-produced worker attractant has been directly linked to the queen's egg-laying ability. Queens treated with the insect growth regulator, fenoxycarb, develop atrophied ovaries and stop egg production. These queens have normal quantities of the attractant phermone in their poison sacs; however, they are no longer attractive to fire ant workers because the pheromone is not being released [17]. The behavior associated with egg-laying appears to be an involuntary event; however, our experiments do not allow us to determine whether release of exocrine gland products is voluntary (manipulative queen control) or involuntary (an informative chemical signal) [18].

We observed the same oviposition behavior described here for *S. invicta* for the Pharaoh's ant, *Monomorium pharaonis* [19]. Interestingly, queens produce a queen-specific attractant pheromone in their Dufour's gland [20]. When Pharaoh's ant queens have a high egg production rate, only worker brood are reared; if egg numbers decrease, however, or if the queens are removed or die, workers respond by rearing male and female sexuals [21]. Workers assess the quantity of eggs in a colony (queen fecundity), most likely through pheromones on the egg surface. Similar reports abound in the ant literature [1, 18, 22]; thus, the oviposition behavior described here and the deposition of biologically active glandular products on eggs may have more general applicability.

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