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Poison Gland of Queen Fire Ants (*Solenopsis invicta*) is the Source of a Primer Pheromone

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The use of pheromones in animal communication is ancient and widespread. These semiochemicals are generally divided into two main groups – releaser pheromones which act on the nervous system to elicit rapid behavioral responses, and primer pheromones which act physiologically to modify the endocrine or reproductive system [1]. Both releaser and primer pheromones play a critical role in regulating the behavior and physiology of social insects. Whereas the glandular source and chemical structure of dozens of social insect releaser pheromones have been documented [2], the glands producing

primer pheromones of social insects have been identified in only three instances: the honey bee queen substance inhibiting queen cell construction by workers [3]; a pheromone that delays reproductive development in workers of the bumble bee, *Bombus terrestris* [4]; and a secretion that suppresses the differentiation of new soldiers in the termite, *Nasutitermes lujae* [5]. Of these only the chemical composition of honey bee queen substance has been elucidated (reviewed in [6]).

Ants are by far the largest group of social insects. Despite the important role that primer pheromones play in regulating many basic features of ant societies [7, 8] information on their glandular source and chemical identity is lacking. The function of pheromones in colonies of the fire ant *Solenopsis invicta* has received considerable study, with three effects of queen-produced primer pheromones having been documented: inhibition of dealation (wing shedding) and ovary development in winged virgin queens [9]; inhibition of the production of winged male and female sexuals [10]; and mutual inhibition of egg production among egg-laying queens in polygyne (multiple-queen) colonies [11]. I report here the identification of the queen poison gland as the source of the pheromone inhibiting dealation and ovary development in virgin queens of *S. invicta.*

"Queen" is defined as the dealated, reproductively active members of the queen caste. "Virgin queens" are young members of the queen caste that have not yet shed their wings or become reproductively active. Bioassays were performed following the method of Fletcher and Blum [12], which involves placing two winged virgin queens in a small chamber with several hundred nestmate workers and brood, and monitoring the virgin queens for dealation. Whole body extracts were obtained by extraction in a Soxhlet extractor for 72 h. Extracts of the sting apparatus were prepared by removing the sting, the attached poison sac containing the poison gland, the Dufour's gland (a gland associated with the sting in aculeate Hymenoptera) and small bits of associated cuticle, and then homogenizing these in a tissue grinder. Extracts of the poison sac and the Dufour's gland were prepared by excising the glands and homogenizing them in a tissue grinder. The extracts were tested by applying them to glass cover slips (18×18 mm), allowing the solvent to evaporate for at least 30 min, and then introducing the treated cover slips to the test chambers at the interval indicated for each experiment. Controls did not receive cover slips because several experiments had shown that introduction of cover slips treated with solvent had no effect. The virgin queens were inspected every 8 h for dealation (wing shedding). The colonies used in the bioassays were monogyne (single-queen), while the source of queens for preparation of extracts was polygyne colonies collected in Austin, Texas. All extracts were prepared and stored in hexane, because of several solvents tested hexane yielded the most active material of whole body extracts (data not shown). The results of each experiment were analyzed by ANOVA followed by the Tukey test (P<0.05) for multiple comparison of treatment groups.

To narrow down the glandular source of the inhibitory pheromone, the activity of an extract of whole queen bodies was compared with extracts of queen sting apparatus and virgin queen sting apparatus. Extracts were applied at a rate of 5 queen equivalents (QE) at 24-hour intervals (0, 24, and 48 h). The experiment was started on 9 May 1994 using a single colony collected from Calcasieu Parrish, Louisiana, in April 1994 as the source of virgin queens. As shown in Fig. 1, there was a significant effect of treatment ($F_{3,44}$=13.8, P<0.0001). Whole body extract of queens was highly inhibitory, causing virgin queens to retain their wings more than 40 h longer than the controls, a duration close to the 48-h period during which the extract was applied. Moreover, extract of queen sting apparatus was as inhibitory as the whole body extract, indicating that one of the glands associated with the sting apparatus is the source of the pheromone. In contrast, extract of the sting assembly of virgin queens was not inhibitory.

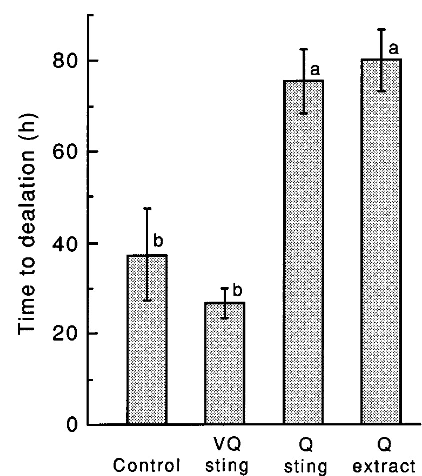

Fig. 1. Ability of hexane extracts of whole queen body (Q extract), queen sting apparatus (Q sting), and virgin queen sting apparatus (VQ sting) to inhibit dealation in virgin queens. Results are given as mean±SE. Treatments with different lower case letters differed significantly (P<0.05, Tukey test; n=12 in each case)

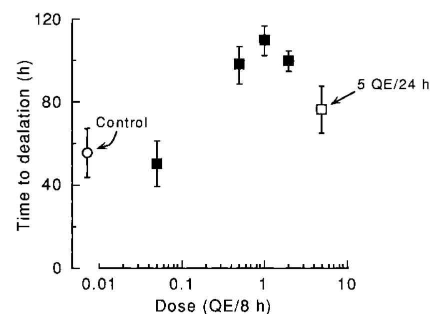

Fig. 2. Effect of different dosages of extract of queen sting apparatus on dealation in virgin queens ($F_{5,66}$=6.9, P<0.0001). Shown are means±SE (n=12 of each dose)

To test the effect of dose, the following dilutions of queen sting extract were compared: 0.05, 0.5, 1, and 2 QE applied at 8-h intervals for 48 h, as well as 5 QE at 24-h intervals for 48 h. This experiment began on 13 June 1994; the source of virgin queens was a colony collected from Calcasieu Parrish, Louisiana, in April 1994. The results are seen in Fig. 2. There was no discernible activity in the 0.05 QE treatment, whereas the 0.5, 1, and 2 QE treatments were highly active, differing significantly from the controls. In this case, application of 5 QEs once every 24 h showed intermediate activity, but this

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Table 1. Effect of glandular extracts on dealation by virgin queens

Treatment	n	Time to dealation (h)	Results of Tukey test
Control	12	26.7± 9.2	b
Dufour's gland	12	51.3±37.4	b
Poison sac	12	102.0±21.6	a
Dufour's gland + poison sac	10	104.0±16.9	a
Whole sting apparatus	12	111.3± 9.9	a

treatment did not differ significantly from any of the other treatments. Because the application of 1 QE at 8-h intervals gave the greatest activity, this dose was used in the next experiment.

The glandular source of the pheromone was located by testing extracts of queen poison sac and Dufour's gland. These were compared with extract of the queen sting apparatus. A colony collected from Austin, Texas, in early Dec. 1994 served as the source of virgin queens, and the experiment was started on 20 Dec. 1994. The extracts were applied at a rate of 1 QE/8 h for 72 h. The results presented in Table 1 show that there was a significant difference among treatments ($F_{4,53}=35.4$, $P<0.0001$). The poison sac extract contained the full activity of the whole sting extract, both causing a delay in dealation of more than 72 h, the full period during which the extracts were made available. In contrast, the Dufour's gland extract exhibited only minor activity, not differing significantly from the control. Addition of Dufour's gland extract did not enhance the activity of the poison sac extract, indicating that the poison gland alone is the major source of the inhibitory substance.

The results of the present study, together with previous investigations showing that the queen poison gland of *S. invicta* is the source of a releaser pheromone that elicits attraction and queen tending activities by workers [13], demonstrate that this gland has both primer and releaser functions. In addition, the poison gland secretion, which is rich in alkaloids, is the source of potent antimicrobial agents that are applied to the eggs during oviposition [14].

The poison gland is ideally situated for dispensing queen pheromone. The glandular secretions are stored in the poison sac which empties into the sting apparatus [15]. During the process of oviposition poison sac contents are exuded [14]. This mechanism of pheromone dispersal directly links the quantity of pheromone secreted with egg production, provided that the same amount of poison gland product is released with the laying of each egg. Such a relationship between pheromone secretion and fecundity is predicted [16] if queen pheromones are "honest signals" that accurately convey the reproductive condition of the queen. Indeed, previous studies of *S. invicta* queens found [17, 18] a positive association between egg production and pheromone release as measured by bioassay.

The primer pheromone is relatively nonvolatile and must be transmitted through the colony by direct contact among colony members [9]. Because the primer and queen attractant are produced by the same gland, the primer is assured of being transferred from the queen to the workers during queen tending and distributed through the colony to the virgin queens by surface contact and/or trophallaxis. A previous study using radiolabeled markers [19] indicated that both surface contact and trophallaxis are efficient means of transmitting queen-derived substances within *S. invicta* colonies.

The results of the present study indicate that queen poison sac extract exhibits dose-dependent inhibition of dealation by virgin queens in small colony fragments, with effective dosages of 0.5–2 QE/8 h, or 1.5–6 QE/day. At the present time it is not possible to relate this dosage to physiologically relevant quantities within *S. invicta* colonies. However, comparable dosages of 1 QE/day were found to be active in the bees *A. mellifera* [20] and *B. terrestris* [4], the two other hymenopteran species in which the glandular source of primer pheromones has been identified. Queen pheromone transmission in *A. mellifera* has been studied in detail by Naumann et al. [21], who found that secretion of 1 QE/day appears to be well within the physiological limits of both pheromone production by the queen and pheromone perception by workers.

S. invicta, a member of the myrmicine subfamily, is the only ant species in which a function has been demonstrated for the poison gland in reproductively active queens. The poison gland of worker ants is generally used in defensive activities [7], but queens of the more specialized subfamilies, including the Myrmicinae, rarely engage in defense. In virgin queens of three other myrmicine genera the poison gland has been shown to serve as the source of a sex attractant [22–24]. Studies of reproductively active queens of other species are needed to determine whether the poison gland commonly produces primer pheromones in the Formicidae, but so far the lack of effective bioassays has slowed research in this area [8].

The few glands that have been implicated in primer pheromone production in other social insects also serve as the source of releaser pheromones. In the honey bee secretions from the queen mandibular gland prevent the development of new queens through inhibition of queen cell construction by workers and elicit several releaser responses including queen tending behavior by workers, stimulation of foraging and brood rearing, and attraction of drones [6]. The queen mandibular gland of the bumble bee, *B. terrestris*, produces both a primer pheromone that inhibits young workers from developing their ovaries [4] and a substance that releases copulatory behavior by males [25]. In the termite *N. lujae* the frontal glands of soldiers produce primer secretions that inhibit the production of new soldiers in the colony [5] as well as defensive compounds and probably alarm pheromones [26].

The occurrence of multiple functions in a single gland raises the question

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of whether the separate responses are elicited by the same or different glandular products. Only in the honey bee has the chemistry been thoroughly studied, and in this species the same mix of five compounds elicits several releaser responses and a primer response (reviewed in [6]). It is not yet possible to say whether a single mixture of compounds is responsible for queen attraction and the primer effect in the fire ant. The queen attractant of *S. invicta* appears to be a multicomponent blend; two of the constituent compounds have reportedly been identified [27, 28], but the active mixture has not been fully characterized. Recent results (E. Vargo, S. Baird, and K. Slessor, unpublished data) indicate that there is a mixture of compounds responsible for inhibiting dealation and ovary development in *S. invicta* virgin queens, and work is currently in progress to isolate and identify the active components.

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