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Multiple glandular origins of queen pheromones in the fire ant *Solenopsis invicta*

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Abstract

The poison sac of the fire ant *Solenopsis invicta* is the only identified glandular source of pheromones produced by a functional ant queen. This structure, which contains the poison gland, has previously been shown to be the source of a releaser pheromone that mediates queen recognition and tending by workers. The poison sac has also been demonstrated to be the source of a primer pheromone that inhibits winged, virgin queens from shedding their wings (dealating) and developing their ovaries. To determine if the poison sac was the only source of these pheromones, we excised the poison sac from queens and observed whether operated queens retained their pheromonal effects. In a first experiment, the poison sac was removed from functional (egg-laying) queens which were then paired with unoperated nestmate queens in small colonies. Counts of the workers surrounding each queen two weeks after the operation showed that queens without poison sac were as effective as their unoperated nestmates in attracting worker retinues. In a second experiment, we removed the poison sacs of virgin queens which had not yet begun laying eggs and thus had not begun producing queen pheromone. After allowing them to develop their ovaries, these individuals produced amounts of queen recognition pheromone comparable to those secreted by unoperated or sham operated virgin queens as determined by bioassay. Testing the head, thorax and abdomens of functional queens separately revealed that the head was the most attractive region in relation to its relative surface area. Bioassays of extracts of two cephalic glands—the mandibular gland and postpharyngeal gland—showed that the postpharyngeal gland is a second source of the queen recognition pheromone. Finally, we found that virgin queens whose poison sacs were removed before they began producing queen pheromone initiated production of a primer pheromone that inhibits winged virgin queens from dealating, indicating that this pheromonal effect also has an additional but as yet undetermined source. These results parallel those on the honey bee in which several of the pheromonal effects of functional queens appear to have multiple glandular sources.

Keywords: Communication; Poison gland; Postpharyngeal gland; Mandibular gland; Dealation; Queen recognition pheromone

1. Introduction

Queens of many social insect species produce a variety of pheromones that profoundly influence the behavior, development and physiology of colony members (Hölldobler and Wilson, 1990; Winston and Slessor, 1992; Vargo, 1998). Despite the important role these royal substances play in colony function, progress in understanding social insect queen pheromones has been slow, particularly concerning their chemical composition and glandular sources. Characterization of a queen pheromone has only been accomplished in a single species, the honey bee, *Apis mellifera* (reviewed in Free, 1987; Winston, 1987; Winston and Slessor, 1992). Glandular sources of queen pheromones have been identified in only two species: *A. mellifera* and the fire ant, *Solenopsis invicta* (Vander Meer et al., 1980; Vargo, 1997).

The best-studied queen pheromone system is that of the honey bee, *A. mellifera*. The honey bee queen's mandibular gland produces a complex of five compounds that have several different effects, including formation of a retinue of workers around the queen (Slessor et al., 1988), inhibition of emergency queen cell construction by workers (Winston et al. 1989, 1990), suppression of swarming (Winston et al., 1991), delaying the onset of

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worker foraging behavior (Pankiw et al., 1998), and stimulation of foraging and brood rearing (Higo et al., 1992).

Although the honey bee queen mandibular gland is clearly established as a source of release and biosynthesis of queen pheromone (Nedel, 1960; Plettner et al., 1996), a number of studies indicate there is an additional source or sources of queen pheromones as well. Gary and Morse (1962) found queens whose mandibular glands had been excised were still capable of inhibiting workers from constructing queen cells, although not as strongly as unoperated queens. Velthuis and van Es (1964) and Velthuis (1970) reported that queens whose mandibular glands had been removed at a young age attracted and maintained a normal-sized retinue for at least four months following the operation, but Zmarlicki and Morse (1964) found that queens without mandibular glands were no longer attractive after 11 months. Velthuis (1970) has proposed an additional glandular source of queen pheromone in the abdomen, but so far no second gland has been identified. Studies of the honey bee therefore suggest that the queen pheromones of social insects are likely to be composed of complex chemical mixtures and potentially derived from multiple glandular sources.

Among ants, queen pheromones of *S. invicta* have received the most attention. Although functional (egg-laying) queens of *S. invicta* contain several exocrine glands, the poison sac, which houses the poison gland, is the only demonstrated source of queen pheromones. Vander Meer et al. (1980) showed that the queen poison sac was a source of a releaser pheromone that attracts workers to the queen. Rocca et al. (1983a,b) identified three compounds which, when tested in combination, were reported (Glancey et al., 1984) to elicit a behavioral response from workers. Vargo (1997) demonstrated that the poison sac also was the source of a queen primer pheromone that inhibits virgin queens from shedding their wings (dealating) and developing their ovaries. In the present paper we excised the poison sac from queens to determine if it was the sole glandular source of the queen attractant and the pheromone inhibiting dealation. We also attempted to identify additional glandular sources of queen attractant pheromone.

2. Materials and methods

2.1. Source and maintenance of ants

All ants used in these experiments originated from Travis County, Texas or Calcasieu Parish, Louisiana, USA. Mounds containing the ants were excavated and the ants were separated from the soil by flooding (Jouvenaz et al., 1977). Ants of both the monogyne (single queen per colony) and the polygyne (multiple-queen) forms were used in the experiments. The social form of the experimental colonies as well as the dates and location of collection are given below under the experimental descriptions. Colonies were housed in plastic trays (40×52×8 cm) supplied with three to four nests (14-cm diameter Petri dishes half filled with damp dental plaster) and maintained at 29±2°C and natural photoperiod. The ants were fed crickets (*Acheta domesticus*) daily and given sugar water and tap water ad libitum.

2.2. Surgical removal of poison sac

Functional queens were placed under a dissecting microscope and immobilized using modeling clay. The fifth and sixth abdominal segments were gently stretched apart using forceps, revealing the location of the poison sac through the intersegmental membrane. Using sharpened forceps, a small incision was made in the intersegmental membrane immediately dorsal to the poison sac. In many cases, the poison sac was exuded through the incision by applying gentle pressure on the abdomen. The poison sac was then removed by grasping it and lifting up. At other times, the poison sac was extracted by means of forceps inserted into the wound. Only ants whose entire poison sac was removed, as determined by inspection of the excised gland, were included in the experiments. Once the poison sac was excised, the abdominal segments were restored to their previously overlapping position using blunt forceps. In a pilot study, we dissected three operated queens 2 weeks after surgery to inspect the poison sac. In no cases was regrowth observed, and the Dufour's gland was intact in all three individuals. Sham operated individuals were treated as above, except after gently grasping the poison sac with forceps, it was released. Unless otherwise noted, operated individuals were placed in small plaster-bottomed cups in groups of 5–10 for at least 24 h before introducing them to nestmate workers.

2.3. Assessment of the ability of functional queens to attract and retain a retinue following removal of the poison sac

The following experiment was performed to determine if mated, reproductively active queens retain their attractiveness to workers after removal of their poison sacs. If queens without poison sacs continued to attract workers, it would suggest that the poison sac was not the only source of queen attractants. Queens used in this experiment originated from three polygyne colonies collected on 16 January and 23 February 1998 from Travis County, Texas and from a polygyne colony collected on 25 November 1997 from Calcasieu Parish, Louisiana. Poison sacs were excised as described above. Operated queens were placed in groups of five nestmates; after 24 h each group was provided with 5 g of nestmate workers

and brood (~2500 workers, 2500 worker pupae and 2500 larvae of all stages) and held in a tray (24×18×7.5 cm) equipped with a single nest. After two weeks, operated queens were marked with a spot of paint (Tex Pen®, Mark-Tex Corp., Englewood, NJ, USA) and weighed to the nearest 0.1 mg. Unoperated queens from the original colonies were also marked and weighed. Two nestmate queens, one operated and one unoperated, were then placed together in a tray containing an observation nest and provided with 5 g nestmate workers and brood from the original colony. The observation nest was similar to the one described by Tschinkel (1988) and consisted of a square plastic nest (10 cm per side) filled with moist dental plaster. The nest was covered with a plate of glass, leaving just enough space for a single layer of ants. The ability of each queen to attract and retain a retinue was assessed by counting the number of workers in each queen's retinue 3 days after pairing the queens. Trays containing the ants were positioned under a dissecting microscope and left undisturbed for at least 30 min before making the counts. Observations were performed under ambient light without the use of an illuminator. The retinue counts for the two queens of a pair were made within 1 min of each other. Following counts, the queens were reweighed.

2.4. Effect of removal of the poison sac from non-reproductive, alate queens on their ability to produce a queen attractant

To investigate whether removal of the poison sac from non-attractive, alate queens before the onset of reproduction would prevent them from producing the queen recognition pheromone, the following experiment was performed. Eleven queenright monogyne colonies collected from Calcasieu Parish, Louisiana in October and January 1997, and April and May 1998 served as the source of all alates and workers. Alates were removed from their natal colony, under which conditions they rapidly dealate, develop their ovaries and produce queen pheromones, including the queen recognition signal (Glancey et al., 1981; Willer and Fletcher, 1986; Vargo, 1999). At the time of isolation from the colony, alates were divided into one of three experimental groups: (1) the treatment group consisted of individuals whose poison sac was removed as described above; (2) sham operated control group; and (3) an unoperated control group. Following surgical manipulation, 10 nestmate alates of each group were placed in a small cup and held without workers or food. On the fifth day, by which time most of the individuals had dealated, one individual from each cup was removed to assess attractiveness, and another individual (nestmate) was used to determine degree of ovary development. Alates that had been maintained in the queenright colony during the five days of experimental treatment also were included. For each source colony, at most two individuals per treatment were used, one for assessment of attractiveness, and one for determination of degree of ovary development. Presence of a queen recognition pheromone was assayed as described below using whole corpses killed by freezing tested with nestmate workers. Degree of ovary development was determined by dissecting the ovaries in 70% ethanol under a dissecting microscope and counting the number of fully developed (chorionated) eggs present in the ovaries and common oviduct (Vargo and Laurel, 1994).

2.5. Extract preparation of body sections and glands

All body sections and glands were removed from previously unoperated individuals. Extract quantities are reported in fractions of a queen equivalent. Body parts and extracted glands were placed immediately into 1.5 ml hexane and pulverized with a tissue grinder. Extracts were held at −20°C until used in bioassays.

To narrow down the location of additional glandular sources of the queen recognition pheromone, bioassays were performed using extracts of the head, thorax, and abdomen. Dealate, reproductively active queens were taken from polygyne colonies collected on 15 October and 3 November, 1997 from Travis County, Texas. Extracts were made by homogenizing a pooled sample of queens. Testing serial dilutions of extracts (1, 0.1, 0.01 and 0.001 queen equivalents), we found (data not shown) that 1 queen equivalent was most active for each of the body regions. Consequently, we used one queen equivalent for all subsequent experiments. One queen equivalent for each treatment was tested with workers from each of 10 colonies, for a total of 10 replicates per treatment. In the adjustment of attraction by relative surface area (see below), the score for the control replicate was subtracted from the score for each of the treatment replicates obtained from the same colony. Adjustments were made on these differences.

In one experiment (see Section 3.4), we compared the activity of the mandibular gland and postpharyngeal glands of both functional and alate queens. These glands were removed from functional queens originating from polygyne colonies collected on 7 and 14 April, 1998 in Travis County, Texas. Glands from alates were removed from individuals originating from three monogyne colonies collected from Calcasieu Parish, Louisiana on 12 April, 1998. The 10 colonies serving as the source of workers used in the attraction bioassays were collected from Travis County, Texas on 23 February, 1998. Glands were excised from queens and were placed in one of four treatments for homogenization: 12 postpharyngeal glands only, 24 mandibular glands only, 12 postpharyngeal glands and 24 mandibular glands together, and 12 poison sacs. Four similar extracts were prepared from alate queen glands. Following homogenization, the hexane containing the extracts was evaporated to 300 μl,

and the extracts were stored at −20°C until the next day when they were tested. A single postpharyngeal gland was considered one queen equivalent, whereas a pair of mandibular glands was counted as one queen equivalent. We also included a poison sac extract and a hexane control in the bioassay.

2.6. Queen recognition bioassay of corpses and extracts

A modification of the 'surrogate queen' bioassay of Vander Meer et al. (1980) was used to test workers' response to corpses or extracts of queens. Twenty workers were placed in a small glass Petri dish (9-cm diameter) whose bottom was scored with sandpaper to provide traction for the workers and whose sides were coated with fluon to prevent escape. An observation square (2.5×2.5 cm) was marked on the bottom center. Corpses were thawed for 20 min and then introduced to the center of the observation square. Extracts were applied to small boat-shaped glass lures (~8×3 mm), and the solvent was allowed to evaporate for 5 min before introduction to the dish. Virgin queen corpses were tested for attraction using nestmate workers from the queenright fraction of their common natal colony. The number of workers inside the square was counted at 1-min intervals for 5 min. At the end of the 5-min test period, the number of workers inside the test square were summed for the five counts, giving a total possible score of 100 for worker attraction.

2.7. Calculations of the surface area of queens

The surface area of queens' bodies was estimated and used to adjust the attractiveness of the head, thorax, and abdomen by their relative surface areas. Using reproductively active queens from multiple queen colonies, we calculated the surface area of the different body parts by dividing them into many different geometric shapes and measuring the dimensions of all shapes using a dissecting microscope fitted with a reticle. The surface area of the head, which includes the head capsule and antennae, was estimated to be 4.2 mm^2 (8.3% of the total surface area). For the purposes of this study, the thorax was comprised of the alitrunk and the legs. We estimated the surface area of the thorax at 24.7 mm^2 (48.6% of total). We use the term 'abdomen' here to refer to the gaster+petiole+postpetiole. Their combined surface area was estimated to be 21.9 mm^2 (43.1% of total).

2.8. Bioassay for primer pheromone inhibiting dealation

The six colonies used in this experiment were taken from monogyne colonies collected in Calcasieu Parish, Louisiana on either 12 April or 4 May, 1998. Alates were taken from their natal colony and their poison sac removed as described above. They were then placed in groups of five in small plaster-bottomed cups. The following day, the dissected females were placed in groups of five into a rearing tray containing a nest and 2.5 g of nestmate workers and brood, all of which had been set up 24 h earlier. Four days later, all of the dissected females had dealated. At this time all but one were removed, leaving a small monogyne colony fragment headed by a virgin replacement queen without a poison sac. Ten female alates from the queenright natal colony were then introduced to the colony fragment with the virgin replacement queen. Ten female alates were also introduced to a paired colony fragment containing no replacement queen which was also set up five days earlier. There was one pair of colony fragments—one with a virgin replacement queen without a poison sac and one with no queen of any kind—per colony. The numbers of alates and dealates in each colony fragment were counted daily for 3 d. At the end of the third day, all females alates and dealates were removed and dissected to assess ovary development. We also included in the ovary assessment 10 alates from each of the natal colonies that had been kept in the presence of their mother queen for the duration of the experiment.

3. Results

3.1. Ability of living queens to attract and retain a retinue following removal of the poison sac

Removal of the poison sac did not weaken the queens' ability to attract a retinue, as operated queens had as many workers in their retinues as did their unoperated nestmates (Fig. 1). There was no significant difference in the weights of the operated (mean±SD=12.3±2.4 mg) and unoperated queens (12.3±2.9 mg) in each pair ($P>0.9$, $t_5=0.04$, two-tailed paired test), nor did the weights of either group of queens change significantly during the course of the experiment ($P>0.2$, $t_{11}=1.28$, two-tailed paired test). Because body weight is strongly correlated with fecundity in reproductively active queens of *S. invicta* (Tschinkel, 1988; Vargo and Fletcher, 1989), these results suggest that operated queens continued to function normally with respect to their reproduction and attraction to workers.

3.2. Effect of poison sac removal on the onset of queen pheromone production

The previous experiment suggests there is a source of queen recognition pheromone in addition to the poison sac. However, it is possible that the active compounds are produced by the poison sac and can remain on the surface of the queens' bodies for at least two weeks, the

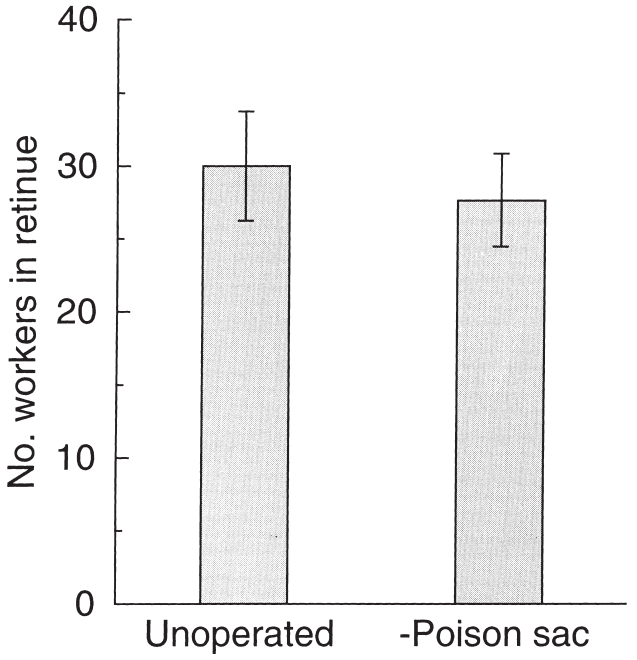

Fig. 1. Ability of queens whose poison sacs had been removed to attract a retinue of workers compared with an unoperated nestmate queen. Each operated queen was paired in a small colony with an original nestmate queen containing an intact poison sac. Shown is the mean (±SE) number of workers in the retinue around each queen. There was no significant difference between treatments (t_5=1.6, P>0.2, n=6 of each treatment).

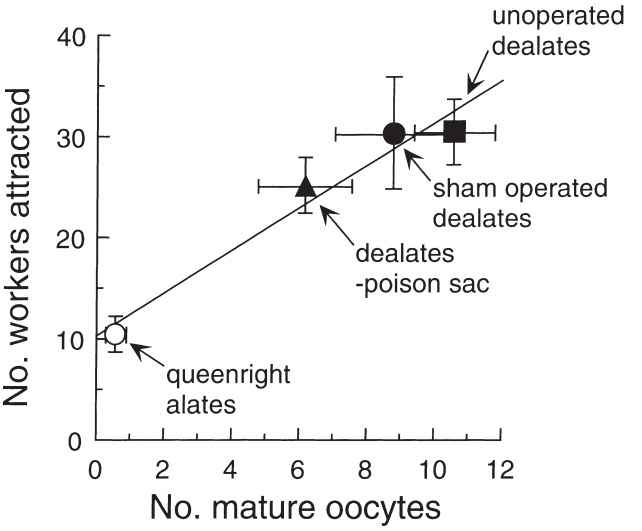

Fig. 2. Relationship between ovary development and attractiveness of virgin queens with regard to presence or absence of wings and presence or absence of the poison sac. The slope of the line differs significantly from 0 (R^2=0.98, $F_{1,2}$=94.0, P<0.02). Sample sizes were as follows: ovary development: alates from queenright colonies (n=11), unoperated dealates (n=10), sham operated dealates (n=10), dealates without poison sacs (n=9); attraction: alates from queenright colonies (n=9), unoperated dealates (n=8), sham operated dealates (n=9), dealates without poison sacs (n=9).

interval between the operation and the retinue counts in the previous experiment. If so, the attractiveness of the operated queens observed above could be due to residual pheromone present before the operation rather than coming from a second source(s). To eliminate any possible effect of residual pheromone, the poison sacs were removed from alate virgin queens which are reproductively inactive and do not produce queen pheromones (Vander Meer et al., 1980; Willer and Fletcher, 1986; Vargo, 1999). They were then allowed to initiate reproductive development and subsequently tested for their ability to attract workers.

As shown in Fig. 2, alates lacking their poison sacs initiated reproductive development, although they had somewhat less developed ovaries than did either the unoperated or sham-operated controls. Nonetheless, the poison sacless alates were attractive to workers, signaling the presence of the queen recognition pheromone that accompanies the initiation of reproduction (Vargo, 1999). These results thus demonstrate the presence of a second source of queen recognition pheromone.

3.3. Attractiveness of different body regions

Based on previous work of others (Vander Meer et al., 1980) and our own, we reasoned that the queen recognition pheromone should be distributed over the body of the queen, and that in the absence of any glandular reservoirs, the activity of each body region should be in proportion to its surface area. Disproportionate activity exhibited by any body region would suggest the presence of a glandular source. Fig. 3 shows a comparison of the attractiveness of the body regions with and without adjustment for relative surface area. Before adjustment, the abdomen is clearly the most active region, attracting significantly more workers than any other body region, even though it comprises slightly less relative area than the thorax. The head, which contains only about 8% of the total surface area of the queen was not significantly more attractive than the solvent control. However, a very different result emerges once these attraction values are adjusted for relative surface area. After adjustment, the head is the most active region, with 50% more activity than the abdomen, although this difference was not significant. The abdomen was still more active than the thorax, but not significantly so. These results suggest that a second glandular source of queen recognition is located in the head.

3.4. Activity of the postpharyngeal and mandibular glands

Two main exocrine glands located in the heads of fire ant queens are the mandibular gland and the postpharyngeal gland (Phillips and Vinson, 1980). Extracts of these glands from dealated, functional queens and from alate, reproductively inactive queens were tested for their

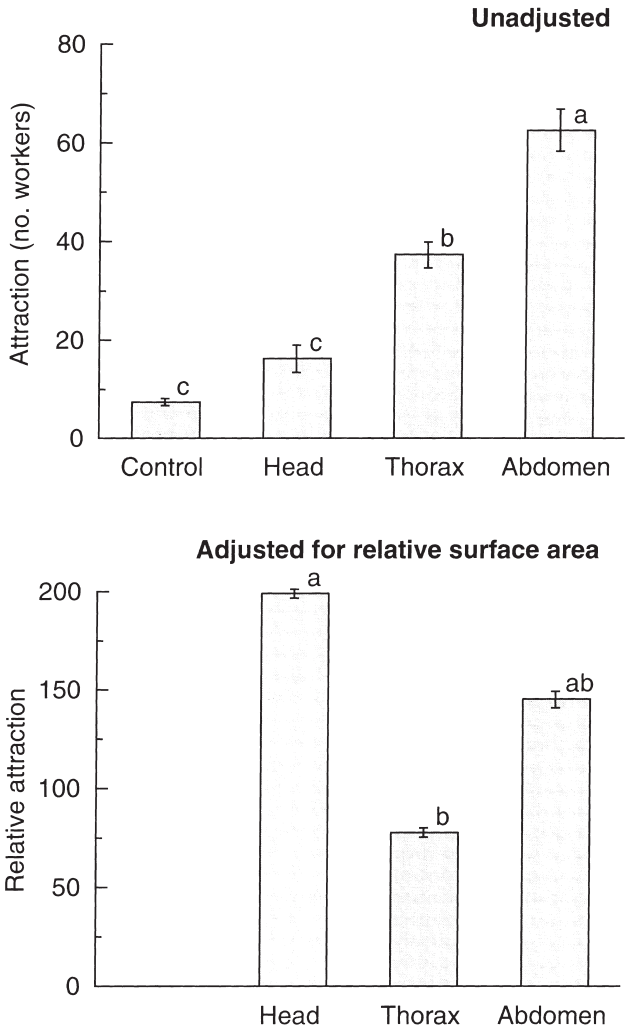

Fig. 3. The attractiveness of different body regions of queens before and after adjustment for relative surface area. The adjusted values were obtained by subtracting the corresponding control value from each experimental replicate and then dividing that value by its proportional surface area. There was a significant difference among treatments for both the unadjusted (F=, P<0.0) and adjusted values (F=, P<0.0); treatments with different letters differed significantly (P<0.05, Tukey test).

attraction. As shown in Fig. 4, extracts of alate queen glands were not attractive. In contrast, the postpharyngeal gland of functional queens was nearly as active as the poison sac, whereas the mandibular gland alone was not significantly more attractive than the hexane control. The mixture of the mandibular and postpharyngeal glands was not significantly more attractive than the postpharyngeal gland by itself, suggesting that the activity of the postpharyngeal gland alone accounts for the attractiveness of the head.

3.5. Effect of poison sac removal on primer pheromone production

To determine if there might be additional glandular sources of the primer pheromone inhibiting dealation

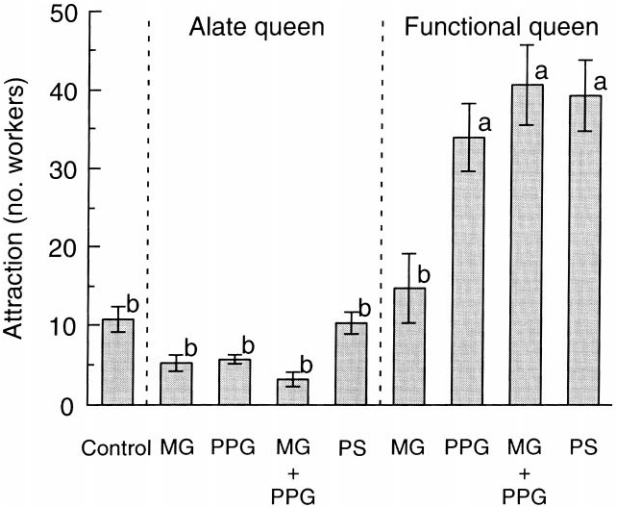

Fig. 4. Attractiveness of head exocrine glands of functional queens and of alates. Extracts of the mandibular gland (MG) and postpharyngeal gland (PPG) were tested separately and in combination. Poison sac extract (PS) was included for comparison. There was a significant difference among treatments ($F_{8,91}$=23.9, P<0.0001); treatments with different letters differed significantly (P<0.05, Tukey test). Extract were made from pooled glands of 12 individuals. There were 10 replicates of each treatment, except for the control which had 20.

and ovary development in virgin queens, we removed the poison sac from alate virgin queens. We then tested the ability of these females without poison sacs to develop into replacement queens capable of inhibiting the dealation of other female alates from their natal colony.

Even without their poison sacs, virgin replacement queens were capable of inhibiting alates from dealating and developing their ovaries (Table 1). Only a single alate out of 60 tested (3%) dealated in the presence of a virgin replacement queen lacking a poison sac. By contrast, 20 of 60 (33%) of the controls dealated with no queen present. The one individual that dealated in the presence of a virgin replacement queen had very little ovary development compared to the dealates in the controls.

4. Discussion

Our results demonstrate that the poison sac is not the only source of either queen recognition pheromone or the primer pheromone inhibiting reproductive development in alates. Removal of the poison sac from functionally reproductive, attractive queens or from non-attractive alates before they initiated reproduction did not diminish the ability of these queens to attract workers. Alates with their poison sacs removed also were able to develop into functional queens capable of producing a primer pheromone that inhibits alates from dealating. The postpharyngeal gland seems to be a second source

Table 1
Ability of virgin replacement queens (VRQ) without poison sacs to inhibit nestmate female alates from dealating and developing their ovaries[a]

Treatment	Status	*n*	Ovary development (mean ± SD)	Results of Tukey test ($P<0.05$)
VRQ-PS	Alate	59	0.2±0.1	c
	Dealate	1	0	
	VRQ	6	25.2±18.6	a
Q−	Alate	36	0.2±0.6	c
	Dealate	15	6.7±4.5	b
Q+	Alate	60	0.1±0.2	c

[a] Ovary development was assessed by counting the number of mature oocytes present in the ovaries and common oviduct. Q+: queenright monogyne colony; VRQ-PS: colony fragment containing a virgin replacement queen whose poison sac had been surgically removed; Q−: queenless colony fragment. There was a significant difference among treatments in degree of ovary development ($F_{5,176}$=69.0, $P<0.0001$).

of the recognition pheromone, whereas the possible second source(s) of the inhibitory primer pheromone remains to be identified.

Thus, as in the honey bee (Velthuis, 1970; Winston, 1987), there is apparent redundancy in the glandular sources of *S. invicta* queen pheromones. It is not known in either species whether the different exocrine glands release the same active compounds, or whether each gland secretes unique compounds that produce similar behavioral and physiological effects. In the latter case, it is possible that the different pheromone blends produced by each gland are not totally redundant. Rather, they may have slightly different but overlapping functions that were not evident in the context of the present study.

Studies of the postpharyngeal gland in *S. invicta* have revealed this gland to be dynamic in its contents and have suggested that it may have multiple functions. Of the different cephalic exocrine glands in this species, only the postpharyngeal gland is most developed in the queen caste (Phillips and Vinson, 1980). Vinson et al. (1980) proposed that the queen postpharyngeal gland serves as a cephalic caecum, absorbing fatty acids and their glycerol esters from food and then releasing them to the hemolymph. The lipids absorbed by the postpharyngeal gland appear to be important in sustaining the reproductive activity of the queen. These authors found that queens whose postpharyngeal glands were excised suffered depletion of the fat body, gradually lost weight and died. Interestingly, Vinson et al. (1980) noted that operated queens appeared to function normally and were treated normally by workers. To the extent that the postpharyngeal gland secretion constitutes redundancy in the queen pheromone system, normal treatment of glandectomized queens by workers is expected.

Calling attention to the postpharyngeal gland's apparent biosynthetic abilities, Vander Meer et al. (1982) suggested that the queen postpharyngeal gland serves social functions. The postpharyngeal gland of the queen contains large amounts of hydrocarbons, and the hydrocarbon composition and quantity changes after queens begin to reproduce (Thompson et al., 1981; Vander Meer et al., 1982). Thompson et al. (1981) proposed that the queen postpharyngeal gland might play a role in colony organization, including queen tending. Our results indicate that the gland does mediate queen tending, and it may well serve other important social functions.

Given the absorptive (Vinson et al., 1980) and biosynthetic (Vander Meer et al., 1982) capabilities of the postpharyngeal gland, there are two possible sources of the active pheromone components. First, the queen recognition pheromone could be produced in the postpharyngeal gland itself. Alternatively, the active compounds could be synthesized in other sites and absorbed by the gland, either directly from the hemolymph or from the surface of the queen's body after secretion to the cuticle. The results of our experiment showing that non-attractive alates were able to produce the pheromone after their poison sacs were removed suggest that it is unlikely that the postpharyngeal gland absorbs the retinue pheromone from the poison sac. The postpharyngeal gland must produce the active compounds itself, or it absorbs them from yet another gland.

The social role of postpharyngeal glands in other ants is not well known, but they appear to be involved in reinforcing and homogenizing colony-specific odors. In a comparative study, Bagnères and Morgan (1991) found that in each of five ant species the postpharyngeal glands of workers were rich in hydrocarbons that closely matched the profile of cuticular hydrocarbons. Soroker et al. (1995) showed in workers of *Cataglyphis niger* that the hydrocarbon contents of the postpharyngeal gland are synthesized internally and secreted onto the cuticle. From the cuticle, these hydrocarbons are sequestered in the postpharyngeal gland through self-grooming. Workers then distribute the hydrocarbons to nestmates via allogrooming and trophallaxis, where they are subsequently absorbed in the recipients' postpharyngeal glands, forming a homogeneous, colony-specific blend. Queens of *C. niger* appear to function as repositories and distribution centers of colony specific hydrocarbons, because their relatively large postpharyngeal glands

primarily contain secretions of worker postpharyngeal glands (Lahav et al., 1998). These secretions are then spread around the colony through grooming and feeding of the queen. Thus the postpharyngeal gland of ant queens is well situated for distribution of queen pheromones, and our results demonstrating a social function for this gland in reproductively active queens of *S. invicta* support the possibility that the postpharyngeal gland plays a communicative role in queens of other species.

Little is known about the glandular sources of queen pheromones in other ant species. Investigating the queen attraction pheromone of *M. rubra*, Cogliotore and Cammaerts (1981) were unable to pinpoint a single glandular source; these authors found that the head, thorax and abdomen were all equally attractive in relation to their relative surface areas. Edwards and Chambers (1984) identified neocembrene produced in the Dufour's gland of *Monomorium pharaonis* queens, which the authors speculated may serve as a queen recognition compound. Cariou-Etienne et al. (1992) found that the thorax of the queens of *L. humile* were more attractive than the head or abdomen, and they suggested that the metapleural gland or dispersed epidermal cells in the thorax could be the source of the queen attractant.

Brian and Blum (1969) showed that free fatty acids isolated from extracts of *Myrmica rubra* queen heads suppressed larval growth when applied topically, and these authors concluded that the source of the active material was most likely the postpharyngeal or mandibular glands. However, in a subsequent study, Brian (1973) found that queens who had either the postpharyngeal gland or mandibular glands removed retained the normal queen effect on larvae. He concluded that neither of these cephalic glands was the source of queen pheromone. If *M. rubra* queens also secrete pheromones from multiple sources, then it is possible that either the mandibular and/or postpharyngeal glands could be one source of queen pheromone with another source located elsewhere in the body.

Our results clearly demonstrate that two distinct effects of queen pheromones in *S. invicta* have multiple glandular sources. This work together with previous studies on the honey bee, suggest diverse sites of release and possibly biosynthesis may be a general feature of social insect queen pheromones.

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