



Studies on the Mode of Action of a Queen Primer Pheromone of the Fire Ant *Solenopsis invicta*

EDWARD L. VARGO,* MICHELE LAUREL*

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Studies were conducted on the physiological mode of action and mode of perception of a queen pheromone that inhibits dealation (wing-shedding) and ovary development in virgin queens of the fire ant *Solenopsis invicta*. Winged virgin queens were removed from the pheromonal signal (queen) to compare the response time for dealation, a behavioral response, and ovary development, a physiological response. Dealation was always accompanied by some degree of ovary development, whereas some individuals exhibited slightly developed ovaries before dealation occurred, suggesting that ovary development precedes dealation by several hours to a day. The response time of virgin queens following extinction of the pheromonal signal was highly variable and was related to colony source and body weight. Individuals from monogyne (single functional queen) colonies were more responsive than those from polygyne (multiple functional queens) colonies, a result consistent with the high cumulative levels of queen pheromone in the latter colonies. Virgin queens treated topically with the juvenile hormone (JH) analogue, methoprene, dealated and developed their ovaries in the presence of the pheromone (queen), suggesting that the pheromonal mode of action involves the suppression of JH titers. To obtain more precise information on the physiological processes underlying the inhibition of ovary development, vitellogenin titers were determined for virgin queens and functional (egg-laying) queens. Despite having undeveloped ovaries, virgin queens had vitellogenin titers that were as elevated as those of functional queens. This suggests that the effect of the low JH titers resulting from the primer pheromone is on the uptake of vitellogenin by the oocytes rather than on vitellogenin synthesis. The possible mode of perception of the inhibitory pheromone was also investigated. Virgin queens whose antennae had been removed dealated in the presence of the queen, suggesting that the pheromone acts by stimulating sensory cells in the antennae. The results are incorporated into a general model for the mode of action of the queen primer pheromone.

Formicidae Reproduction Primer pheromone Queen Juvenile hormone

INTRODUCTION

The study of insect chemical communication is a rapidly expanding field. The last 30 years have seen tremendous progress in our understanding of the chemistry of insect pheromones, their mode of perception, and the regulation of their biosynthesis and release (Mayer and Mankin, 1985; Tamaki, 1985; Kaissling, 1986; Prestwich and Blomquist, 1987; Mayer and McLaughlin, 1991; Schneider, 1992; Raina and Menn, 1993). However, nearly all of this progress has concerned releaser pheromones, i.e. intraspecific chemical signals that elicit behavioral responses, such as sex attractants. In contrast, progress has been much slower in our understanding of primer pheromones,

which have less rapid but more profound effects on the physiology of the target individuals (Wilson and Bossert, 1963).

Primer pheromones are thought to be widespread among the social insects. They are produced by the primary reproductives (queens in the social Hymenoptera) and serve to regulate ovary development in adult female members of the colony and influence the developmental fate of larvae (Wilson, 1971). We currently have little information on the chemical nature of social insect queen primer pheromones, their mode of perception, or their physiological mode of action. The only social insect primer pheromone that has been identified is that of the queen honey bee, which originates primarily in the mandibular gland [reviewed in Winston and Slessor (1992)]. Originally, this queen mandibular pheromone, especially its major constituent, (E)-9-keto-2-decenoic acid, was reported to inhibit both

*Department of Zoology, University of Texas, Austin, TX 78712, U.S.A.

the rearing of new queens (Butler, 1959a, 1960, 1961; Butler and Paton, 1962; Butler and Callow, 1968) and ovary development in workers (de Groot and Voogd, 1954; Butler 1959b; Butler *et al.*, 1962; Butler and Fairey, 1963). However, recent studies using synthetic components in natural proportions suggest that, although this pheromone complex inhibits the rearing of new queens (Winston *et al.*, 1989, 1990), it does not inhibit worker ovary development (Willis *et al.*, 1990).

There are few data concerning the mode of perception of the honey bee queen pheromone. Kaissling and Renner (1968) found that (E)-9-keto-2-decenoic acid elicits firing by specialized sensory cells in the antennae of worker honey bees. However, the response of worker antennae to the other components of the queen mandibular gland secretion has not been investigated. Information is also lacking concerning the physiological mode of action of the honey bee queen pheromones. Kaatz *et al.* (1992) recently found that (E)-9-keto-2-decenoic acid inhibits juvenile hormone (JH) secretion by the corpora allata in young workers. The consequences of lower JH synthesis in response to (E)-9-keto-2-decenoic acid in young bees are unclear, however, because this pheromone component may not inhibit ovary development (Willis *et al.*, 1990). Furthermore, developed ovaries in queen and worker honey bees is not related to elevated JH titers (Robinson *et al.*, 1991, 1992), suggesting that in honey bees JH does not play the role it typically assumes in regulating insect ovary development (Koeppel *et al.*, 1985).

In the bumble bee, *Bombus terrestris*, ovary development appears to follow the more general insect scheme involving regulation by JH (Röseler, 1977; Röseler and Röseler, 1978). In this species queens produce a pheromone of known composition in the mandibular glands that inhibits ovary development in workers by suppressing JH synthesis (Röseler *et al.*, 1981). No information is available on the possible mode of transmission or perception of this bumble bee queen pheromone.

Several effects of queen pheromones have been documented in the fire ant *Solenopsis invicta*. These include the development of male and female sexuals (Vargo and Fletcher, 1986), dealation (wing shedding) and ovary development in winged virgin queens (Fletcher and Blum, 1981a), and ovary development among cohabiting egg-laying queens in polygyne (multiple-queen) colonies (Vargo, 1992). Although there is little specific information available on the mode of action of these pheromones, evidence to date suggests that pheromonal regulation of ovary development involves JH.

Results of studies in which virgin queens in isolation were treated topically with synthetic JH, either by itself (Kearney *et al.*, 1977) or in combination with allatectomy (Barker, 1978, 1979), indicate that both dealation and ovary development are under the control of JH. Using topical application of the JH analogue, methoprene, Vargo (1992) obtained evidence that the rate of oviposition among queens in polygyne colonies is regulated by JH. Fletcher and Blum (1983) and Vargo (1992)

hypothesized that fire ant queen primer pheromones prevent ovary development in winged queens and reproductively active queens, respectively, by inhibiting JH synthesis. However, the possible JH-suppressing effect of the pheromone has not been tested.

In this paper, we investigate several aspects of the mode of action and mode of perception of the pheromone inhibiting dealation and ovary development in *S. invicta* virgin queens. Specifically, we address four questions. First, how closely linked are the responses of ovary development and dealation? These two responses are very different; dealation is a behavioral response in which an individual breaks off her wings with the hind legs, whereas ovary development is a physiological response. Although these two responses occur in individuals removed from the influence of the queen, it is not known whether they occur simultaneously or whether there is a delay separating them. Second, can the inhibitory effects of the pheromone be overcome with topical application of a JH analogue? Third, is ovary development in virgin queens blocked at the vitellogenin-synthesis or vitellogenin-uptake stage? Finally, are antennal receptors in the virgin queens involved in the perception of the pheromone? Answers to these questions give a more complete picture of the physiological mode of action of this queen primer pheromone.

MATERIALS AND METHODS

Source and maintenance of ants

All ants used in these experiments originated from Travis and Hays Counties, TX. The colonies were collected by excavation of the mound, and the ants were removed from the soil by flooding (Jouvenaz *et al.*, 1977). Dates of collection are given below. Colonies were housed in plastic trays (40 × 52 × 8 cm) equipped with four nests (14 cm dia Petri dishes half-filled with damp plaster) and maintained in the laboratory at 29 ± 2°C and natural photoperiod. The ants were fed crickets (*Acheta domesticus*) daily and given sugar water and tap water *ad libitum*.

Timing of dealation and ovary development

Monogyne colonies ($n = 9$) used in this experiment were collected on 6 May 1991 and polygyne colonies ($n = 9$) were collected on 13 May 1991. All colonies had numerous winged virgin queens present at the time of collection.

Dealation and ovary development were studied using two winged virgin queens placed in small colony fragments (2.5 ml workers and brood) as described by Fletcher and Blum (1981b). Virgin queens were monitored every 12 h for dealation, defined as having lost at least three of the four wings. The time elapsed for dealation to occur in at least one of the two virgin queens present in each fragment was recorded. Ovary development was assessed by dissecting virgin queens in 70% ethanol under a dissecting microscope and counting the

total number of chorionated eggs (fully-formed eggs, i.e. those ≥ 0.4 mm wide \times 0.28 mm long) present in the ovarioles and common oviduct. At the beginning of each experiment, a sample of virgin queens from each colony was weighed and dissected to assess ovary development.

In this experiment, the timing of dealation and ovary development was investigated in virgin queens originating from monogyne and polygyne colonies as well as the effect of the weights of individual queens on response time. Ten virgin queens from each colony were weighed and then dissected to assess ovary development. Ten others were weighed and placed in pairs into small colony fragments ($n = 5$ fragments per colony); each individual was marked with one of two colors of paint (Tex Pen[®], Mark-Tex Corp., Engelwood, NJ) for later identification. The fragments were examined every 12 h for the presence of dealates. As soon as at least one dealate was observed in a fragment, both virgin queens present were removed, dissected and their ovary development assessed. The experiment ran for three weeks, after which time all remaining virgin queens were dissected to determine the degree of ovary development.

Methoprene treatment

The ants used for this experiment were of the monogyne form and were collected 26 and 30 May 1992 from Hays and Travis Counties, TX. All colonies contained numerous alate queens. Approximately one month after collection, the colonies were sieved to remove all sexual forms [adults, pupae and large fourth instar larvae; Vargo (1988)] and reduced to a size of 10 g adult workers and brood (approx. 5500 adult workers, 4000 worker pupae and 8500 larvae of all stages). The colonies were housed in rearing trays (40 \times 52 \times 8 cm), each containing a single nest as described above. The mother queen of the colony was placed in the fragment. Twenty virgin queens, all field-reared, were returned to each colony. Half of these were topically treated with 0.5 μ g methoprene in 0.5 μ l acetone by placing the solution on the abdominal dorsum. The other half of the virgin queens was treated with only the acetone carrier. Each of the queens was marked with a spot of Texpen ink identifying it to group. The colony fragments were monitored daily for three days for the presence of any dealated virgin queens. All dealates present on a given day were removed and dissected to assess ovary development. At the end of three days, all remaining winged virgin queens were removed and their ovaries examined.

Vitellogenin titers

Vitellogenin titers were determined by SDS-PAGE based on the methods of Martínez and Wheeler (1991a, b). Hemolymph from individual queens was collected in 0.5 μ l capillary tubes through a small incision on the thoracic dorsum and immediately transferred into a tube containing 10 μ l SDS sample buffer (62 mM Tris-HCl, pH 6.8, 10% glycerol, 5% 2-mercaptoethanol, 2% SDS, 0.005% Bromophenol Blue). Freshly-laid eggs were homogenized directly into 10 μ l

SDS sample buffer. Samples were centrifuged for 3 s at 8000 *g* and stored at -20°C . Just prior to loading, the samples were thawed, boiled for 5 min, centrifuged for 3 s at 8000 *g*, and transferred to ice. Aliquots from each sample were used for SDS-PAGE analyses.

Samples were subjected to SDS-PAGE using a 7% polyacrylamide gel with a 4% stacking gel. Electrophoresis was performed at constant voltage with a starting current of 20 mA. Gels were stained with 0.24% Coomassie Brilliant Blue R 250 in 45.6% methanol, 9.2% acetic acid and destained in 7.5% acetic acid, 5% methanol.

The density of the vitellogenin bands was quantified for each sample using a video densitometer designed by L. L. Poulsen (U.S. Patent No. 5194949). The band corresponding to the most abundant protein in freshly-laid eggs (approx. 180 kDa) was determined for each sample. The quantity of vitellogenin present was determined by comparison with the density of a known quantity of myosin standard (200 kDa).

All queens sampled were dissected to assess ovary development. The ovaries were removed and the maximum number of opaque vitellogenic oocytes per ovariole was determined (Tschinkel, 1988) by examining several ovarioles.

Antennectomy

Five monogyne colonies, containing numerous winged queens, were collected 26 and 30 May 1992. Approximately four weeks after collection, the colonies were sieved to remove sexuals, and the workers and brood were divided into two equal halves based on weight (mean \pm SD weight of colony halves = 11.5 \pm 2.8 g). The mother queen was returned to one of the halves (queen-right halves). Each colony half was given a total of 20 nestmate winged virgin queens, all of which were field reared and at least one month post emergence age. The antennae were removed from 10 of the virgin queens in each colony half by cutting the scape near the insertion point with fine dissecting scissors. The 10 control virgin queens in each colony half were amputated at a middle leg by cutting the proximal region of the femur. The colony halves were monitored daily for the presence of dealates. Any dealates observed were removed and dissected to assess ovary development as above. Ovary development was also assessed for 10 winged virgin queens from each colony at the time of colony division. The experiment was terminated after 15 days.

RESULTS

Timing of dealation and ovary development

There was a large difference between monogyne and polygyne colonies in the frequency of virgin queen dealation; by 72 h dealation had occurred in all 25 fragments containing virgin queens from monogyne colonies, whereas dealation had occurred in only three (12%) of the 25 fragments from polygyne colonies ($X^2 = 25.1$, $P < 0.0001$). The number of polygyne

fragments containing dealates had risen to only 7 (28%) by the end of the experiment. Virgin queens from polygyne colonies that dealated took nearly four times longer to do so than did those from monogyne colonies (mean = 120.0 ± 156.0 and 31.7 ± 15.8 h for polygyne and monogyne, respectively; $t_{30} = 2.90$, $P < 0.01$, two-tailed test). Among the monogyne colonies, there was a significant effect of colony on the time to dealation ($F_{4,20} = 4.49$, $P < 0.01$).

Dealation was related to both higher weights and increased ovary development. For the colony fragments in which only one of the two virgin queens dealated, the dealated individual had an initial weight that was on average 4% more than the alate (mean difference = 0.58 ± 0.85 mg; $t_{21} = 3.22$, $P < 0.005$, two-tailed paired test). When the virgin queens from monogyne and polygyne colonies were analyzed separately, the dealates in the polygyne form had an initial weight that was 8.6% greater than alates (mean difference = 1.1 ± 0.9 mg, $t_5 = 3.0$, $P < 0.05$), whereas in the monogyne form dealates weighed only 2.8% more (mean difference = 0.4 ± 0.8 mg, $t_{15} = 2.0$, $P > 0.05$). The difference in ovary development between the dealate and alate in the same fragment was even more pronounced. When virgin queens from monogyne and polygyne colonies were taken together, the dealated females had an average of 7.6 ± 2.0 chorionated oocytes compared to 3.2 ± 2.8 for the alates ($t_{21} = 6.85$, $P < 0.0001$, two-tailed paired test). Again, when analyzed separately, this difference was greater among females of the polygyne form, where dealates had 6.2 ± 2.0 more chorionated oocytes than did alates ($t_5 = 7.4$, $P < 0.001$); in fragments from monogyne colonies dealates had 3.8 ± 3.2 more ($t_{15} = 4.8$, $P < 0.0002$).

A comparison of the degree of ovary development in relation to dealation status was performed by means of a two-way ANOVA, with social form and dealation status as the independent variables. In order to detect changes in ovary development under queenless conditions, winged virgin queens from the queenright colonies at the start of the experiment were also included in this analysis. Both social form ($F_{1,191} = 75.4$, $P < 0.0001$) and dealation status ($F_{2,191} = 105.1$, $P < 0.0001$) had highly significant effects, and the interaction term was also significant ($F_{2,191} = 3.1$, $P < 0.05$). Therefore, the effects of dealation status were analyzed separately for each form. In both social forms, there was a highly significant difference in the degree of ovary development among all three groups, i.e. dealates and alates in the queenless colony fragments and alate virgin queens in the queenright colonies at set-up (Table 1; $F_{2,97} = 80.3$, $P < 0.0001$ and $F_{2,94} = 72.8$, $P < 0.0001$, for the monogyne and polygyne forms, respectively). In both forms, dealates showed the most ovary development, whereas alate virgin queens in the queenless colony fragments had significantly more developed ovaries than did those in the presence of the queen at the start of the experiment ($P < 0.05$, Tukey test). These results suggest that the responses of dealation and ovary

TABLE 1. Ovary development (mean \pm SD) of virgin queens in response to queenlessness and dealation

Form/condition	<i>n</i>	No. chorionated oocytes	Results of the Tukey test
<i>Monogyne</i>			
Q+	50	2.1 ± 2.1	A
Alate	16	4.3 ± 2.4	B
Dealate	34	8.7 ± 2.7	C
<i>Polygyne</i>			
Q+	50	0.2 ± 0.5	A
Alate	39	1.0 ± 1.2	B
Dealate	8	5.4 ± 2.8	C

Queenright condition (Q+) refers to condition of the virgin queens in their natal colonies at the time the experiment was started. Virgin queens were set up in pairs in small queenless colony fragments. As soon as at least one individual in each fragment dealated, both virgin queens present were dissected to assess ovary development. Included in the polygyne form are those fragments in which no dealation occurred during the 3-week experiment. Treatments with different letters within each form differed significantly ($P < 0.05$, Tukey test).

development are tightly linked, but that ovary development begins slightly before dealation.

Methoprene treatment

Of the 50 methoprene treated virgin queens, 44 (88%) individuals dealated by the end of the 3-day period (Fig. 1), with a mean of 8.8 ± 1.3 individuals per colony

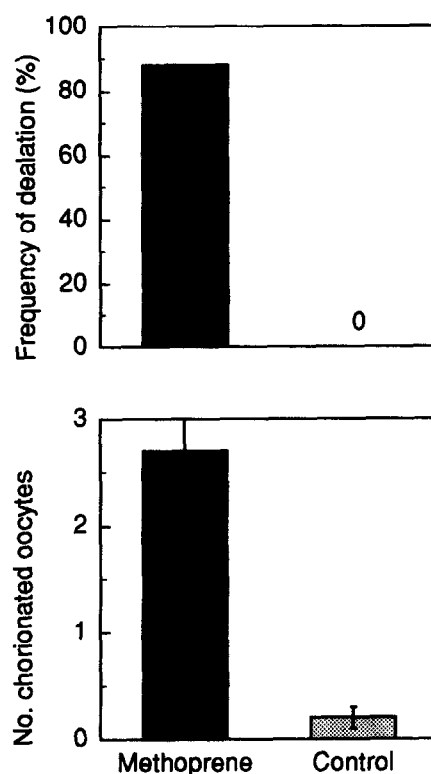


FIGURE 1. Effect of topical treatment of methoprene ($0.5 \mu\text{g}$ in $0.5 \mu\text{l}$ acetone; $n = 50$) on dealation and ovary development (mean \pm SE) in the presence of queen inhibitory pheromone. Controls ($n = 45$) were treated with $0.5 \mu\text{l}$ of the acetone carrier.

(range = 7–10). In contrast, none of the control virgin queens shed their wings during this time. The difference between the two groups was highly significant when analyzed either by pooling individuals from all colonies ($P < 0.0001$, Fisher exact test) or by examining each colony separately (all $P < 0.0025$, Fisher exact test). A total of five control individuals from three different colonies were found dead of unknown causes.

Individuals in the methoprene treatment had far more developed ovaries (Fig. 1). A two-way ANOVA showed a significant effect of both treatment ($F_{1,85} = 74.6$, $P < 0.0001$) and colony ($F_{4,85} = 3.2$, $P < 0.02$) but not of the interaction term ($F_{4,85} = 1.3$, $P > 0.25$).

Vitellogenin titer determinations

As shown in Fig. 2, there was no significant difference among the three categories of queens in their titers of vitellogenin ($F_{2,21} = 0.6$, $P > 0.5$). This is in sharp contrast to the large differences in ovary development (Fig. 2; $F_{2,21} = 221.5$, $P < 0.0001$), in which all categories differed significantly from the others ($P < 0.05$, Tukey test). Thus, inhibited virgin queens with undeveloped ovaries had vitellogenin titers comparable to those of

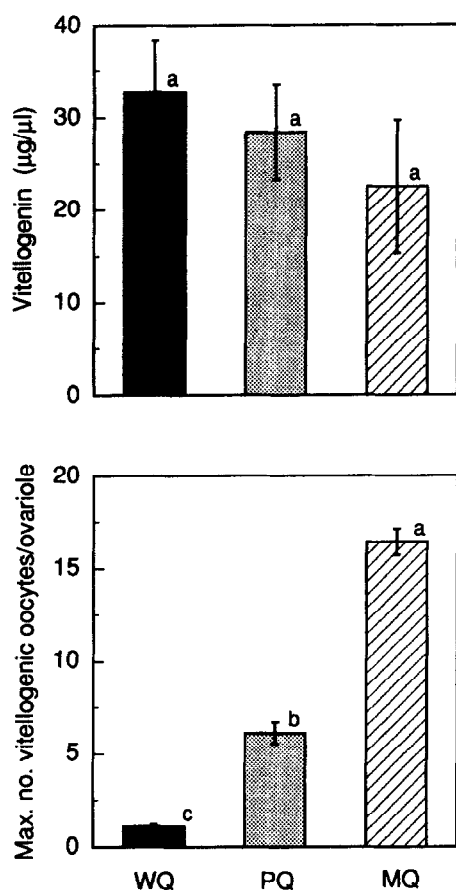


FIGURE 2. Relationship between vitellogenin titers ($\mu\text{g}/\mu\text{l}$ hemolymph, expressed as myosin equivalents) and ovary development in *S. invicta* queens. Shown are means \pm SE. WQ, winged virgin queens in the presence of a pheromone producing queen ($n = 10$); PQ, egg-laying dealated queens from polygyne colonies ($n = 9$); MQ, egg-laying dealated queens from monogyne colonies ($n = 5$). Groups with different lower case letters differed significantly ($P < 0.05$, Tukey test).

actively laying queens with highly developed ovaries. These results suggest that the effect of the inhibitory pheromone is to block ovary development primarily by suppressing the uptake of vitellogenin rather than affecting its rate of synthesis.

Antennectomy

There was considerable mortality of the virgin queens whose antennae had been removed. Of the 50 treated individuals, 25 (50%) died in the queenless colony halves during the experiment (range = 3–9 per colony half). In contrast only a single control individual died in the queenless halves. Mortality was higher in the queenright halves, in which only nine of the antennaeless individuals survived (range = 4–0 survivors per colony half), as opposed to 38 survivors in the control group. In some cases, workers were seen attacking individuals, all of which were wingless. It is not known whether dealation occurred before attack by workers or as a result of attack. However, since no alates were seen to be attacked, it is likely that worker attack followed dealation rather than causing it.

Among the surviving virgin queens, there was a strong difference in the frequency of dealation in the queenright halves but not the queenless halves (Fig. 3). In the queenright halves, all nine of the surviving individuals

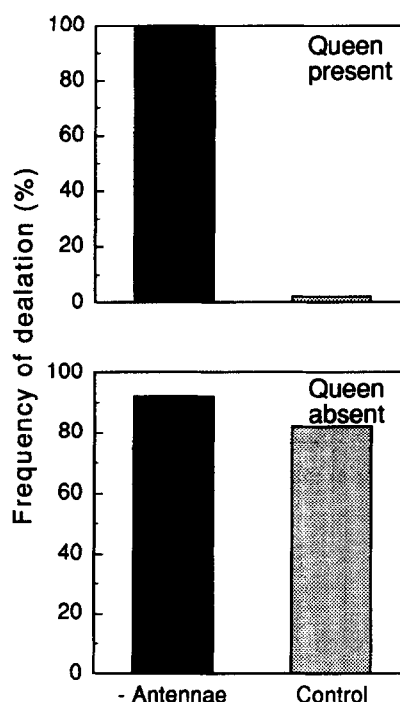


FIGURE 3. Effect of removing antennae on dealation in the presence and absence of queen inhibitory pheromone. The antennectomized (-antennae) group ($n = 9$ and 25 in the presence and absence of the queen, respectively) had their antennae removed by cutting them off at the proximal end of the scape. The control group ($n = 41$ and 49 in the presence and absence of the queen, respectively) had one middle leg removed by cutting at the proximal end of the femur. A significantly higher frequency of antennectomized individuals dealated in the presence of the queen ($P < 0.001$, Fisher exact test) but not in her absence ($P = 0.15$, Fisher exact test).

whose antennae had been removed dealated, whereas only one (2.6%) of the controls shed her wings ($P < 0.001$, Fisher exact test). In contrast, the frequency of dealation in the two groups was similar in the queenless halves ($P = 0.145$, Fisher exact test).

In comparing ovary development, individuals were grouped according to whether queen pheromone was present or absent (queenright vs queenless colony halves), and whether they had dealated in the course of the experiment (alate vs dealate). Out of the eight possible groups (2 pheromone conditions \times 2 treatments \times 2 states for wing presence), three groups were not included because one group (antennaeless alates in queenright colony halves) had no members and two groups (antennaeless alates in queenless colony halves and control dealates in queenright colony halves) had very few members ($n = 2$ and 1, respectively). Individuals that were in the queenright colonies on the day the experiment started also were included in the analysis. An ANOVA showed a significant difference among the groups ($F_{5,162} = 1.3$, $P < 0.0001$). As seen in Table 2, this difference was due to the greater degree of ovary development in the control dealates in the queenless colony halves than in those of all the other groups. Thus, although antennectomy caused virgin queens to dealate, even when in the presence of a pheromone producing queen, development of the ovaries that normally accompanies wing shedding did not occur.

DISCUSSION

The finding that topical application of a JH analogue to virgin queen fire ants overcomes the inhibitory effects of the queen pheromone strongly suggests that the pheromone inhibiting dealation and ovary development acts by suppressing JH titers. The precise mechanism of JH titer suppression was not investigated, but a likely candidate is inhibition of JH synthesis by the corpora allata. There is growing evidence from social insects that queen primer pheromones affect JH titers by suppressing the activity of the corpora allata. In the bumble bee, *B. terrestris*, Röseler *et al.* (1981) found that extracts of queen mandibular glands prevented ovary development

in workers by inhibiting JH production by the corpora allata (Röseler, 1977; Röseler and Röseler, 1978). Recently, Kaatz *et al.* (1992) showed in the honey bee that synthetic (E)-9-oxo-2-decenoic acid, the principal component of the honey bee queen mandibular gland secretion, inhibits JH synthesis by the corpora allata in young (8 day-old) workers. However, the effects of lower rates of JH synthesis in young bees is not clear, because JH does not appear to be the gonadotropic hormone in this species (Robinson *et al.*, 1991, 1992). In the termite, *Zootermopsis angusticollis*, Greenberg and Tobe (1985) reported that larvae had lower rates of JH synthesis by the corpora allata in the presence of the king and queen than in their absence. It is likely that a pheromone produced by the royal pair is responsible for this effect, but the existence of an inhibitory pheromone in *Z. angusticollis* has not been conclusively demonstrated (Stuart, 1979).

Although it is well established that social insect queens inhibit ovary development in nestmates, little is known about the process of oogenesis and the physiological mechanisms underlying its control. The present results demonstrate that virgin queens of *S. invicta*, whose ovary development is blocked by the queen inhibitory pheromone, have vitellogenin titers comparable to those of egg-laying queens. Thus in fire ants, the presence of vitellogenin in the hemolymph is not sufficient to induce yolk deposition, a phenomenon noted in other insects (Bell and Barth, 1971), including the honey bee (Engels, 1974) and the ant *Camponotus festinatus* (Martinez and Wheeler, 1991a). Evidently, in many insects a specific trigger is necessary for vitellogenin uptake to occur, and it is such a trigger that is blocked by the fire ant queen inhibitory pheromone. As discussed below, the trigger in *S. invicta* queens may be a relatively high titer of JH. Although the main effect on ovary development appears to be inhibition of oogenesis, it is nevertheless possible that the queen pheromone reduces the rate of vitellogenin synthesis in inhibited virgin queens compared to reproductively active queens. However, because vitellogenin is not incorporated in the former group, it accumulates and eventually reaches titers as elevated as those in egg laying queens, which may be synthesizing

TABLE 2. Effect of antennectomy and queen pheromone on ovary development in virgin queens

Treatment	Dealated	<i>n</i>	No. chorionated oocytes (mean \pm SD)	Results of Tukey test ($P < 0.05$)
Queenright colony at start	—	50	1.5 \pm 2.4	B
<i>Queenright colony half</i>				
Antennae removed	+	9	1.2 \pm 2.9	B
Control	—	37	0.9 \pm 1.5	B
<i>Queenless colony half</i>				
Antennae removed	+	23	1.9 \pm 2.6	B
Control	—	9	2.6 \pm 3.6	B
Control	+	40	7.3 \pm 4.6	A

Treatments with different letters differed significantly ($P < 0.05$, Tukey test).

vitellogenin at higher rates. Studies of the rate of vitellogenin synthesis and turnover in inhibited and reproductively active queens are needed to determine possible effects of the pheromone on vitellogenin synthesis.

In this study, we found that virgin queens amputated of their antennae dealated in the presence of a queen producing the primer pheromone. This result is expected if the primer pheromone is perceived through sensory cells in the antennae, and the inhibitory signal is then transmitted via the nervous system to the target tissues, presumably the corpora allata. In many insects, the activity of the corpora allata is regulated by neurosecretory cells in the brain [reviewed in Feyereisen (1985)]. Results from extirpation experiments on *S. invicta* virgin queens (Barker, 1978) are consistent with this general model and suggest that the median neurosecretory cells regulate the activity of the corpora allata. In line with the present results, Sorensen *et al.* (1985) found that the pheromone components, which appear to be relatively non volatile (Fletcher and Blum, 1981a), could be quickly and efficiently transported throughout fire ant colonies via surface contact.

A puzzling result of the antennectomy experiment was that ovary development in amputated individuals did not coincide with dealation. This is unexpected if inhibition of both dealation and ovary development is achieved simply by pheromonal stimulation of sensory cells in the antennae, which in turn act on the corpora allata to inhibit JH synthesis. The reasons for the uncoupling of dealation and ovary development in this case are not clear, but possible explanations include:

- (1) the presence of other sensory cues perceived by the antennae that are needed to stimulate oogenesis; and
- (2) antennectomy induces dealation by affecting the nervous system via some alternate route not involving JH.

We do not yet have sufficient data to exclude either of these possibilities.

Based on the results of the present study, we propose a general model, illustrated in Fig. 4, for the mode of action of the fire ant queen pheromone that inhibits dealation and ovary development. The pheromone is perceived by sensory cells in the antennae. Stimulation of these sensory cells inhibits the brain from releasing chemical and/or neural signals that stimulate the corpora allata to synthesize JH. Consequently, the JH titer is maintained at relatively low levels in the presence of the pheromone. While the JH titer is kept relatively low, dealation and oogenesis by virgin queens are prevented. Presumably, JH acts directly on the nervous system to elicit dealation, whereas it acts indirectly on ovary development by stimulating the oocytes to absorb previously synthesized vitellogenin. The uncoupling of dealation and ovary development following antennectomy, as discussed above, suggests the physiological control of these processes is more complicated than

indicated here, but the precise nature of these complications has yet to be determined.

We also hypothesize that JH controls vitellogenin synthesis by the fat body, as occurs in many insects (Koeppel *et al.*, 1985), but that vitellogenin synthesis has a lower JH threshold than does vitellogenin uptake by the ovaries. Thus the pheromone does not completely suppress JH synthesis, but reduces it to a low level, keeping the JH titer below the vitellogenin absorption threshold but above the vitellogenin synthesis threshold. Further studies are needed to test and refine this model. Of critical importance in this regard will be the identification of the active pheromone components and JH titer determinations.

Once removed from the queen primer pheromone, ovary development in virgin queens appears to begin slightly ahead of dealation. Although several studies have shown that dealation by virgin queens of *S. invicta* is associated with eventual oogenesis and oviposition (Kearney *et al.*, 1977; Barker, 1978; Tschinkel and Howard, 1978; Fletcher and Blum, 1981a, 1983; Fletcher *et al.*, 1983), the precise timing of these events was previously unknown. Fletcher and Blum (1981a) dissected dealate virgin queens one week after queenlessness and found some with developed ovaries. Fletcher *et al.* (1983) reported that some dealate virgin queens examined 72 h after queenlessness exhibited ovary development. The present study, in which virgin queens were dissected within 12 h post-dealation, offers a more detailed view of the timing. All of the dealates showed some signs of ovary development, whereas only some of the alates, which had been free from the queen pheromone for the same period of time as the dealates, exhibited signs of ovary development. The finding that ovary development appears to precede dealation suggests that either dealation has a slightly higher JH threshold than vitellogenin absorption by the ovaries, or dealation proceeds more slowly after the JH threshold is crossed. Histolysis of the flight muscles, another response of virgin queens to queenlessness, takes longer to occur than does dealation; it begins just after wing shedding and is complete after about 10 days (Barker, 1979).

In the present study, polygyne virgin queens were much less responsive than monogyne virgin queens to removal of the pheromone-producing queens. Keller and Ross (1993a) also found polygyne queens to be less responsive. These authors reported that genotype greatly affects the response of virgin queens from polygyne but not monogyne colonies. They showed that in polygyne colonies from Georgia, mature virgin queens with the genotype *Pgm-3^a/-3^a* weighed 13% more than virgin queens of the other two genotypes that occur at this locus (*Pgm-3^a/-3^b* and *Pgm-3^b/-3^b*) and, following removal of all reproductively active queens, were 5 times more likely to dealate and begin laying eggs during the 3-day test period. Although the frequency of the *Pgm-3* alleles has not been investigated in our Texas study population, the small number of responsive individuals

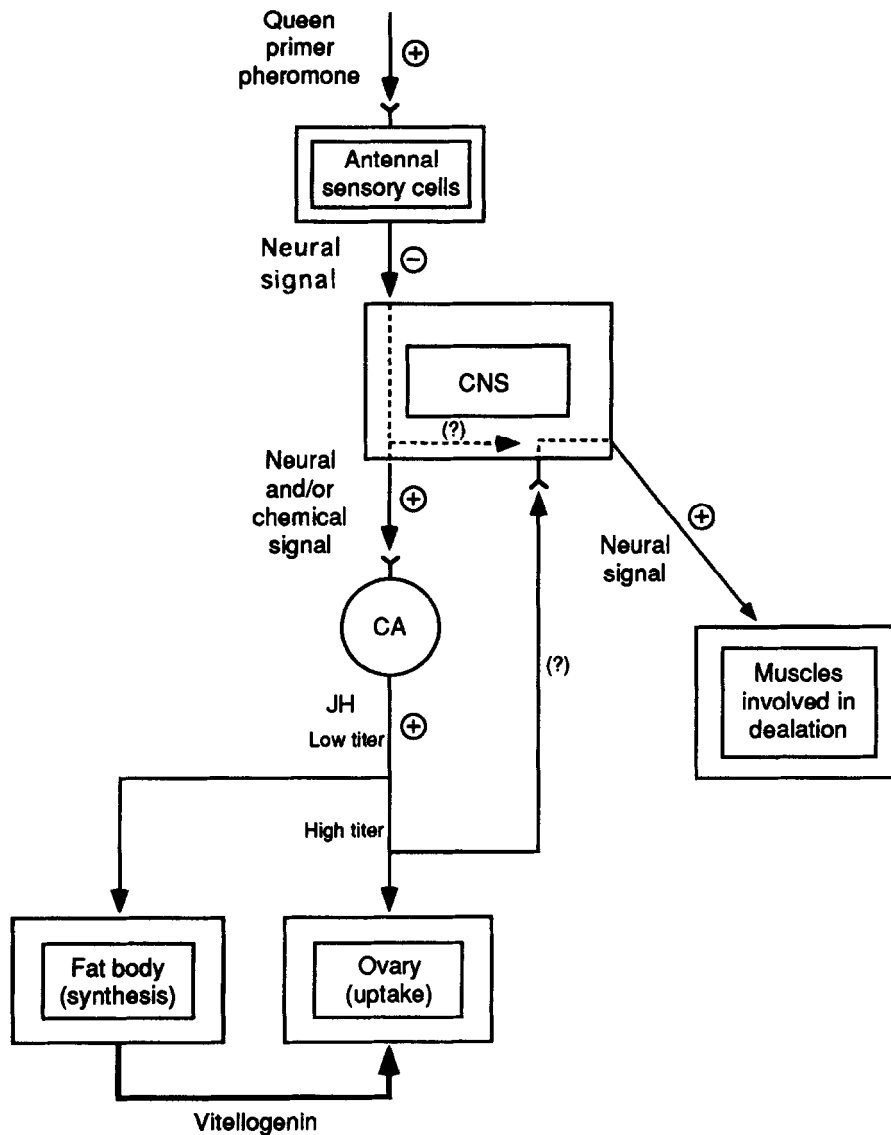


FIGURE 4. Proposed general model for the mode of action of the primer pheromone of queen fire ants that inhibits dealation and ovary development in virgin queens. In this model, the pheromone triggers antennal receptors which send inhibitory signals to the median neurosecretory cells in the brain. Largely inhibited, the median neurosecretory cells only weakly stimulate the corpora allata to synthesize JH, maintaining low titers of this hormone. At low levels, JH stimulates vitellogenin synthesis in the fat body. In the absence of the pheromone, the disinhibited neurosecretory cells send a stronger chemical and/or neural signal that triggers the corpora allata to produce larger quantities of JH. At higher titers, JH stimulates vitellogenin uptake by the ovaries and dealation, the latter process possibly involving an effect of JH on the nervous system. Dealation may result from a JH-independent pathway in the nervous system in lieu of or in addition to the JH-mediated pathway. These two possible pathways for control of dealation are flagged with question marks.

from polygyne colonies found here (12% after 3 days) is similar to the low frequency of the more responsive *Pgm-3^a/-3^a* individuals occurring in a Georgia polygyne population [11%; Keller and Ross (1993a)], suggesting that the frequency of the *Pgm-3^a/-3^a* genotype may be similar in the two populations. Interestingly, this genotypic effect on reproductive maturation does not occur in monogyne colonies, in which all three genotypic classes are equally likely to undergo reproductive development under queenless conditions (Keller and Ross, 1993a).

As hypothesized by Keller and Ross (1993a), the low responsiveness of virgin queens in polygyne colonies

probably results from the pheromonal milieu of the colony. Because of the relatively high levels of queen pheromone that likely accumulate in polygyne colonies (Vargo and Fletcher, 1987), polygyne virgin queens are subjected to more intense inhibition than virgin queens in monogyne colonies. This increased pheromonal influence likely affects the rate of virgin queen reproductive maturity in a genotype-specific manner. Evidence for such a social environment-genotype interaction has been recently documented by Keller and Ross (1993b), in which queen pupae from monogyne and polygyne colonies were cross-fostered in colonies of the alternate type. In addition to an effect of social form on

reproductive development of virgin queens, the results of the present study indicate there is a strong effect of source colony in the monogyne form. The causes of this variability are not known but could involve one or more of the following: age differences, genotype, social environment and ecological factors.

REFERENCES

- Barker J. F. (1978) Neuroendocrine regulation of oocyte maturation in the imported fire ant *Solenopsis invicta*. *Gen. comp. Endocrinol.* **35**, 234–237.
- Barker J. F. (1979) Endocrine basis of wing casting and flight muscle histolysis in the fire ant *Solenopsis invicta*. *Experientia* **35**, 552–554.
- Bell W. J. and Barth R. H. (1971) Initiation of yolk deposition by juvenile hormone. *Nature, New Biol.* **230**, 220–222.
- Butler C. G. (1959a) Queen substance. *Bee World* **40**, 269–275.
- Butler C. G. (1959b) The source of the substance produced by a queen honey bee (*Apis mellifera* L.) which inhibits development of ovaries of the workers of her colony. *Proc. Roy. ent. Soc. London (A)* **34**, 137–138.
- Butler C. G. (1960) The significance of queen substance in swarming and supersedure in honeybee (*Apis mellifera* L.) colonies. *Proc. Roy. ent. Soc. London (A)* **35**, 129–132.
- Butler C. G. (1961) The scent of queen honeybees (*A. mellifera* L.) that causes partial inhibition of queen rearing. *J. Insect Physiol.* **7**, 258–264.
- Butler C. G. and Callow R. K. (1968) Pheromones of the honeybee (*Apis mellifera* L.): the “inhibitory scent” of the queen. *Proc. Roy. ent. Soc. London B* **43**, 62–65.
- Butler C. G. and Fairey E. M. (1963) The role of the queen in preventing oogenesis in worker honey bees. *J. apic. Res.* **2**, 14–18.
- Butler C. G. and Paton P. N. (1962) Inhibition of queen rearing by queen honey-bees (*Apis mellifera* L.) of different ages. *Proc. Roy. ent. Soc. London (A)* **37**, 114–116.
- Butler C. G., Callow R. K. and Johnston N. C. (1962) The isolation and synthesis of queen substance, 9-oxodec-trans-2-enoic acid, a honey bee pheromone. *Proc. Roy. ent. Soc. London (B)* **155**, 417–432.
- Callow R. K., Chapman J. R. and Paton P. N. (1964) Pheromones of the honey bee: chemical studies of the mandibular gland secretion of the queen. *J. apic. Res.* **3**, 77–89.
- Engels W. (1974) Occurrence and significance of vitellogenins in female castes of social Hymenoptera. *Amer. Zool.* **14**, 1229–1237.
- Feyereisen R. (1985) Regulation of juvenile hormone titer: synthesis. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 7: Endocrinology I* (Eds Kerkut G. A. and Gilbert L. I.), pp. 391–429. Pergamon Press, Oxford.
- Fletcher D. J. C. and Blum M. S. (1981a) Pheromonal control of dealation and oogenesis in virgin queen fire ants. *Science* **212**, 73–75.
- Fletcher D. J. C. and Blum M. S. (1981b) A bioassay technique for an inhibitory primer pheromone of the fire ant, *Solenopsis invicta* Buren. *J. Ga. ent. Soc.* **16**, 352–356.
- Fletcher D. J. C. and Blum M. S. (1983) The inhibitory pheromone of queen fire ants: effects of disinhibition on dealation and oviposition by virgin queens. *J. comp. Physiol. A* **153**, 467–475.
- Fletcher D. J. C., Cherix D. and Blum M. S. (1983) Some factors influencing dealation by virgin queen fire ants. *Insectes soc.* **30**, 443–454.
- Greenberg S. and Tobe S. S. (1985) Adaptation of a radiochemical assay for juvenile hormone biosynthesis to study caste differentiation in a primitive termite. *J. Insect Physiol.* **31**, 347–352.
- de Groot A. P. and Voogd S. (1954) On the ovary development in queenless worker bees (*Apis mellifica* L.). *Experientia* **10**, 384–385.
- Jouvenaz D. P., Allen G. E., Banks W. A. and Wojcik D. P. (1977) A survey for pathogens of fire ants, *Solenopsis* spp., in the south-eastern United States. *Fla. Ent.* **60**, 275–279.
- Kaatz H., Hildebrandt H. and Engels W. (1992) Primer effect of queen pheromone on juvenile hormone biosynthesis in adult worker honey bees. *J. comp. Physiol. B* **162**, 588–592.
- Kaissling K.-E. (1986) Chemo-electrical transduction in insect olfactory receptors. *A. Rev. Neurosci.* **9**, 121–145.
- Kaissling K.-E. and Renner M. (1968) Antennale Rezeptoren für Queen Substance und Sterzelduft bei der Honigbiene. *Zeit. verg. Physiol.* **59**, 357–361.
- Kearney G. P., Toom P. M. and Blomquist G. L. (1977) Induction of de-alation in virgin female *Solenopsis invicta* with juvenile hormones. *Ann. ent. Soc. Am.* **70**, 699–701.
- Keller L. and Ross K. G. (1993a) Phenotypic basis of reproductive success in a social insect: genetic and social determinants. *Science* **260**, 1107–1110.
- Keller L. and Ross K. G. (1993b) Phenotypic plasticity and “cultural transmission” of alternative social organizations in the fire ant *Solenopsis invicta*. *Behav. Ecol. Sociobiol.* **33**, 121–129.
- Koeppe J. K., Fuchs M., Chen T. T., Hunt L. M., Kovalick G. E. and Briers T. (1985) The role of juvenile hormone in reproduction. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 8: Endocrinology II* (Eds Kerkut G. A. and Gilbert L. I.), pp. 165–203. Pergamon Press, Oxford.
- Martinez T. and Wheeler D. (1991a) Effect of the queen, brood and worker caste on haemolymph vitellogenin titre in *Camponotus festinatus* workers. *J. Insect Physiol.* **37**, 347–352.
- Martinez T. and Wheeler D. (1991b) Identification of vitellogenin in the ant, *Camponotus festinatus*: changes in hemolymph proteins and fat body development in workers. *Arch. Insect. Biochem. Physiol.* **17**, 143–155.
- Mayer M. S. and McLaughlin J. R. (1991) *Handbook of Insect Pheromones and Sex Attractants*. CRC Press, Boca Raton, FL.
- Mayer M. S. and Mankin R. W. (1985) Neurobiology of pheromone perception. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 9: Behaviour* (Eds Kerkut G. A. and Gilbert L. I.), pp. 95–144. Pergamon Press, Oxford.
- Prestwich G. D. and Blomquist G. J. (1987) *Pheromone Biochemistry*. Academic Press, New York.
- Raina A. K. and Menn J. J. (1993) Pheromone biosynthesis activating neuropeptide: from discovery to current status. *Arch. Insect. Biochem. Physiol.* **22**, 141–151.
- Robinson G. E., Strambi C., Strambi A. and Feldlaufer M. F. (1991) Comparison of juvenile hormone and ecdysteroid haemolymph titres in adult worker and queen honey bees (*Apis mellifera*). *J. Insect Physiol.* **37**, 929–935.
- Robinson G. E., Strambi C., Strambi A. and Huang Z. (1992) Reproduction in worker honey bees is associated with low juvenile hormone titers and rates of biosynthesis. *Gen. comp. Endocrinol.* **87**, 471–480.
- Röseler P. (1977) Juvenile hormone control of oögenesis in bumble bee workers, *Bombus terrestris*. *J. Insect. Physiol.* **23**, 985–992.
- Röseler P. and Röseler I. (1978) Studies on the regulation of the juvenile hormone titre in bumblebee workers, *Bombus terrestris*. *J. Insect. Physiol.* **24**, 707–713.
- Röseler P., Röseler I. and van Honk C. G. J. (1981) Evidence for inhibition of corpora allata activity in workers of *Bombus terrestris* by a pheromone from the queen’s mandibular glands. *Experientia* **37**, 348–351.
- Schneider D. (1992) 100 years of pheromone research. *Naturwissenschaften* **79**, 241–250.
- Sorensen A. A., Fletcher D. J. C. and Vinson S. B. (1985) Distribution of inhibitory queen pheromone among virgin queens of an ant, *Solenopsis invicta*. *Psyche* **92**, 57–69.
- Stuart A. M. (1979) The determination and regulation of the neotenic reproductive caste in the lower termites (Isoptera): with special reference to the genus *Zootermopsis* (Hagen). *Sociobiology* **4**, 223–237.
- Tamaki Y. (1985) Sex pheromones. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 9: Behaviour* (Eds Kerkut G. A. and Gilbert L. I.), pp. 145–191. Pergamon Press, Oxford.
- Tschinkel W. R. (1988) Social control of egg-laying rate in queens of the fire ant, *Solenopsis invicta*. *Physiol. Ent.* **13**, 327–350.

- Tschinkel W. R. and Howard D. F. (1978) Queen replacement in orphaned colonies of the fire ant, *Solenopsis invicta*. *Behav. Ecol. Sociobiol.* **3**, 297–310.
- Vargo E. L. (1988) A bioassay for a primer pheromone of queen fire ants (*Solenopsis invicta*) which inhibits the production of sexuals. *Insectes soc.* **35**, 382–392.
- Vargo E. L. (1992) Mutual pheromonal inhibition among queens in polygyne colonies of the fire ant *Solenopsis invicta*. *Behav. Ecol. Sociobiol.* **31**, 205–210.
- Vargo E. L. and Fletcher D. J. C. (1986) Evidence of pheromonal queen control over the production of sexuals in the fire ant, *Solenopsis invicta*. *J. comp. Physiol. A* **159**, 741–749.
- Vargo E. L. and Fletcher D. J. C. (1987) Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta*. *Physiol. Ent.* **12**, 109–116.
- Willis L. G., Winston M. L. and Slessor K. N. (1990) Queen honey bee mandibular pheromone does not affect worker ovary development. *Can. Ent.* **122**, 1093–1099.
- Wilson E. O. (1971) *The Insect Societies*. Belknap Press of Harvard University Press, Cambridge, MA.
- Wilson E. O. and Bossert W. H. (1963) Chemical communication among animals. *Rec. Prog. Hormone Res.* **19**, 673–716.
- Winston M. L. and Slessor K. N. (1992) The essence of royalty: honey bee queen pheromone. *Am. Sci.* **80**, 374–385.
- Winston M. L., Slessor K. N., Willis L. G., Naumann K., Higo H. A., Wyborn M. H. and Kaminski L. A. (1989) The influence of queen mandibular pheromones on worker attraction to swarm clusters and inhibition of queen rearing in the honey bee (*Apis mellifera* L.). *Insectes soc.* **36**, 15–27.
- Winston M. L., Higo H. A. and Slessor K. N. (1990) Effect of various dosages of queen mandibular gland pheromone on the inhibition of queen rearing in the honey bee (Hymenoptera: Apidae). *Ann. ent. Soc. Am.* **83**, 234–238.

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