

## Mutual pheromonal inhibition among queens in polygyne colonies of the fire ant *Solenopsis invicta*

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**Summary.** Decrease in individual reproductive output with increasing numbers of reproductives is a general feature of social insect colonies. The previously described negative relationship between the fecundity of individual queens and number of resident queens in polygyne (multiple-queen) colonies of the fire ant *Solenopsis invicta* appears to result from mutual pheromonal inhibition. In an experimental test for the presence of fecundity reducing pheromones, corpses of functional (egg-laying) queens were found to effectively inhibit the fecundity of functional queens, suggesting that queen-produced pheromones suppress egg production in such queens. Evidence concerning a possible mechanism mediating this inhibition was also obtained. Treatment of queens with methoprene, a juvenile hormone (JH) analog, increased ovary development, suggesting that fecundity in functional queens may be mediated by the level of endogenous JH. These findings are consistent with the occurrence of mutual pheromonal inhibition among queens achieved by suppression of endogenous JH titers.

### Introduction

A general feature of hymenopteran societies is that as group size increases within a species, reproductive output per individual decreases, a relationship known as the "reproductivity effect" (Michener 1964). This relationship holds for both the size of a colony (essentially, the number of workers) and the number of queens in polygyne (multiple-queen) colonies. Among ants, in which polygyny is common (Buschinger 1968; Rissing and Pollock 1988), a negative effect of queen number on the fecundity (oviposition rate) of queens has been shown in several species (for review see Vargo and Fletcher 1989; Passera et al. 1991), but no mechanism for this effect has so far been demonstrated for any social insect species. There are two likely mechanisms by which increasing queen number in polygyne colonies could depress fecundity of the resident queens (Fletcher and

Blum 1983; Mercier et al. 1985). First, as the number of queens in a colony increases, colony resources could be divided more ways, resulting in each queen receiving less of the food required for egg production. Second, there could be mutual inhibition among queens, possibly involving pheromones. I report here the results of experiments that strongly suggest the presence of mutually inhibitory pheromones in polygyne colonies of the fire ant, *Solenopsis invicta*.

*S. invicta* occurs in both monogyne (single egg-laying queen per colony) and polygyne forms within its range in the United States (Vargo and Fletcher 1989). Queens from polygyne colonies are less physogastric and lay fewer eggs per individual than queens from monogyne colonies (Fletcher et al. 1980; Greenberg et al. 1985; Vargo and Fletcher 1989; see also Tschinkel and Howard 1978). Within polygyne colonies there is a strong negative relationship between the number of resident queens and the number of eggs laid per queen (Greenberg et al. 1985; Vargo and Fletcher 1989). The fecundity of queens in monogyne colonies is determined by the number of fourth instar larvae, which are believed to provide an unknown stimulatory substance to queens (Tschinkel 1988). If reduced fecundity in polygyne colonies of *S. invicta* were simply a matter of colony resources being split more ways as queen number increases, then the fecundity of queens in such colonies should be strongly related to the number of fourth-instar larvae. Although this has not been specifically tested, Vargo and Fletcher (1989) found no significant effect of total colony size (presumably related to the number of larvae) on queen fecundity, suggesting that the fecundity of queens in polygyne colonies is not strongly dependent on stimulatory factors provided by larvae. These results raise the possibility that some form of mutual inhibition among queens may be involved.

The possibility of mutual pheromonal inhibition among *S. invicta* queens gains support from the fact that queen pheromones influence reproductive development in both adults (Fletcher and Blum 1981, 1983a) and larvae (Vargo and Fletcher 1986a). Functional (egg-

laying) queens of this species produce a pheromone that inhibits ovary development in winged virgin queens (Fletcher and Blum 1981, 1983a; Willer and Fletcher 1986), presumably by suppressing the production of juvenile hormone (JH) (Fletcher and Blum 1983a), which is known to stimulate vitellogenin synthesis and ovary development in a variety of insects (Engelmann 1983), including *S. invicta* (Barker 1978). Thus it is possible that mutual inhibition of ovary development occurs among cohabiting queens in polygyne colonies of this ant and that this inhibition is mediated by pheromones that affect JH production. In this study I conducted experiments to determine whether pheromonal inhibition of fecundity occurs in *S. invicta* and whether JH might be involved in regulating fecundity in this species.

## Methods

**Test units.** The source of the queens, workers and brood was colonies of the polygyne form of *Solenopsis invicta* collected from the Brackenridge Field Laboratory of the University of Texas at Austin. Colonies, defined here as the inhabitants of a single mound, were excavated and maintained in the laboratory according to standard procedures (Vargo 1988). Changes in queen fecundity were studied by dividing colonies into small standardized units (Vargo 1988). A portion of each source colony was sieved (Vargo and Fletcher 1986b) to remove dealate (functional) queens and any alates or sexual brood. The queens were returned to the unsieved portion of their source colonies, and the workers and brood removed from each colony were mixed together to form a homogeneous pool. From this pool standardized units were drawn, each consisting of 5.8 ml workers (c. 3500) and 2.8 ml brood (c. 1000 worker pupae, 300 prepupae, 1000 fourth instar larvae, 2000 first-third instar larvae and 1000 eggs). Each unit was housed in a small nest as described by Vargo (1988). The test units were kept queenless for 48 h in order to maximize acceptance of queens by workers (Fletcher and Blum 1983b), after which period a single queen was introduced into each unit.

In a series of preliminary experiments (unpublished data), it was determined that queens increase in fecundity under such conditions. Queen weight was used to assess fecundity, because the weight of functional *S. invicta* queens is highly correlated with ovary development (Fletcher and Blum 1983a; Willer and Fletcher 1986; Tschinkel 1988) and oviposition rate (Ross 1988; Tschinkel 1988; Vargo and Fletcher 1989). Experiments were run for 5–6 days (see below). Change in weight (final weight–initial weight) was used to assess change in fecundity of individual queens. Following the final weighing, queens were dissected under a dissecting microscope to determine insemination status and to assess ovary development directly. Insemination status was determined by noting whether the spermatheca was white or clear, the former condition indicating the presence of sperm. The number of vitellogenic follicles in ten representative ovarioles was used to assess ovary development in order to verify that final weight reflected the degree of ovary development.

**Ability of queen corpses to inhibit fecundity.** The possible role of queen pheromones in inhibiting fecundity was tested by introducing into the experimental test units the corpses of three dealate queens each day. These queens, killed by freezing and held in a freezer ( $-20^{\circ}\text{C}$ ) until use, originated from various polygyne colonies foreign to those used as the source of the test units. The control treatment was given daily the corpses of three freshly killed alate queens whose wings had been mechanically removed (Vargo and Fletcher 1986a; Vargo 1988). Such dewinged virgin queens are ideal controls for tests of queen pheromones (Vargo and Fletcher 1986a; Vargo 1988; Vargo and Passera 1991), because

they have the same form and presumably tactile cues as do the corpses of functional queens, therefore differing from functional queens primarily in the activity of the reproductive system and associated endocrine and exocrine glands.

Four colonies collected on 26 January 1990 were used to set up 30 test units, which were divided equally ( $n=15$ ) between the experimentals and controls. The experiment was set up on 7 May 1990. The single queen tested in each unit was weighed before set-up (mean  $\pm$  SD =  $11.5 \pm 1.2$  mg, range = 10.0–14.7 mg). The experiment was allowed to run for 6 days, because it was determined in a preliminary experiment that there was no significant change in fecundity after this time (see also Tschinkel 1988). Queens were weighed on days 1, 2, 3, 4 and 6. Upon dissection, nine queens in each treatment were found to be inseminated and six were found to be uninseminated. Although there was no significant difference in the weights of the inseminated and uninseminated queens tested ( $t_{28}=0.4$ ,  $P>0.6$ ), uninseminated queens in polygyne colonies of this species tend to be less fecund than nestmate inseminated queens (Vargo and Fletcher 1989), suggesting that the response of queens to factors affecting fecundity varies with insemination status. Therefore, the data were analyzed by a two-way analysis of variance (ANOVA) with treatment and insemination status as independent variables and change in weight as the dependent variable.

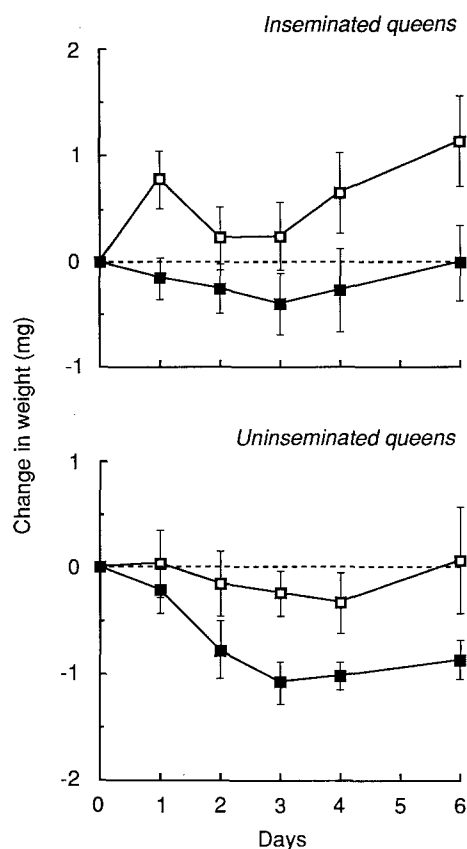
**Effect of methoprene on queen fecundity.** In order to obtain information on a possible mechanism mediating mutual pheromonal inhibition among queens, the effect of methoprene on fecundity was tested in the following manner. Queens were set up individually in standardized units. Source colonies were collected on 11 ( $n=2$ ) and 20 ( $n=2$ ) September 1990, and the experiment was begun on 6 November 1990. Queens received one of three treatments: topical application of 1  $\mu\text{g}$  methoprene in 1  $\mu\text{l}$  acetone daily, topical application of 1  $\mu\text{l}$  acetone daily, or untreated controls. There were 12 replicates of each treatment. The mean initial weight of queens was 9.7 mg (SD = 0.2 mg, range = 9.5–10.0 mg). Queens were weighed daily and the experiment was terminated after five days, because queen weights had leveled off by this time. Following the final weighing, queens were dissected to assess insemination status and ovary development. Only 3 of the 36 queens were found to be uninseminated; all three belonged to the untreated control treatment and were eliminated from the analysis. The data were then analyzed by a one-way ANOVA.

## Results

### *Ability of queen corpses to inhibit fecundity*

The addition of dealate queen corpses significantly depressed weights compared to controls (Fig. 1), the difference between treatments being significant from day 3 on (for days 3, 4 and 6,  $F_{1,26} \geq 5.8$ ,  $P < 0.04$ ). However, the response varied with insemination status; weights of uninseminated queens were depressed below that of inseminated queens in both treatments. The difference between inseminated and uninseminated queens was significant on the last 2 days (for days 4 and 6,  $F_{1,26} \geq 5.8$ ,  $P > 0.025$ ). The interaction between insemination status and treatment was not significant on any day (all  $F_{1,26} \leq 0.1$ ,  $P > 0.7$ ).

Among inseminated queens, the controls had gained an average of 1.1 mg, about 10% of their original weight, by the end of the experiment, whereas queens given dealate queen corpses showed little change from their starting weights. Among uninseminated queens, the controls had regained their initial weights by the 6th day,



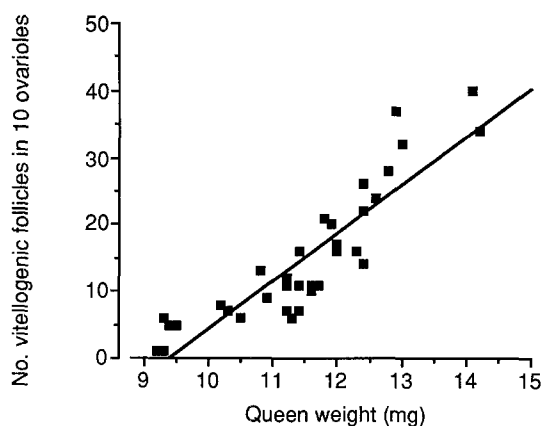
**Fig. 1.** Inhibition of fecundity of queens by the corpses of functional queens. Experimentals (■) received the corpses of 3 dealate queens from polygyne colonies each day. Controls (□) received the corpses of nonfunctional winged virgin queens with wings removed. Shown are the mean ( $\pm$ SE) changes in weight (weight on a particular day – initial weight) after the queens were removed from their natural polygyne colonies and set up individually in standardized units of workers and brood. *Inseminated queens* ( $n=9$  of each) and *uninseminated queens* ( $n=6$  of each) are shown separately

whereas the presence of dealate queen corpses resulted in an average loss of 0.9 mg (7.5%).

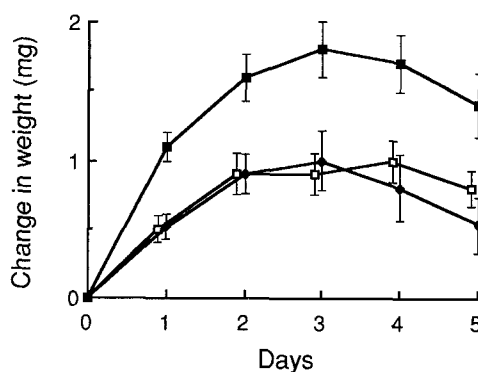
Queen weight was a good indicator of queen fecundity (Fig. 2), confirming that the observed changes in weight corresponded to changes in fecundity.

#### *Effect of methoprene on queen fecundity*

Queens in all treatments gained weight (Fig. 3). Consistent with juvenile-hormone regulated fecundity, there was a significant difference among the treatments from the 1st day on (for days 1–5,  $F_{2,31}=4.48$ ,  $P<0.02$ ), with the methoprene treated queens having gained significantly more weight than either of the other two treatments ( $P<0.05$ , Newman-Keuls test). By day 5 these queens showed a 14% increase over their initial weights, compared to 8.5% for the acetone treatment and 6% for the untreated queens. The difference between the acetone-treated queens and the untreated controls was not significant on any day. As in the previous experiment, queen weight was a good indicator of queen fecun-



**Fig. 2.** Relationship between queen weight and fecundity among queens tested in the queen corpse experiment. The line is described by the equation:  $y=7.17x-67$  ( $r^2=0.79$ ,  $F_{1,28}=104.03$ ,  $P<0.0001$ )



**Fig. 3.** Effect of methoprene on queen fecundity. ■, Queens treated daily with 1  $\mu$ g methoprene in 1  $\mu$ l acetone ( $n=12$ ). □, Queens treated daily with 1  $\mu$ l acetone ( $n=12$ ). ♦, Untreated controls ( $n=9$ ). Shown are the mean ( $\pm$ SE) changes in weight (weight on a particular day – initial weight) after queens were removed from polygyne colonies and set up individually in standardized units of workers and brood

dity, accounting for 61% of the variation in the number of vitellogenic follicles in 10 representative ovarioles for queens of all groups combined ( $F_{1,31}=47.56$ ,  $P<0.0001$ ).

#### **Discussion**

Functional queens of *S. invicta* evidently produce a pheromone that effectively suppresses fecundity in other functional queens. This phenomenon results in mutual pheromonal inhibition among cohabiting egg-layers in polygyne colonies. Thus the negative relationship between colony queen number and individual fecundity previously found in polygyne colonies of this species (Vargo and Fletcher 1989) most likely arises because the presence of more queens results in higher levels of inhibitory pheromone due to the queens' combined pheromone production. To my knowledge this is the first report of a queen pheromone affecting the fecundity of functional reproductives as well as the first demonstration of a mechanism mediating the decline in per capita

egg production with increasing queen number in polygyne colonies of a social insect.

These findings raise the possibility that mutual pheromonal inhibition occurs in other ants, because queen reproductive output is negatively correlated in polygyne colonies of a number of species (Vargo and Fletcher 1989; Passera et al. 1991). That pheromones may not be the responsible mechanism in all species is suggested by Bourke's (1992) recent experimental study of queen fecundity in relation to queen number in *Leptothorax acervorum*, in which no evidence of mutual pheromonal inhibition was found. However, as admitted by Bourke (1992), this does not necessarily mean that pheromonal inhibition of fecundity does not exist in *L. acervorum*, only that his experiment did not detect it.

The present findings together with previous work on the exocrine and endocrine controls of initiation of ovary development in *S. invicta* virgin queens provide a working hypothesis for the mode of action of the pheromone inhibiting fecundity of functional queens. Fletcher and Blum (1983a) proposed that pheromonal inhibition of ovary development in virgin queens is achieved by suppression of endogenous JH titers. This hypothesis is made plausible by the finding (Barker 1978) that JH is necessary for initiation of ovary development in virgin queens of this species. The fact that methoprene increases fecundity in functional queens, as shown here, suggests that the degree of ovary development is also under the control of JH. Thus one hypothesis for the mode of action of the fecundity-reducing pheromone is that it acts by lowering endogenous JH titers, possibly by affecting the activity of the corpora allata. If *S. invicta* queen pheromones do in fact control both the onset of ovary development in virgin queens and the degree of ovary development in functional queens by regulating JH titers, it is possible that the same pheromone is responsible for both effects.

If the pheromone works directly on queen physiology, it would suggest that reproductive competition among egg-layers in *S. invicta* colonies is the driving force behind mutual inhibition of cohabiting queens. This possibility is supported by the fact that nestmate queens are generally not closely related in this species (Ross and Fletcher 1985; Ross 1992), and that nestmate queens vary considerably both in relative fecundity (Vargo and Fletcher 1989) and in their production of sexual offspring (Ross 1988, 1992). By inhibiting reproductive activity in rival nestmate queens, a queen could be expected to increase her personal fitness, provided this allows her to secure a greater share of the colony's resources for rearing her own progeny. However, this explanation is not so straightforward, because it is unclear how differential egg production in these colonies translates into differential reproductive success over the long term. Nestmate queens vary substantially in the proportion of viable eggs laid (Vargo and Ross 1989) and in the ability of their viable eggs to be reared into sexuals (Ross 1988, 1992). Moreover, individual variability in both egg viability and production of sexuals appear only weakly related to rates of egg production (Ross 1988, 1992; Vargo and Ross 1989). The relationship between differential

fecundity, differential egg viability, and variation in sexual production, and the effects of these on long-term reproductive success deserve further study. Nevertheless, competition for egg production could be one of several levels at which cohabiting *S. invicta* queens compete for reproductive privileges in polygyne colonies.

One difficulty for the reproductive competition hypothesis is that in order for pheromonal inhibition to prove advantageous to a queen, she would have to inhibit other queens from laying eggs without herself being affected by the pheromone she produces, or at least not affected to the same degree as the other queens. It is not obvious how a queen could avoid being influenced by her own pheromone. One possibility is that the pheromone produced by each queen bears a unique signature identifying the producer, and workers preferentially direct the pheromone away from the producer to other queens. Another possibility that could operate in the absence of individually distinguishable pheromones is that the quantity of pheromone secreted by a queen is positively correlated with fecundity, whereas her sensitivity to the effects of the pheromone is negatively correlated with fecundity. If this were true, queens of higher fecundity would contribute more to the colony "pheromone pool" but would be less affected by the total amount of circulating pheromone. Additional studies are needed to rule out one or both of these possibilities. On the other hand, demonstration of one of them would provide strong support for pheromonally mediated competition for egg production among nestmate queens in *S. invicta*.

Keller and Nonacs (1992) argue that direct pheromonal control of oviposition by rival queens would not be evolutionarily stable, because selection would be strong on target individuals to "escape" control. An alternative mode of action is that the pheromone acts indirectly by causing workers to feed queens less food and/or substances required for sustained egg production. Such an indirect effect may evolve by selection acting on workers to maximize colony reproductive efficiency. In this scenario, workers would use the information derived from queen pheromone production to match the queens' reproductive output to the number of brood the colony can rear (Keller and Nonacs 1992). In light of the present results with methoprene, it is possible that differential feeding rates by workers influences the queens' oviposition rates by affecting JH titers, because nutrition affects JH production in adult females of a number of insects (reviewed in Feyereisen 1985). Distinguishing between the reproductive competition and worker control hypotheses will require detailed understanding about the precise mode of action of the inhibitory pheromone, including possible effects of the pheromone and nutrition on JH titers.

The increase in fecundity in response to topical application of methoprene found in the present study is at odds with two previous studies (Troisi and Riddiford 1974; Vinson and Robeau 1974) in which monogyne fire ant colonies were treated with JH analogs. In the previous studies a variety of JH analogs, including methoprene, administered to whole fire ant colonies through

feeding, contact, or fumigation resulted in reduced egg production by queens. However, these treatments also affected worker behavior and larval development (Troisi and Riddiford 1974; Vinson and Robeau 1974). Because queens in monogyne colonies are dependent on substances provided by fourth instar larvae for stimulating and maintaining ovary development, and because these substances are passed from the larvae to the queens by workers (Tschinkel 1988), treatment of the entire colony with JH analogs could have a strong indirect impact on queen fecundity over and above any direct physiological effects on the queen. Direct treatment of the queen, such as the topical application performed in the present study, minimizes the effects of JH analogs on other colony members and should therefore give a more accurate assessment of their direct effects on queen fecundity. Alternatively, the discrepancy between the present results and those of the previous studies could be due to differences in the dosages of JH analog received by the queens.

An interesting result of this study was that inseminated and uninseminated queens responded differently when set up individually; unlike inseminated queens, uninseminated control queens failed to increase in fecundity. Apparently uninseminated queens are less capable of responding to whatever factors stimulate fecundity, presumably nutritional substances provided by fourth instar larvae (Tschinkel 1988), or are provided less of these factors by workers. This result is consistent with the finding (Vargo and Fletcher 1989) that uninseminated queens in field colonies tend to be less fecund than their nestmate inseminated queens, weighing some 10% less on average. Despite this difference, the presence of queen corpses still inhibited the uninseminated queens, and the magnitude of inhibition was similar in both types of queens. Thus, although uninseminated queens were less stimulated to reproduce, they were as much affected by the inhibitory pheromone.

The apparent occurrence of a queen-produced pheromone that affects queen fecundity brings to four the number of effects that queen pheromones have on reproduction in *S. invicta* (reviewed in Vargo 1990). In addition to the inhibition of dealation and ovary development in virgin queens, queen pheromones inhibit the production of both male and female sexuals (Vargo and Fletcher 1986a; Vargo 1988), probably by affecting the behavior of workers toward developing larvae. Also, queen pheromones are instrumental in enforcing the occurrence of a single functional queen in monogyne colonies (Fletcher and Blum 1983b). Thus, there is extensive pheromonal queen influence in this species, affecting processes as diverse as larval development, the onset of reproductive activity in female sexuals, the number of egg-laying queens in a colony, and the extent of reproduction by cohabiting functional queens. Determination of the number of pheromones responsible for these different effects awaits chemical identification of the active compounds.

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