

Pheromonal and behavioral queen control over the production of gynes in the Argentine ant *Iridomyrmex humilis* (Mayr)

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Summary. Both field observations and laboratory experiments have suggested that queens of *I. humilis* inhibit the production of new queens (gynes). Using small colony fragments, laboratory experiments were conducted to determine the means by which this inhibition is achieved. The addition of queen corpses to queenless fragments effectively inhibited the production of gynes, suggesting that a queen inhibitory primer pheromone is involved. This inhibitory influence was removed when corpses were washed in pentane, lending further support to the pheromonal hypothesis. Adult gynes (winged virgin queens) were not inhibitory, whereas young dealated mated queens of the same age were, suggesting that only inseminated queens produce the pheromone. Daily addition of eggs to queenless units did not appear to have a strong inhibitory influence, indicating that the lower worker/larva ratios associated with the presence of an egg-laying queen in such colony fragments does not greatly influence the production of sexuals. Pheromonal inhibition of gyne development appears to be achieved mainly by preventing the sexualization of bipotent female larvae, probably by affecting the brood-rearing behavior of workers. In addition, queens may also cause the execution of female larvae after they have become sexualized. In nearly all cases, the addition of a living queen to previously queenless units containing gyne larvae caused workers to execute one or more of these larvae within 24 h. In some cases queens were also seen attacking gyne larvae. The addition of queen corpses resulted in the execution of gyne larvae, suggesting that a queen pheromone mediates, at least in part, this execution behavior of workers. These results show that *I. humilis* queens exert control over the production of gynes in two ways: (1) by preventing the sexualization of female larvae and (2) by killing female larvae after they have become sexualized. A queen primer phero-

mone appears to be involved in both processes. Queen behavior also plays a role, at least in the execution of gyne larvae. This queen control over the production of gynes, probably mostly pheromonal, appears to operate strongly in the field where gynes are produced only in spring just after a sharp drop in the inhibitory queen influence due to the massive execution of queens by the workers.

Introduction

Queen control, the ability of queens to influence reproductive development in nestmates, plays a central role in regulating colony functioning in insect societies. Information on the extent and nature of this control is therefore expected to provide important insights into insect social organization and its evolution (see, e.g., West Eberhard 1977, 1981; Fletcher and Ross 1985; Wheeler 1986). In ants, queens are known to influence a variety of reproductive processes (reviewed in Brian 1980; Fletcher and Ross 1985; Hölldobler and Wilson 1990), but, in most cases, the mechanisms underlying this influence are not known. The extensive nature of this control together with the general lack of physical aggression on the part of the queen suggest that queen control in ants is largely mediated by primer pheromones (Wilson 1971; Hölldobler and Wilson 1990); however, no such ant primer pheromone has been chemically identified and there have been few decisive demonstrations of pheromonally mediated queen control in this group, owing to the difficulty of assaying for this class of semiochemicals.

The most conclusive evidence to date of pheromonal queen control in the Formicidae comes from work on the myrmicine, *Solenopsis invicta*. Using a bioassay in which queen corpses could be tested, Fletcher and Blum (1981 a, b) demonstrated that a queen primer pheromone inhibits dealation and oogenesis in virgin queens. A similar technique was employed by Vargo and Fletcher

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(1986) and Vargo (1988) to show that the production of both males and gynes (female sexuals) in *S. invicta* is also under pheromonal control of the queen.

More circumstantial evidence favoring pheromonal queen influence has been obtained in other myrmicine ants, e.g., over the sexualization of female larvae in *Myrmica rubra* (Carr 1962; Brian and Hibble 1963; Brian and Blum 1969) and over worker oviposition in *Leptothorax recondens* (Dejean and Passera 1974). Among the Formicinae, evidence suggesting pheromonal mediation of queen control comes from studies of worker oviposition and production of gynes in *Plagiolepis pygmaea* (Passera 1980) and worker oviposition in *Oecophylla longinoda* and *O. smaragdina* (Hölldobler and Wilson 1983).

As in the other subfamilies of ants outside the Myrmicinae and Formicinae, study of queen control in the Dolichoderinae has received little attention. Nevertheless, there is some evidence that queens exert control over nestmates in members of this subfamily. For example, in *Dolichoderus quadripunctatus* the queen appears to inhibit workers from laying reproductive eggs (Torosian 1968), but the mechanism of this control is not known. Also, cohabiting queens in young colonies of *Iridomyrmex purpureus* form temporary behavioral hierarchies in which the dominant queen inhibits egg-laying in subordinates (Hölldobler and Carlin 1985).

In the Argentine ant, *I. humilis*, observations from both the field and laboratory suggest that queens exert strong control over the production of gynes, possibly by means of pheromones. In the field, gynes of this highly polygyne species (i.e., many functional queens per nest) are produced annually in spring just after the workers eliminate by execution some 90% of the resident queens (Markin 1970; Benois 1973; Bartels 1983; Keller et al. 1989a). The timing of these events suggests that the production of gynes in nature coincides with a sharp decrease in inhibitory queen influence associated with the sudden reduction in queen number.

Additional support for queen control over gyne production in *I. humilis* comes from laboratory studies (Bartels 1988; Passera et al. 1988a), where it has been found that gynes are often reared in queenless colony fragments but never in fragments containing one or more queens.

In this study we used queen corpses in small colony fragments to investigate the possibility of pheromonal control over gyne production in *I. humilis*.

Methods

All colonies of *I. humilis* used in these studies originated from Port-Leucate on the western Mediterranean coast of France. The ants were collected by digging and brought to the laboratory where they were slowly drawn out of the soil with the aid of a 100-W incandescent lamp (Passera et al. 1988a; Keller et al. 1989a). Colonies were housed in artificial nests as described by Passera et al. (1988a). Both these and the experimental units were maintained in the laboratory (28 ± 2° C, 16 h/8 h light/dark regime) and fed ad libitum on sugar water and an artificial diet (Keller et al. 1989b).

Experimental units consisted of 2 cm³ of workers (ca. 1200), 200–300 larvae of all stages, and 30 or so eggs. These were housed in a smaller version of the artificial nests used for the colonies (Passera et al. 1988a). The units were examined weekly with the aid of a dissecting scope for the presence of female sexual (gyne) larvae (1.9–3.6 mm long) and pupae, which were counted without removal. Gyne larvae are easily distinguished from worker larvae (maximum length 1.9 mm) by their size and from the large shiny male larvae by their matte appearance (Passera et al. 1988b). CO₂ gas was used to slow the ants while setting up the experimental units and during the weekly examinations.

Overwintering has a strong positive effect on the production of gynes in *I. humilis* (unpublished data), which under natural conditions takes place only in spring (Markin 1970; Benois 1973; Bartels 1983; Keller et al. 1989a). In order to maximize the number of gynes reared in the experimental units, only ants collected in late winter and spring of 1988 and 1989 were used, and the units were set up within 2 weeks of the date of collection. To eliminate intercolonial variability, all units involved in a particular experiment were drawn from the same source colony.

Except where otherwise mentioned, queens serving as corpses were taken haphazardly from several source colonies maintained in the laboratory. These were killed by freezing (ca. 15 min at –20° C), allowed to thaw for several minutes, and were then introduced to the nests through a small hole in the plastic nest cover.

Experiments and results

Effect of queen corpses on the production of gynes

Corpses of queens were used to test the hypothesis that a queen pheromone is involved in the inhibition of gyne production. The use of dead queens is a powerful way of testing for the presence of pheromones since it eliminates any behavioral influence associated with a living queen. In this experiment, the number of queen larvae and pupae produced in units receiving daily a fresh queen corpse was compared to those containing a living nestmate queen and to queenless controls.

There was a dramatic difference among treatments in the production of gynes (Fig. 1). While gyne larvae and pupae steadily increased in numbers in the queenless units, resulting in some 15/unit by week 6, the addition of queen corpses caused complete inhibition, as did the presence of a living queen. The queenless treatment differed significantly from the other two treatments after only 2 weeks. That queen corpses are strongly inhibitory is good evidence for the presence of a queen inhibitory pheromone. These results do not, of course, rule out the possibility that behavioral cues or other factors associated with the queen also play a role in inhibiting gyne production.

One such factor associated with a living queen that may also inhibit the production of gynes is a relatively low worker/larva ratio compared to equivalent queenless units due to the continual production of eggs and their subsequent hatching into larvae (see Vargo and Fletcher 1986). The following experiment was designed to compare the relative strength of such a possible influence with that of the inhibitory pheromone. To queenless units were added 20 eggs/day, about the daily rate of oviposition of such queens (Keller 1988; unpublished data). Thus, these units were deprived of queen pheromone,

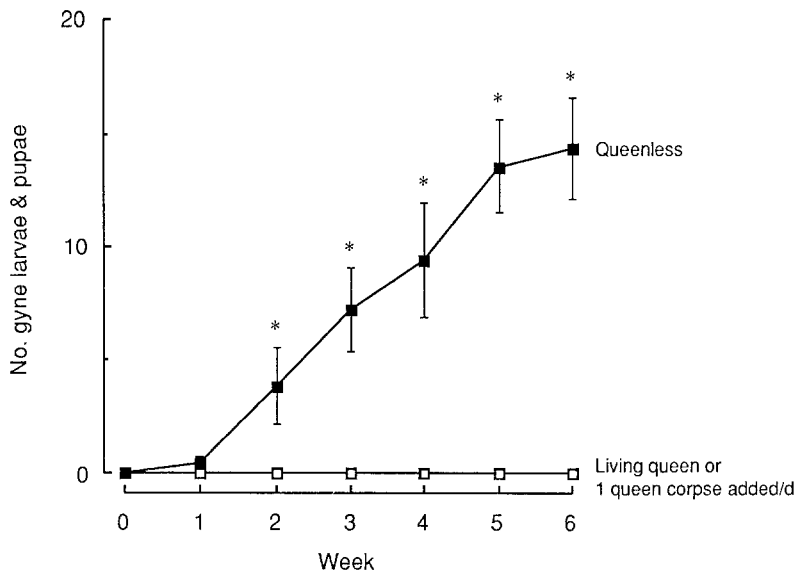


Fig. 1. Inhibitory effect of queen corpses on the production of gynes in small experimental units in comparison with living queens and with queenless controls. Shown are means \pm SE. Asterisks indicate that the queenless controls differed significantly from 0 at a particular week (all $t_4 \geq 4.23$, $P < 0.01$; one-tailed one-sample t -test, square-root transformation); $n = 5$ in each case

but the worker/larva ratio was kept relatively low by the addition of eggs. The production of gynes in these units was compared with that in queenless units receiving daily the fresh corpse of a queen killed by freezing. This latter group was provided with the physical presence of a queen, but the worker/larva ratio was free to rise as in the queenless units of the previous experiment.

As a rough measure of the relative volatility of the putative pheromone, a third treatment was included to determine whether direct contact with the queen was important in transmitting the inhibitory influence. A living queen and 20 workers were placed in a small plastic tube (1 cm diameter \times 4 cm long) sealed at one end. To provide adequate moisture, water was added to the tube and a cotton plug was inserted about two-thirds of the way down. The open end of the tube was fitted with a cap made of fine wire mesh (width 0.23 mm). The small size of the mesh severely limited physical contact of the encaged queen and her worker entourage by the workers in the experimental unit, but it did not prevent the possible transmission of volatile substances from the queen to the workers outside the cage. During each weekly examination, the cages were replaced by new ones containing a fresh queen and workers.

The results are shown in Fig. 2. There was a large difference among treatments in the number of gynes produced, being significant from the second week on (all $F_{2,14} \geq 13.14$, $P < 0.001$, square-root transformation). The presence of queen corpses was again strongly inhibitory. The units containing a caged queen differed significantly from those receiving eggs only at week 4. This may have been because some of the eggs added had developed into gynes by this time and/or there was some inhibitory effect of the caged queens.

These results are consistent with the presence of a queen inhibitory pheromone that is transmitted primarily by contact among colony members. Although not compared directly to queenless units not receiving eggs, the relatively high production of gynes in the units to which eggs were added suggests that the inhibitory effect

associated with a living queen in such units is not related to the worker/larva ratio, or if such an effect exists, it is of minor importance compared with the strong pheromonal influence. These results also suggest that there is no significant inhibitory effect directly associated with the eggs, as has been reported for *Monomorium pharaonis* (Edwards 1987).

Effect of queen corpses rinsed in pentane

Even stronger evidence for the presence of a queen inhibitory pheromone could be obtained if rinsing corpses in an organic solvent were to remove the inhibitory effect. This is because washing in a solvent should remove only chemical cues, while tactile cues should not be affected. In addition, such efforts are a necessary step in the preparation of biologically active extracts and the eventual isolation and identification of the active substances.

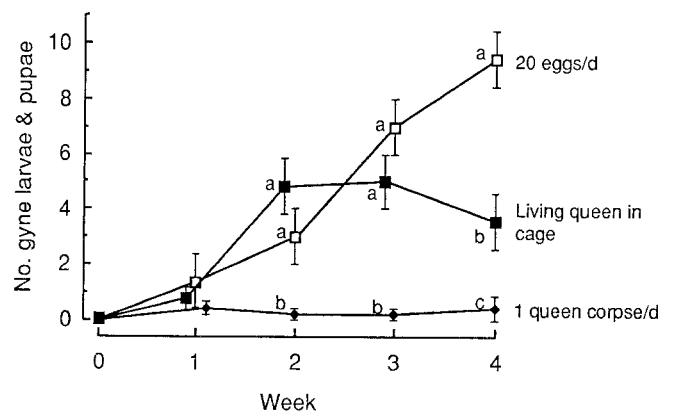


Fig. 2. Inhibitory effect of queen corpses on the production of gynes in in comparison with queenless units receiving eggs and those containing a living queen enclosed in a wire cage. Shown are means \pm SE. Treatments with different lower case letters differed significantly at a particular week ($P < 0.05$, Newman-Keuls test, square-root transformation); $n = 5$ in each case

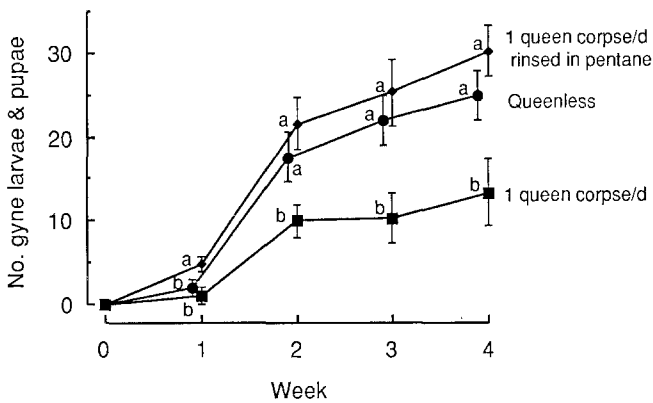


Fig. 3. Effect of queen corpses rinsed in pentane on the production of gynes in comparison with unwashed corpses and with queenless controls. Shown are means \pm SE. Treatments with different lower case letters at a particular week differed significantly ($P < 0.05$, Newman-Keuls test, square-root transformation); $n = 10$ in each case

To see if the effect of the putative pheromone could be removed by rinsing queen corpses in an organic solvent, the following experiment was performed. Experimental units were set up and allocated to one of three treatments. One treatment received daily the fresh corpse of a queen rinsed in two 10-min baths of pentane (1 ml solvent/queen). One treatment received daily the fresh unwashed corpse of a queen. The remaining treatment did not receive any queens and served as the queenless control.

As seen in Fig. 3, there were significant differences among treatments from the first week on (all $F_{2,29} \geq 4.56$, $P < 0.02$, square-root transformation). The units receiving queen corpses rinsed in pentane contained significantly more gyne larvae and pupae than those receiving unwashed corpses by the first week. The units receiving the untreated corpses contained significantly more gyne larvae and pupae than the queenless controls at week 1, but these two treatments did not differ significantly for the remainder of the experiment. Although unrinsed queen corpses were inhibitory compared to both of the other treatments, unlike the previous experiments in which queen corpses were tested (see Figs. 1 and 2), there were considerable numbers of gyne larvae and pupae produced in their presence. This corresponds

to an overall greater production of gynes by the units in this experiment, probably reflecting variation among the source colonies used in the different experiments in their tendency to produce gynes.

The results of this experiment are consistent with the presence of an inhibitory queen pheromone that is extractable in an organic solvent. However, we cannot rule out the possibility that the pentane did not remove the inhibitory pheromone, but instead removed one or more chemicals instrumental in attracting workers to the corpse and thereby reduced contact between workers and any pheromone on the surface of the queen's body (see, e.g., Vander Meer 1983; Vargo 1990).

Inhibitory effect of virgin queens

The following experiment was performed to determine whether young adult gynes (virgin queens) also produce the inhibitory pheromone. This experiment was designed to compare the inhibitory capability of young virgin queens with young mated queens. The gynes used in this experiment were reared in the laboratory from a single queenless colony. These were removed as pupae and placed into small rearing units containing workers either with or without males. This procedure yielded both inseminated and uninseminated young queens of similar age (not more than 2–3 days apart). Within 5 days of eclosion, a single queen of each category was placed in an experimental unit. Units containing an old inseminated queen and queenless units were also included in the experiment. Dissection of all queens at the end of the experiment confirmed their mating status.

The results (Fig. 4) show that young winged virgin queens were not inhibitory, whereas young mated delated queens were, suggesting that unmated queens do not produce the inhibitory pheromone.

Effect of temporary queen presence

To determine whether the temporary presence of a living queen would completely inhibit the production of gynes, the following experiment was performed. Five experimental units were given a living nestmate queen for 24 h every seventh day. The number of gyne larvae and pupae

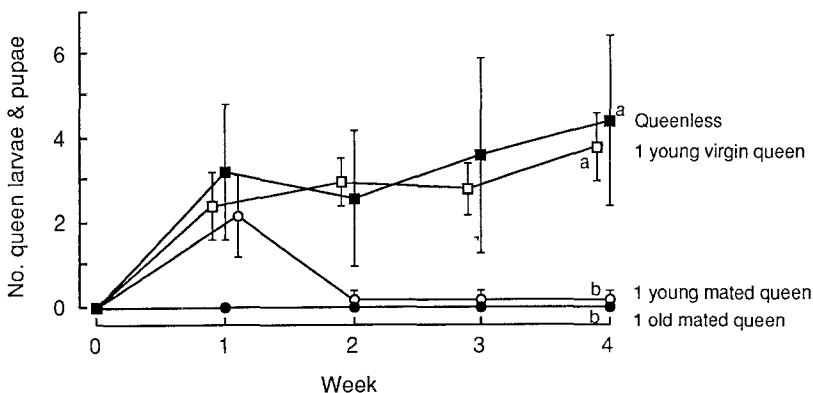


Fig. 4. Effect of insemination on the ability of young queens to inhibit the production of gynes. A queenless control treatment is included for comparison. Shown are means \pm SE. Treatments with different lower case letters at week 4 differed significantly ($P < 0.05$, Newman-Keuls test, square-root transformation); $n = 5$ in each case

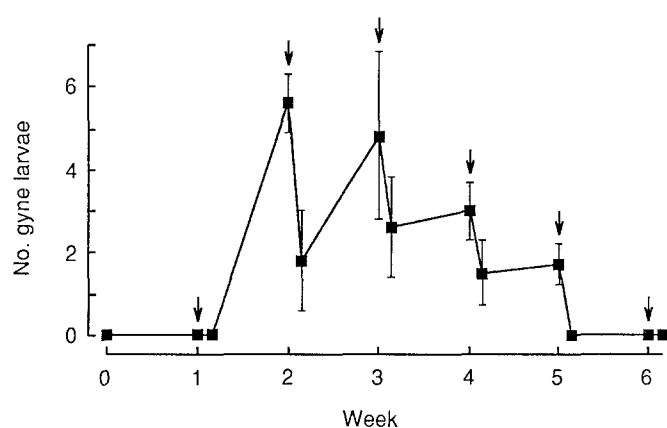


Fig. 5. Effect of queen presence 1d/week on the survival of gyne larvae. The number of larvae present was counted before and after each 24 h stay by a queen. Arrows indicate the beginning of the queenright period. Shown are means \pm SE. For all weeks in which gyne larvae were present (weeks 2–5), there was a significant reduction in their numbers 24 h after introduction of a queen (weeks 2–3, $t_4 \geq 3.14$; week 4 $t_3 = 4.24$; week 5, $t_2 = 5.10$; all $P < 0.02$, paired t -test, square-root transformation)

present in each unit was counted just before the introduction of the queen and then again upon her removal 24 h later.

For all weeks in which gyne larvae were present, there was a reduction in their numbers the day following the introduction of the queen (Fig. 5). Treating each unit and each week independently, there were 17 cases in which gyne larvae were present at the time that the queen was introduced, and at least some of these larvae had disappeared by the next day in 15 (88%) cases. In the most extreme case, all seven of the gyne larvae present upon introduction of the queen were gone 24 h later.

The presence of a living queen for 1 day/week was not enough to inhibit completely the production of gynes, as there were gyne pupae ($x \pm SD = 2.4 \pm 2.5$) present at week 6. Gyne pupae were present from week 4, and their numbers were never reduced the day following queen placement, suggesting that they are not susceptible to this form of queen control.

In many cases in which there were reductions in the number of gyne larvae, workers could be seen biting severely damaged larvae (unrecognizable to sex or caste), suggesting that workers executed some gyne larvae in response to the queen's presence. This possibility was later confirmed by direct observation.

Execution of gyne larvae

These experiments were designed to shed light on the mechanism by which the presence of a queen results in the death of gyne larvae. This was accomplished using queenless units containing gyne larvae originating from various source colonies. The units were always paired by source colony and date of set-up (i.e., length of time queenless). To one unit of a pair was introduced a living queen taken at random from one of several source colonies. The other unit remained queenless. The number

Table 1. Effect of a living queen on the execution of gyne larvae. Paired experimental groups of workers and brood (test units) were allowed to produce some gyne larvae. To one unit of each pair was added a living queen (Q+) and the other remained queenless (Q-). The number of gyne larvae present was counted before and 24 h after addition of the queen. The two treatments differed significantly ($\chi^2_1 = 9.31$, $P < 0.005$; McNemar's test)

Treatment	No. units with gyne larvae	No. units in which gyne larvae were executed within 24 h
Q+	14	13 (93%)
Q-	14	1 (7%)

of gyne larvae and pupae was counted in both units before and 24 h after the introduction of the queen.

The results are shown in Table 1. The presence of a living queen resulted in the elimination within 24 h of at least female sexual larvae in 13 (93%) of 14 cases, whereas only one of these larvae disappeared from one (7%) of the queenless units.

In seven cases, casual observations were made of both queen and worker behavior. To facilitate observations, the nests of the experimental units were equipped with a clear glass lid rendering visible the entire brood chamber. A red plastic box was placed over the unit, and, with a dissecting scope, observation bouts of 1–5 min were made periodically during the first 5 h following queen introduction. Observations were carried out in the rearing chamber at 28° C.

In all units receiving queens, workers were seen aggressively biting some female larvae during the first observation period, which occurred in all cases within 3 h of queen introduction. In the most dramatic case, five of the six gyne larvae present were under attack by groups of 4–9 workers just 3 h after introduction of the queen. In the three units observed 1 h after queen introduction, workers were seen already attacking some gyne larvae. In all, 23 gyne larvae were observed to be aggressively bitten.

Typically, 1–2 workers continually bit a larva. Their mandibles did not seem to pierce the cuticle, but the biting forced body fluids out the larva's mouth and anus. These expelled liquids were highly attractive to other workers, which eagerly collected them. This process continued for several hours until all body fluids were removed and only a shrivelled cuticle remained. One male larva and a smaller larva (unrecognizable to sex or caste) were also seen attacked by workers after the introduction of the queen. No such aggressive behavior toward gyne or other larvae was observed in the queenless units.

In four of the seven cases observed, an extraordinary queen behavior was seen. Queens were observed to bite gyne larvae, participating directly in their execution; in one case, a queen bit a larva repeatedly for more than 5 min. As with the workers, the queens did not appear to pierce the larval cuticle. Due to their larger and more powerful mandibles, their bites were much stronger than those of the workers and resulted in the expulsion of greater quantities of larval body fluids. After a biting bout, in which a queen bit an individual larva from five

Table 2. Effect of queen corpse on the execution of gyne larvae. Paired experimental groups of workers and brood (test units) were allowed to produce some gyne larvae. To one unit of each pair was added the freshly killed corpse of a queen (Q+) and the other remained queenless (Q-). The number of gyne larvae present was counted before and 24 h after addition of the queen. The two treatments differed significantly ($X^2_1 = 11.1$, $P < 0.001$; McNemar's test)

Treatment	No. units with gyne larvae	No. units in which gyne larvae were executed within 24 h
Q+	24	13 (54%)
Q-	24	2 (8%)

to dozens of times, she would withdraw, and a group of workers would rapidly surround the larva and proceed to drain it of fluids. These queen-attacked gyne larvae appeared especially attractive to workers. Only gyne larvae were attacked by queens.

To investigate the possible involvement of a queen pheromone in promoting this execution of gyne larvae, queen corpses were placed in units containing gyne larvae. These were paired with queenless controls as previously described. The results are shown in Table 2. The addition of a fresh corpse resulted in the execution of gyne larvae in over half the cases, whereas execution occurred in only two (8%) of the controls. Observations were made as before on seven pairs of these units for the first 5 h following introduction of the queen corpses. No aggressive behavior of workers toward gyne larvae was observed during this time in either of the two treatments. However, withered cuticles could be observed 24 h later in many of the units where gyne larvae had disappeared, suggesting that these had been killed by the workers.

These results show that queen control over the production of sexuals can act retroactively after female larvae are sexualized. Queen behavior appears to play an important role in that queens both directly participate in and possibly stimulate execution of gyne larvae by the workers. The fact that queen corpses also cause execution of gyne larvae suggests that queen pheromones play a role as well.

Discussion

The evidence presented here strongly supports the presence of pheromonal queen control over the production of gynes in *I. humilis*; this is the first dolichoderine and one of the few social insects outside the well-documented case of the honey bee, *Apis mellifera* (reviewed in Free 1987; Winston 1987), for which strong evidence favoring pheromonally mediated queen control has been obtained. Although there is considerable circumstantial evidence favoring pheromonal queen control in ants (see Introduction), e.g., the finding (Hölldobler and Wilson 1983) that a single queen corpse inhibits workers from laying reproductive eggs in a small number of laboratory colonies of the weaver ants, *O. longinoda* ($n=4$) and *O. smaragdina* ($n=1$), more conclusive but still indirect

evidence (see Vargo 1986) requires that not only behavioral cues be excluded through the use of corpses, but also that tactile cues associated with the queen be eliminated through the use of proper controls. Previously, such a demonstration had only been achieved in the fire ant, *S. invicta* (Fletcher and Blum 1981a, b; Vargo and Fletcher 1986; Vargo 1988), in which bioassays developed from small colony fragments were used to show that corpses of egg-laying queens are effective in inhibiting the production of sexuals and ovary development in virgin queens. The possibility that tactile cues associated with queen bodies are responsible for this inhibition was eliminated in these experiments on *S. invicta* by using as controls one or both of the following: corpses of reproductively inactive queens (which presumably possess the tactile cues but not the fully developed pheromone system of mature queens) and/or corpses of reproductively active queens rinsed in organic solvent (which presumably have only chemical cues removed). The evidence from the present study on *I. humilis* is similarly strong, since possible tactile cues associated with queen corpses were controlled for using corpses rinsed in pentane. As in the studies of *S. invicta*, the present investigation was greatly facilitated by the use of small colony fragments, which in this case permitted an entire experiment to be conducted with replicates drawn from a single source colony thereby eliminating intercolonial variation as a complicating factor. Of course, definitive evidence for pheromonal mediation of queen control requires the extraction and identification of one or more biologically active compounds. To date only the queen substance of the honey bee, *A. mellifera*, has been so identified (Barbier and Lederer 1960; Butler et al. 1961).

In an earlier study of the mechanisms of queen control over gyne production in *I. humilis*, Bartels (1988) observed that queens tend to usurp worker-produced trophic eggs that are otherwise fed to larvae. From these observations, Bartels concluded that the inhibitory queen influence results from this nutritional deprivation of larvae. He also observed queens smearing their own eggs on small larvae and suggested that this may be an additional means of control. However, Bartels' results are not inconsistent with a pheromonal mechanism. Indeed, the present study demonstrates that queen corpses are nearly as inhibitory as living queens, even in colony fragments considerably larger than those employed by Bartels. This suggests that behavioral intervention by queens through trophic egg deprivation and/or "egg smearing" is not a necessary mechanism mediating this form of queen control. It is, of course, possible that these queen behaviors do play a minor role in augmenting the effect of the pheromone.

One of the most intriguing aspects of our findings is their congruence with the conditions under which gyne production in *I. humilis* occurs naturally; this has been studied in California (Markin 1970; Bartels 1983) and southern France (Benois 1973; Keller et al. 1989a). These field observations together with the results of the present study lead us to propose the following account of how gyne production is regulated in nature (see Fig. 6). For most of the year, queen number is relatively

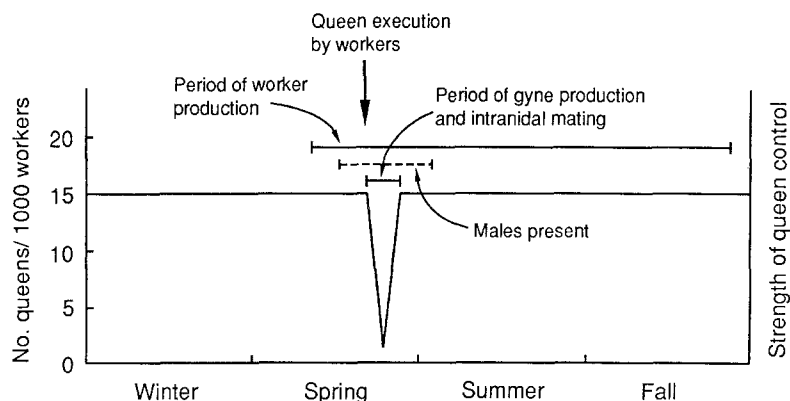


Fig. 6. The annual cycle of gyne production in *I. humilis*. Gynes are produced only during a short period in spring when queen number, and presumably the combined strength of pheromonal queen control, is greatly reduced following the massive execution of queens by workers. See text for details

high (ca. 10–15 queens/1000 workers), resulting in high levels of the inhibitory pheromone; consequently, no gynes are produced. In mid spring, the quantity of pheromone in the colony is sharply reduced when the workers eliminate by execution some 90% of the queens (Keller et al. 1989a). Most likely, caste determination in this species is trophogenic, so that in the absence of a strong pheromonal signal, the workers feed female larvae more, committing them to gyne development. Once mature, these gynes then mate in the nest (Markin 1970; Bartels 1983; Passera et al. 1988b) thereby restoring queen number to its previously high level (Keller et al. 1989a). Since, as the present study has demonstrated, these young queens begin producing the pheromone soon after mating, the inhibitory influence is quickly reinstated, preventing additional gynes from developing. This is achieved by inhibiting the sexualization of the remaining bipotent female larvae and probably by stimulating the execution of any larvae already committed to gyne development. This strong queen control then remains in effect until the following spring when the workers once again lower the level of inhibitory pheromone by execution of queens.

The regulation of gyne production in nature thus appears to involve an unusually complex interplay of social and environmental factors that effectively result in worker control over queen control. Although queen control over the production of gynes is widespread among ants (Fletcher and Ross 1985; Hölldobler and Wilson 1990), the ability of workers to override this control would seem to be rather uncommon. Evasion of queen control by social insect workers may be favored in situations where the genetic interests of the workers and queens are in conflict, such as over direct reproduction by workers and the proportional investment in male versus female sexuals (Trivers and Hare 1976; Oster and Wilson 1978; Bourke 1988). However, because queens and workers should not be in conflict over the timing of the production of sexuals or the fraction of colony resources that goes into their production (Oster and Wilson 1978; Hölldobler and Bartz 1985; Hölldobler and Wilson 1990), it is a moot point whether gyne production in *I. humilis* is controlled by queens or by workers. It thus seems unlikely that queen execution in this species evolved through selection on workers to circumvent queen control over gyne production. Keller et al. (1989a)

have suggested that this execution behavior may have arisen as a result of reproductive competition among queens leading to the elimination of rivals and/or kin selection on workers to eliminate less related queens. For either of these hypotheses to be tenable, however, queen execution would have to be timed such that surviving queens dominate the production of new gynes in a manner similar to that found in the cycles of polygyny and monogyny in some swarm founding polybiine wasps (West Eberhard 1978, 1981). This is not the case in *I. humilis*; gynes arise from brood already present at the time of execution. It is therefore unlikely that queen execution significantly affects the survivors' contributions to the pool of new gynes or the degree of relatedness between workers and gynes. Regardless of its evolutionary origin, queen execution by workers appears to be a key factor regulating gyne production in this species.

An additional factor that comes into play in this annual production of gynes in *I. humilis* is the effect that season has on both larvae and workers. While female larvae taken from the field at any time of the year are capable of gyne development (Passera et al. 1988b; unpublished data), those collected in spring have the highest tendency to become sexualized. In addition, workers appear to be less sensitive to the inhibitory queen pheromone in spring than at other times of the year (unpublished data). The effect of season on gyne development will be presented in a separate paper. Studies of the colony life cycle in native South American populations are needed to assess the importance of season as a general feature of caste determination in this species. Such studies should also be useful in testing the above hypothesized scheme of the social factors regulating gyne production in the field.

Although not specifically tested in the present study, it appears that the production of males is not under strong pheromonal control in *I. humilis*, since, unlike gynes, males are often produced in the presence of queens (Giraud 1983; Bartels 1988; Passera et al. 1988a; unpublished data). Studies by Passera et al. (1988a) indicate that queen-laid male eggs and/or larvae are always present and that adult males are reared when there is an abundance of food available to larvae, such as when worker/larva ratios are high or queen/larva ratios are low. Interestingly, male larvae are sometimes reared to large last instars and then cannibalized (Passera et al.

1988a); however, unlike the execution of gyne larvae, male larvae are killed largely in response to the quantity of available food. It is still possible that male production is influenced by queen pheromones but to a much lesser degree than gyne production. In this respect, queen control over the production of sexuals in *I. humilis* differs from that in the myrmicine *S. invicta*, in which the production of both males and gynes is under strong influence of queen pheromones (Vargo and Fletcher 1986, 1987).

Another possible difference between these two species concerns the mating status of the queens producing the inhibitory pheromone. In *S. invicta*, both inseminated and uninseminated queens that are reproductively active are effective in inhibiting the production of sexuals (Vargo 1988). In the present study, winged virgin queens of *I. humilis* were not inhibitory, whereas dealated inseminated queens of the same age were. These results are consistent with pheromone production being dependent on insemination in this species. Alternatively, pheromone production by *I. humilis* queens may be related to ovary development, as it appears to be in *S. invicta* (Fletcher and Blum 1983; Willer and Fletcher 1986) and as suggested for social insects generally (Fletcher and Blum 1983). When winged virgin queens of *I. humilis* are held in the laboratory and prevented from mating, most remain winged and fail to lay eggs; however, some shed their wings and begin to oviposit (L. Passera, unpublished data). All of the uninseminated gynes used in the present study remained winged and reproductively inactive. It is possible that ovipositing virgin queens also produce the inhibitory pheromone. Additional tests comparing the inhibitory capability of egg-laying virgin queens and comparably aged non-laying virgin queens are needed to determine whether production of this inhibitory pheromone of *I. humilis* is related to insemination status or degree of ovary development.

Despite the differences just mentioned, pheromonal queen influence over the production of sexuals in *I. humilis* and *S. invicta* shows some interesting similarities. The present findings and those of Vargo (1988) suggest that the queen pheromones of both species appear to be relatively non-volatile and are distributed in the colony through contact and/or trophallaxis among workers. In addition, it is likely, but not certain, that these pheromones act mainly by affecting larval feeding behavior of workers rather than by a direct effect on larval development (see Vargo and Fletcher 1986). Also, if the pheromonal signal is strengthened after some female larvae have been committed to gyne development, workers of both species can respond by executing them.

Aggressive behavior of workers toward sexually-potent larvae in response to queen control has been observed in other ants, e.g., the formicine *Plagiolepis pygmaea* (Passera 1969) and the myrmicine *Myrmica rubra* (Brian 1973), although pheromonal mediation of this response has not been demonstrated. In addition to producing a pheromone that stimulates workers to attack gyne larvae, queens of *I. humilis* may participate directly in their execution. Such queen control larval development through direct attack of gyne-potential larvae has

been previously reported only in *M. rubra* (Brian 1970). Biting of larvae by queens in this latter species differs from that in *I. humilis* since it does not kill the larvae but prevents them from becoming sexualized by committing them to worker development.

Larval biting by queens may be more widespread in ants than previously suspected. Masuko (1986) reported that queens of the primitive ponerine, *Amblyopone silvestrii*, feed on larval hemolymph which they obtain by biting larvae. Similar behavior has been observed or inferred (from characteristic scars) by this author in three other Japanese ponerines of the genus *Proceratium*. Although it is not known what effect, if any, such larval hemolymph feeding has on caste determination in these relatively primitive ants, these observations raise the possibility that larval biting by queens is an ancestral formicid trait and may bear some relationship to the control of gyne production via attack of larvae by queens and workers in *I. humilis* and *M. rubra*, two phylogenetically advanced but distantly related taxa.

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References

- Barbier M, Lederer E (1960) Structure chimique de la substance royale de la reine d'abeille (*Apis mellifica* L.). CR Acad Sci Paris, série D 250:4467-4469
- Bartels PJ (1983) Polygyny and reproductive biology of the Argentine ant. PhD dissertation, University of California, Santa Cruz
- Bartels PJ (1988) Reproductive caste inhibition by Argentine ant queens: new mechanisms of queen control. *Insectes Soc* 35:70-81
- Benois A (1973) Incidence des facteurs écologiques sur le cycle annuel et l'activité saisonnière de la fourmi d'Argentine, *Iridomyrmex humilis* Mayr dans la région d'Antibes. *Insectes Soc* 20:267-296
- Bourke AFG (1988) Worker reproduction in the higher eusocial Hymenoptera. *Q Rev Biol* 63:291-311
- Brian MV (1970) Communication between queens and larvae in the ant *Myrmica*. *Anim Behav* 18:467-472
- Brian MV (1973) Caste control through worker attack in the ant *Myrmica*. *Insectes Soc* 20:87-102
- Brian MV (1980) Social control over sex and caste in bees, wasps and ants. *Biol Rev* 55:379-415
- Brian MV, Blum MS (1969) The influence of *Myrmica* queen head extracts on larval growth. *J Insect Physiol* 15:2213-2223
- Brian MV, Hibble J (1963) 9-oxodec-*trans*-2-enoic acid and *Myrmica* queen extracts tested for influence on brood in *Myrmica*. *J Insect Physiol* 9:25-34
- Butler CG, Callow RK, Johnston NC (1961) The isolation and synthesis of queen substance, 9-oxodec-*trans*-2-enoic acid, a honeybee pheromone. *Proc R Soc London Ser B* 155:417-432
- Carr CAH (1962) Further studies on the influence of the queen in ants of the genus *Myrmica*. *Insectes Soc* 9:197-211
- Dejean A, Passera L (1974) Ponte des ouvrières et inhibition royale chez la fourmi *Temnothorax recedens* (Nyl). *Insectes Soc* 21:343-356
- Edwards JP (1987) Caste regulation in the Pharaoh's ant *Monomorium pharaonis*: the influence of queens on the production of new sexual forms. *Physiol Entomol* 12:31-39
- Fletcher DJC, Blum MS (1981a) Pheromonal queen control of

- dealation and oogenesis in virgin queen fire ants. *Science* 212:73–75
- Fletcher DJC, Blum MS (1981 b) A bioassay technique for an inhibitory primer pheromone of the ant, *Solenopsis invicta* Buren. *J Ga Entomol Soc* 16:352–356
- Fletcher DJC, Blum MS (1983) Regulation of queen number by workers in colonies of social insects. *Science* 219:312–314
- Fletcher DJC, Ross KG (1985) Regulation of reproduction in eusocial Hymenoptera. *Annu Rev Entomol* 30:319–343
- Free JB (1987) Pheromones of social bees. Chapman & Hall, London
- Giraud L (1983) Rôle inhibiteur du facteur «groupement de reines» sur l'apparition des mâles chez *Iridomyrmex humilis*, Hymenoptera, Dolichoderinae. *CR Acad Sci Paris Sér III* 296:655–658
- Hölldobler B, Bartz SH (1985) Sociobiology of reproduction in ants. In: Hölldobler B, Lindauer M (eds) *Experimental behavioral ecology and sociobiology*. Sinauer Associates, Sunderland, MA, pp 237–257
- Hölldobler B, Carlin NF (1985) Colony founding, queen dominance and oligogyny in the Australian meat ant *Iridomyrmex purpureus*. *Behav Ecol Sociobiol* 18:45–58
- Hölldobler B, Wilson EO (1983) Queen control in colonies of weaver ants (Hymenoptera: Formicidae). *Ann Entomol Soc Am* 76:235–238
- Hölldobler B, Wilson EO (1990) *The ants*. Harvard University Press, Cambridge
- Keller L (1988) Evolutionary implications of polygyny in the Argentine ant, *Iridomyrmex humilis* (Mayr) (Hymenoptera: Formicidae): an experimental study. *Anim Behav* 27:839–844
- Keller L, Passera L, Suzzoni J-P (1989a) Queen execution in the Argentine ant, *Iridomyrmex humilis*. *Physiol Entomol* 14:157–163
- Keller L, Cherix D, Ulloa-Chacon P (1989b) Description of a new artificial diet for rearing ant colonies as *Iridomyrmex humilis*, *Monomorium pharaonis* and *Wasmannia auropunctata* (Hymenoptera, Formicidae). *Insectes Soc* 36:348–352
- Markin GP (1970) The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae), in southern California. *Ann Entomol Soc Am* 63:1238–1242
- Masuko K (1986) Larval hemolymph feeding: a nondestructive parental cannibalism in the primitive ant *Amblyopone silvestrii* Wheeler (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 19:249–255
- Oster GF, Wilson EO (1978) *Caste and ecology in the social insects*. Princeton University Press, Princeton
- Passera L (1969) Biologie de la reproduction chez *Plagiolepis pygmaea* Latreille et ses deux parasites sociaux *Plagiolepis grassei* Le Masne et Passera et *Plagiolepis xene* Stärcke (Hymenoptera, Formicidae). *Ann Sci Nat Zool Paris 12^{ème} série* 11:327–482
- Passera L (1980) La fonction inhibitrice des reines de la fourmi *Plagiolepis pygmaea* Latr: rôle des phéromones. *Insectes Soc* 27:212–225
- Passera L, Keller L, Suzzoni J-P (1988a) Control of brood male production in the Argentine ant *Iridomyrmex humilis* (Mayr). *Insectes Soc* 35:19–33
- Passera L, Keller L, Suzzoni J-P (1988b) Queen replacement in dequeened colonies of the Argentine ant *Iridomyrmex humilis* (Mayr). *Psyche* 95:59–65
- Torossian C (1968) Recherches sur la biologie et l'éthologie de *Dolichoderus quadripunctatus* (L.) (Hym. Form. Dolichoderidae). VII. Etude des mécanismes permettant l'inhibition de la ponte des ouvrières en présence de leur reine: rôle des phéromones. *Insectes Soc* 15:105–144
- Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insects. *Science* 191:249–263
- Vander Meer RK (1983) Semiochemicals and the red imported fire ant (*Solenopsis invicta* Buren) (Hymenoptera: Formicidae). *Fla Entomol* 66:139–161
- Vargo EL (1986) Queen control over the production of sexuals in the fire ant, *Solenopsis invicta*. PhD dissertation, University of Georgia
- Vargo EL (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 EL (1990) Social control of reproduction in fire ant colonies. In: Vander Meer RK, Jaffe K, Cedeno A (eds) *Applied myrmecology: a world perspective*. Westview Press, Boulder, CO, pp 158–172
- Vargo EL, Fletcher DJC (1986) Evidence of pheromonal queen control over the production of male and female sexuals in the fire ant, *Solenopsis invicta*. *J Comp Physiol A* 159:741–749
- Vargo EL, Fletcher DJC (1987) Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta*. *Physiol Entomol* 12:109–116
- West Eberhard MJ (1977) The establishment of reproductive dominance in social wasp colonies. *Proc VIIIth Int Congress, IUSSI, Wageningen, 5–10 Sept*, pp 223–227
- West Eberhard MJ (1978) Temporary queens in *Metapolybia* wasps: nonreproductive helpers without altruism? *Science* 200:441–443
- West Eberhard MJ (1981) Intragroup selection and the evolution of insect societies. In: Alexander RD, Tinkle DW (eds) *Natural selection and social behavior: recent research and new theory*. Chiron, New York, pp 3–17
- Wheeler DE (1986) Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *Am Nat* 128:13–34
- Willer DE, Fletcher DJC (1986) Differences in inhibitory capability among queens of the ant *Solenopsis invicta*. *Physiol Entomol* 11:475–482
- Wilson EO (1971) *The insect societies*. Harvard University Press, Cambridge
- Winston ML (1987) *The biology of the honey bee*. Harvard University Press, Cambridge