

FINAL REPORT, 1999
Texas Imported Fire Ant Research and Management Plan

**ELIMINATION OF QUEENS BY WORKERS:
A MECHANISM OF REGULATION IN FIRE ANT COLONIES**

Richard Deslippe
Department of Biological Sciences, Texas Tech University

Abstract

The polygyne form of the red imported fire ant (*Solenopsis invicta*) has in its colonies many egg-laying queens. Workers generally tend and groom these queens meticulously, but sometimes they attack and kill them. Workers also regularly adopt foreign queens into their nests, and this combination of executions and adoptions generates turnover in the reproductive units of colonies. I examined whether workers execute and adopt queens in response to queen pheromones, and tested whether they first kill the poorest pheromone producers and leave the best producers unharmed. Execution of nestmate queens was common, particularly when queen abundance was high. In colonies with 10 or 15 queens, in which workers killed at least three queens within 30 days, time to first execution was significantly greater than were times between first to second and second to third executions. In paired-queen experiments, executions were higher in old-old and young-old queen pairings than in young-young pairings. Execution rates were also significantly higher in virgin-functional queen pairings than in functional-functional queen pairings. Colonies exposed to concentrated queen extracts killed their queens significantly more frequently than did control colonies. Although foreign alate queens were usually killed when introduced to queenless colonies, foreign wingless queens were usually accepted even if they were not inseminated. Colonies accepted wingless queens even if they were queenless for only a few hours, a situation that does not occur in the monogyne form of *S. invicta*. Alate and wingless queens differed clearly in their venom alkaloids and hydrocarbon profiles, suggesting these chemicals play a role in worker discrimination of queens. In short, my evidence supports the ideas that queen abundance is regulated by queen pheromones and that workers readily discriminate among queens. An important development will be to isolate the relevant pheromones. It would enable the exploitation of the communication system, and aid in management efforts by stimulating the self-destruction of colonies.

Keywords: *matricide; pheromones; queen extracts; self-destruction of colonies; Solenopsis invicta; worker adoption of queens; worker execution of queens*

Introduction

Much variation exists in the number of mated queens in ant nests and in the way the variation is produced (Hölldobler and Wilson 1977, 1990; Keller 1993). Within their colonies, ants tend to have only a single, functional queen (monogyny), but some species contain several to many queens (polygyny). *Solenopsis invicta* is most unusual in having distinct monogyne and polygyne forms (Macom and Porter 1996). The polygyne form contains dozens of functional queens and is particularly prevalent in Texas (Porter 1991).

Colonies of both forms experience substantial turnover in their reproductive units. Initially, queens may join forces following their nuptial flights, a strategy that enables them to produce more workers in the first cohort than independent queens (Tschinkel and Howard 1983). Once the first workers pupate, they expel or kill many of their queens and pilfer brood from surrounding nests. Most of the brood accumulates in winning nests, and workers from losing nests abandon their own queens and peacefully join the winners (Adams and Tschinkel 1995; Balas and Adams 1996a, Stamps and Vinson 1991; Tschinkel 1992a,b). These activities produce incipient colonies that contain mixtures of related and unrelated individuals.

In the polygyne form, polygyny arises because workers periodically adopt new queens as the colony develops (Porter 1991). Workers, however, also commonly execute their queens (see results), and this combination of adoption and execution results in frequent change in the reproductive units of colonies. The queens that workers kill may even be their own genetic mothers (Balas and Adams 1996a), and this matricide apparently occurs because workers discriminate among queens by criteria other than relatedness (Balas and Adams 1996b).

The concentration of queen pheromones may dictate whether workers adopt and execute queens. For example, workers might adopt queens when key pheromones drop below some threshold, whereas they might execute them when pheromones exceed a threshold. Presumably queens also differ in their pheromone production and the differences could enable individual recognition, generating a queen hierarchy. Workers might first kill the poorest pheromone producers and leave the best producers unharmed. Pheromone production is apparently related to ovary development (Fletcher 1986), and this development is likely correlated with age.

My project involved testing whether workers execute or adopt queens in response to concentration of queen pheromones, and testing whether they first kill the poorest pheromone producers and leave the best producers unharmed. The ultimate goal is to uncover the pheromones responsible for regulating queen abundance, knowledge that would enable exploitation of the communication system and aid in the management of *S. invicta*. In this report, I summarize my studies on the execution of queens. The focus is on how executions relate to the density, age, status and extracts of queens. I also present data on the venom alkaloids and cuticular hydrocarbons of winged and wingless queens, for they suggest how workers may discriminate among queens.

Materials and Methods

a) Executions in relation to age, density and status of queens

To test whether the frequency of queen executions was associated with queen abundance, I established 68 subset colonies from larger polygyne colonies collected in Texas. The subsets contained several hundred workers, brood and 1, 3, 5, 10 or 15 queens. Plastic boxes (11.0 cm x 11.0 cm x 3.8 cm) housed the ants (Fig. 1), and they were rimmed with a teflon paint, Fluon™, to prevent the escape of ants. Each box contained the bottom of a petri dish filled with plaster. A

cavity was created in the plaster for the ants and brood, and it was mostly covered with a piece of red plexiglass. The colonies were generously fed crickets, mealworms and a honey-water solution, and they were monitored regularly over 30 days for executed queens.

To examine whether executions were associated with age or asymmetries in age, 12 subset colonies each containing two queens were created. Young queens were collected following a nuptial flight, and old queens were functional queens from large colonies that had been maintained in the lab for over a year. Queens were paired in combinations of young-young, young-old or old-old (from different colonies), and they were added along with brood to plastic boxes as described above (my standard setup). All workers came from the transferred brood as they developed, the first ones emerging from their pupal states within two days following the experimental setup. Colonies were fed and monitored regularly over 40 days for executed queens.

To compare mortality rates across queen status, I created 41 subset colonies each containing two queens. The queens originated from the same colonies, and they were added to standard chambers with brood and several hundred workers. A naturally wingless queen was paired with an alate queen, a virgin with her wings either clipped or pulled off at the base, or another dealated queen. The colonies were fed and monitored regularly over 40 days for executed queens.

b) Executions in relation to queen extracts

To test whether queen extracts stimulate worker executions of queens, I established 84 subset colonies each containing three functional queens, brood and several hundred workers. Hundreds of wingless queens were isolated from newly collected colonies, and placed in methanol (highly polar solvent), acetone (solvent of intermediate polarity) or hexane (highly non-polar solvent). Twice a day for 12 days, 25 μ l of each solvent (controls) or each solvent containing queens was added to a piece of filter paper, allowed to dry and then introduced to the brood chamber of the colonies. Colonies were fed and monitored daily for executed queens.

A similar experiment was repeated involving only hexane and queen extracts in hexane (Fig. 1). In this experiment, however, each subset colony contained one queen rather than three functional queens. Fifty colonies were monitored daily over 25 days for dead queens.

c) Executions of introduced queens

To assess worker acceptance of foreign queens, I established 66 subset colonies containing a queen, workers and brood. Once the colonies had adjusted to their new chambers, the queens were removed and a foreign queen was introduced on day 0, 3, 6, 9, 12 or 15 since becoming queenless. Introduced queens were either winged or wingless, and they were monitored for survivorship over three days. Wingless queens were later dissected to determine whether or not they were inseminated.

d) Chemical analyses

Because venom alkaloids and cuticular lipids are involved in chemical communication among fire ants, they may enable workers discrimination of queens. To explore this possibility, chemical analyses of alate and wingless queens were conducted. Individual queens were placed

in 2 ml vials containing hexane (0.7 ml) and an internal standard, eicosane. The vials were then sealed with caps, and the contents analyzed for alkaloids and hydrocarbons after 24 h.

To separate and identify the chemicals, I used a GC-MS system (Hewlett Packard [HP] 6890 Series II Plus gas chromatograph coupled to a HP 5973 Series mass selective detector). The oven was programmed from 150 to 270 °C, respectively. Splitless injection was used, and samples were carried through a 5%-diphenyl-95%-dimethylsiloxane copolymer capillary column (HP-5MS, 30 m x 0.25 mm ID, 0.25 µm film thickness) with helium flowing at 1.2 ml/min. Mass spectra were acquired from 50 to 550 amu (2.94 scans/s), and ChemStation software (HP G1701AA) was used to integrate the area of chromatographic peaks.

e) Statistics

I report descriptive statistics as means \pm SE. Standard inferential statistics were used to analyze data when variances were homogeneous by the F_{\max} test (Sokal and Rohlf 1981). When variances were not homogeneous, a Kruskal-Wallis test was used, and significant differences were located with Duncan's Multiple Range Test. To analyze frequency data, log-likelihood analyses were performed. Finally, principle components analyses involving the five major cuticular hydrocarbons were conducted to determine whether winged and wingless queens contained unique profiles.

Results

a) Executions in relation to age, density and status of queens

Executions of queens were common in both large, mature colonies and in small experimental colonies. Workers normally expelled queens from the brood chamber, and then later attacked them in a frenzied swarm. The queens were often passive during the executions, and their limbs and bodies were usually cut into pieces (Fig. 2). On occasion, workers instead penetrated the gasters and emptied their contents while the queens still lived. I observed both functional queens and alate queens attacked in this manner (Fig. 3). The alates were in captivity for several months and they probably had partially developed ovaries, suggesting a link between reproductive physiology and executions.

The percent of experimental colonies executing at least one queen within 30 days was dependent on queen abundance. Only 4 of 26 (15.4%) colonies executed their only queen whereas 6 of 6 colonies (100%) with 15 queens executed at least one queen. Frequencies of executions were also high in colonies with 3, 5 or 10 queens, ranging from 40 to 90 % (Fig. 4). The association between queen number and executions was significant ($G = 10.10$, $P = 0.039$). Workers were more likely to execute additional queens sooner if they had already executed one. In colonies with 10 and 15 queens in which workers executed at least three queens, time between initiation and first execution, first and second executions, and second and third executions differed significantly ($F_{2, 29} = 3.86$; $P = 0.033$) (Fig. 5).

Although sample sizes were small, workers, within 40 days, were more likely to execute a queen in young-old or old-old queen pairings than in young-young queen pairings (Fig. 6). In the young-old pairings, however, of five colonies killing at least one queen, the young queens were killed first in three cases, and old queens were killed first in the other two cases. Cumulative mortality within 30 days differed significantly in pairings related to queen status

(Fig. 7, Table 1). At least one queen was killed in 11 of 13 colonies (84.6%) when an alate was paired with a naturally wingless queen, whereas 0 of 9 colonies (0%) killed a queen in pairings of two naturally wingless queens. Mortality was intermediate in pairings involving wingless queens with virgin queens that had their wings mechanically removed. Like the findings involving age asymmetry, both functional and virgin queens were killed (Fig. 7, Table 1).

b) Executions in relation to queen extracts

Whole bodies of queens placed in solvents were highly attractive to workers (Fig. 8). Although the attraction may be due simply to a queen attraction pheromone, multiple compounds may be involved. Regardless, the simple assay shows that at least some queen pheromones dissolve in solvents. Frequency of executions within 12 days did not differ between control colonies and colonies receiving daily exposure to queen extracts regardless of the solvent used (Table 2). In this experiment, three queens were placed in each colony, and the number of queens executed even in control colonies was high (6 of 19 colonies, 31.6%). As a result, I terminated the experiment prematurely, and conducted a similar experiment with the most promising solvent, hexane. The colonies involved contained only one queen. Colonies exposed to queen extracts killed their queens significantly more than did control colonies (Table 3).

c) Executions of introduced queens

By dissecting gasters, it was determined that as many as 50% of wingless queens in colonies were unfertilized (Fig. 9). I was thus able to test whether survivorship of introduced foreign queens was contingent upon reproductive status. Introduced wingless queens were generally accepted by queenless colonies whether or not they were fertilized. In contrast, alate queens were usually killed (Table 4, Fig. 10) even if colonies were queenless for two weeks (Table 4).

d) Chemical analyses

Alate and wingless queens differed clearly in their venom alkaloids and hydrocarbon profiles. Wingless queens had more than a seven-fold higher ratio of unsaturated to saturated C₁₁ alkaloids compared to alate queens (Fig. 12). Likewise, principle components of the hydrocarbons of wingless queens clustered differently than those hydrocarbons of alate queens, both when individuals within a colony were compared, and when averages were compared among colonies (Fig. 13).

Discussion

Workers regularly both execute their queens and adopt foreign queens into their nests. Results support the hypothesis that the executions and adoptions occur in response to queen pheromones. Frequencies of executions were associated with queen abundance, and they were greater in old-old and young-old queen pairings than in young-young pairings. Colonies exposed to concentrated queen extract also killed their queens more often than did control colonies. These results are encouraging, and I am striving to develop a more sensitive bioassay to test the relationship between queen extracts and execution rates. A possibility is to test extracts in colonies with young virgin queens. Such queens are acceptable to workers if they have no functional queen in the colony. Once the bioassay is refined, I can repeat them using components of the extracts by separating whole-queen extracts using HPLC with size-exclusion and reverse phase columns.

Workers clearly discriminate among queens. Execution rates were higher in virgin-functional queen pairings than in functional-functional pairings. Further, foreign alate queens were usually killed when introduced to queenless colonies whereas foreign wingless queens were usually accepted. Individual recognition of queens could produce a queen hierarchy with workers killing the poorest pheromone producers and leaving the best producers unharmed (Fletcher and Blum 1983; Fletcher 1986). However, although asymmetrical pairings resulted in more frequent executions, the queens being executed were not necessarily the presumed best pheromone producers.

Worker discrimination of queens may involve venom alkaloids and/or cuticular hydrocarbons. The discovered differences between winged and wingless queens in the ratio of alkaloid components may send different messages to workers, a possibility made more likely as venom serves multiple functions in *S. invicta* (Blum 1996). Likewise, queens had different hydrocarbon profiles, and as hydrocarbons are well-known chemical messengers in insects (Singer 1998), they may be important in individual queen recognition. Other ants are known to respond to different proportions of components. Workers of *Tetramorium caespitum*, for example, lay trails comprising two pyrazines, and they respond maximally to a blend of seven parts 2,5-dimethylpyrazine and three parts 3-ethyl-2,5-dimethylpyrazine (Attygalle and Morgan 1983).

Colonies accepted foreign queens even if they were queenless for only a few hours. Such acceptance does not occur in the monogyne form of *S. invicta* (Fletcher 1986), perhaps because this form has a more precise recognition system. It seems likely that the probability of acceptance of foreign queens would decrease with the number of queens in the colony, and I am in the process of testing this idea. Should the evidence support the hypothesis that acceptance is related to pheromone concentration, I will then examine acceptance rates in queenless colonies that receive daily exposure to queen extracts. The extracts should elevate queen pheromones in the colony to at least levels found in polygynous colonies, and thus acceptance rates should be low. If successful, this technique will provide a second bioassay to enable the search for key pheromones regulating queen abundance.

Acknowledgments

I thank Yu-Jie Guo and Jesus Salazar for their valuable and dedicated assistance with the research. They also helped compile and analyze the information for this report.

Literature cited

- Adams, E.S. and W.R. Tschinkel. 1995. Effects of foundress number on brood raids and queen survival in the fire ant *Solenopsis invicta*. *Behavioral Ecology and Sociobiology* 37:233-242.
- Attygalle, A.B. and E.D. Morgan. 1983. Trail pheromone of the ant *Tetramorium caespitum* L. *Naturwissenschaften* 70:364-365.
- Balas, M.T. and E.S. Adams. 1996a. The dissolution of cooperative groups: mechanisms of queen mortality in incipient fire ant colonies. *Behavioral Ecology and Sociobiology* 38:391-399.

- Balas, M.T. and E.S. Adams. 1996b. Nestmate discrimination and competition in incipient colonies of fire ants. *Animal Behavior* 51:49-59.
- Blum, M.S. 1996. Semiochemical parsimony in the arthropods. *Annual Review of Entomology* 41:353-374.
- Fletcher, D.C. and M.S. Blum. 1983. Regulation of queen number by workers in colonies of social insects. *Science* 219:312-314.
- Fletcher, D.C. 1986. Triple action of queen pheromones in the regulation of reproduction in fire ant (*Solenopsis invicta*) colonies. *Advances in Invertebrate Reproduction* 4:305-316.
- Hölldobler, B. and E.O. Wilson. 1977. The number of queens: an important trait in ant evolution. *Naturwissenschaften* 64:8-15.
- Hölldobler, B. and E.O. Wilson. 1990. The ants. Harvard University Press, Cambridge.
- Keller, L. 1993. Queen number and sociality in insects. Oxford University Press, New York.
- Macom, T.E. and S.D. Porter. 1996. Comparison of polygyne and monogyne red imported fire ant (Hymenoptera: Formicidae) population densities. *Annals of the Entomological Society of America*. 89:535-543.
- Porter, S.D. 1991. Origins of new queens in polygyne red imported fire ant colonies (Hymenoptera: Formicidae). *Journal of Entomological Science* 26:474-478.
- Porter, S.D., A. Bhatkar, R. Mulder, S.B. Vinson and D.J. Clair. 1991. Distribution and density of polygyne fire ants (Hymenoptera: Formicidae) in Texas. *Journal of Economic Entomology* 84:866-874.
- Singer, T.L. 1998. Roles of hydrocarbons in the recognition system of insects. *American Zoologist* 38:394-405.
- Stamps, W.T. and S. Vinson. 1991. Raiding in newly founded colonies of *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Environmental Entomology* 20:1037-1041.
- Sokal, R.R. and J.F. Rohlf. 1981. Biometry. W.H. Freeman, San Francisco, California, USA.
- Tschinkel, W.R. 1992a. Brood raiding in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae): laboratory and field observations. *Annals of the Entomological Society of America* 85:638-646.
- Tschinkel, W.R. 1992b. Brood raiding and the population dynamics of founding and incipient colonies of the fire ant, *Solenopsis invicta*. *Ecological Entomology* 17:179-188.

Tschinkel, W.R. and D.F. Howard. 1983. Colony founding by pleometrosis in the fire ant, *Solenopsis invicta*. *Behavioral Ecology and Sociobiology* 12:103-113.



Fig. 1. Typical experimental setup to test whether queen extracts stimulate queen executions. Each of the 50 chambers contained a fraction of a larger *S. invicta* colony.



Fig. 2. A queen recently executed by her nestmates. One leg had been removed, and workers were still attacking her. Executed queens are often completely dismembered by workers.



Fig. 3. The gaster of this virgin queen was ripped open and emptied by workers while she lived. Virgins unable to undergo nuptial flights due to captivity have partially developed ovaries. Similar executions were observed in some functional queens, suggesting a link between reproductive physiology and executions.

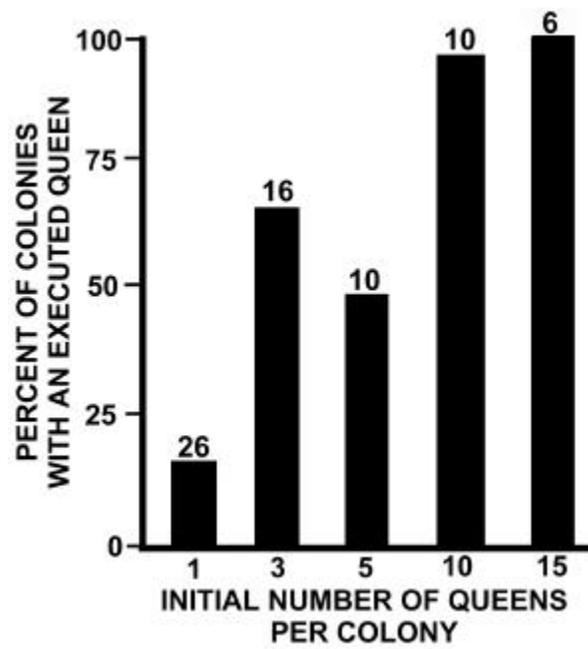


Fig.4. Percent of colonies in which workers executed at least one queen within 30 days. The colonies were created from larger ones, and they initially contained from 1 to 15 queens. Sample sizes are indicated by the numbers above the bars.

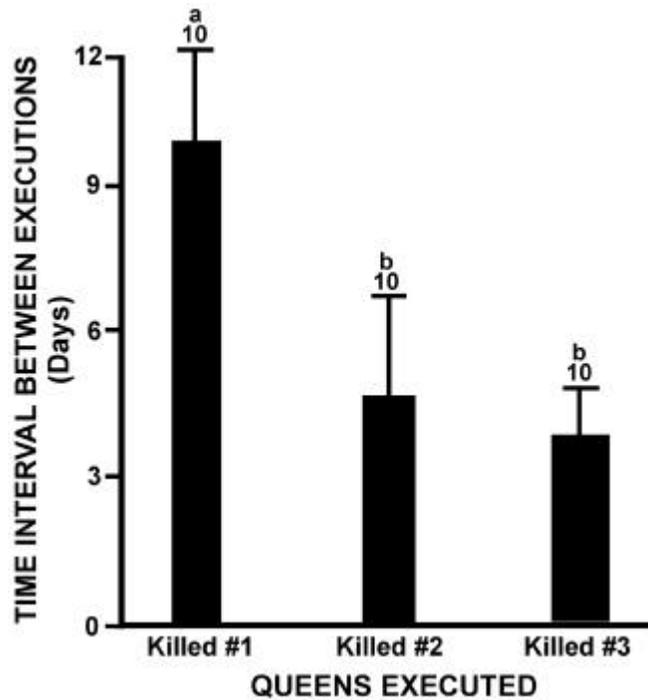


Fig. 5. Time (days) between executions in colonies beginning with 10 and 15 queens and in which workers executed at least three queens within 30 days. Time between initiation and first execution, first and second executions, and second and third executions differed significantly ($F_{2, 29} = 3.86$; $P = 0.033$). Sample sizes are indicated by numbers above error bars. Shared letters within execution order designate means that did not differ significantly (Duncan's Multiple Range Test, $P > 0.05$).

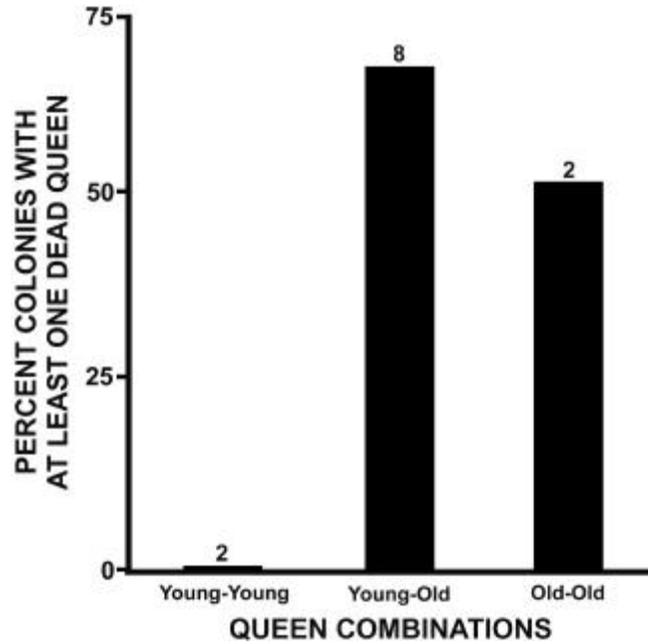


Fig. 6. Percent of colonies with at least one dead queen after 40 days. Colonies contained two queens paired as young-young, young-old or old-old. Sample sizes are indicated by numbers above the bars.

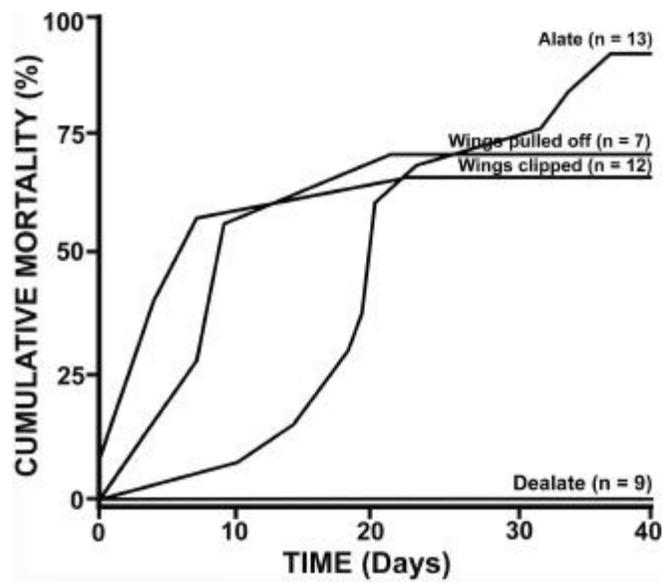


Fig. 7. Cumulative mortality across time in colonies with two queens. A naturally wingless queen was paired with another queen from the same colony. The other queen was an alate queen, a virgin with her wings either clipped or pulled, or another dealated queen.

Table 1. Number and percent of colonies with at least one queen executed within 40 days. Colonies contained two queens, one a functional queen and the other either an alate queen, a virgin with her wings clipped or pulled off at the base, or another functional queen. The frequency of association between executions and queen status was significant ($G = 9.29$, $P = 0.026$).

| Queen status | N colonies | N colonies with a dead queen | Colonies with a dead queen (%) |
|---------------------|-------------------|-------------------------------------|---------------------------------------|
| Alate | 13 | 11 | 84.6 |
| Wings clipped | 12 | 8 | 66.7 |
| Wings pulled off | 7 | 5 | 71.4 |
| Naturally wingless | 9 | 0 | 0 |

Note: The first executed queen in a colony was the functional queen 6 of 18 times (33.3%). I was uncertain of the queen status in six of the pairings involving alate queens, as some alates shed their wings during the experiment. They were not included in the calculation as a result.

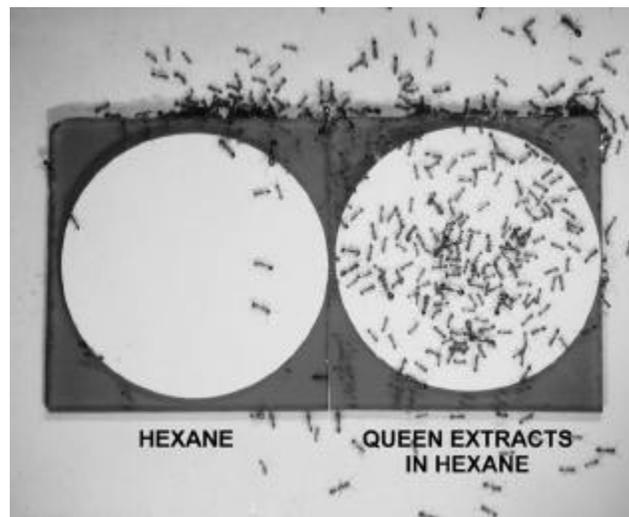


Fig. 8. Workers are highly attracted to queen extracts in hexane (a non-polar solvent), but are not attracted to hexane controls. Workers remain attracted for several days to a drop of extract allowed to evaporate on filter paper.

Table 2. Percent colonies with at least one queen executed by workers within 12 days. Twice a day liquid was added to a piece of filter paper, allowed to dry and then introduced to colonies. The liquids were solvents ranging from highly polar (methanol) to highly non-polar (hexane), and the solvents containing the extracts of many queens. Each colony initially contained three functional queens. There were no significant differences among treatments.

| Treatment | Total number of colonies | Number of colonies with executed queens | Percent colonies with executed queens |
|-------------------|---------------------------------|--|--|
| Methanol | 12 | 5 | 41.7% |
| Methanol + queens | 12 | 5 | 41.7% |
| Acetone | 12 | 5 | 41.7% |
| Acetone + queens | 12 | 5 | 41.7% |
| Hexane | 12 | 3 | 25.0% |
| Hexane + queens | 12 | 6 | 50.0% |
| No solvent | 12 | 4 | 33.3% |

Table 3. Frequency of association between queen executions and treatments ($G = 4.55$, $P = 0.03$). Each colony contained a single queen and was monitored for executions for 30 days. Twice a day, 25 μ l of either hexane or queen extracts in hexane was added to a piece of filter paper, allowed to dry and then introduced to colonies.

| | | QUEEN | |
|-----------|---------------|----------|--------------|
| | | Executed | Not executed |
| TREATMENT | Control | 1 | 24 |
| | Queen extract | 6 | 19 |

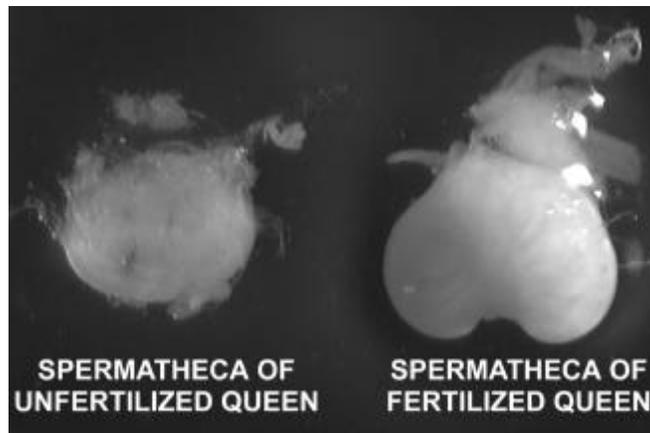


Fig. 9. Wingless queens in colonies are often virgins. They can be readily distinguished from mated queens by examination of the spermatheca.

Table 4. Survival of foreign queens introduced to queenless colonies. Survivorship was monitored for three days and the introduced queens were either fertilized, wingless virgins or alates. The colonies were queenless from 0 to 15 days.

| Treatment | Survivorship of introduced foreign queen | | | | | |
|-----------------------|---|----------|------------------------|----------|--------------------|----------|
| | Fertilized queen | | Wingless virgin | | Alate queen | |
| | Percent | n | Percent | n | Percent | n |
| Queenless for 0 days | 83 | 6 | 67 | 3 | 0 | 2 |
| Queenless for 3 days | 67 | 6 | 33 | 3 | 100 | 2 |
| Queenless for 6 days | 83 | 6 | 75 | 4 | 0 | 1 |
| Queenless for 9 days | 100 | 3 | 80 | 5 | 67 | 3 |
| Queenless for 12 days | - | 0 | 100 | 9 | 0 | 2 |
| Queenless for 15 days | - | 0 | 80 | 5 | 20 | 5 |

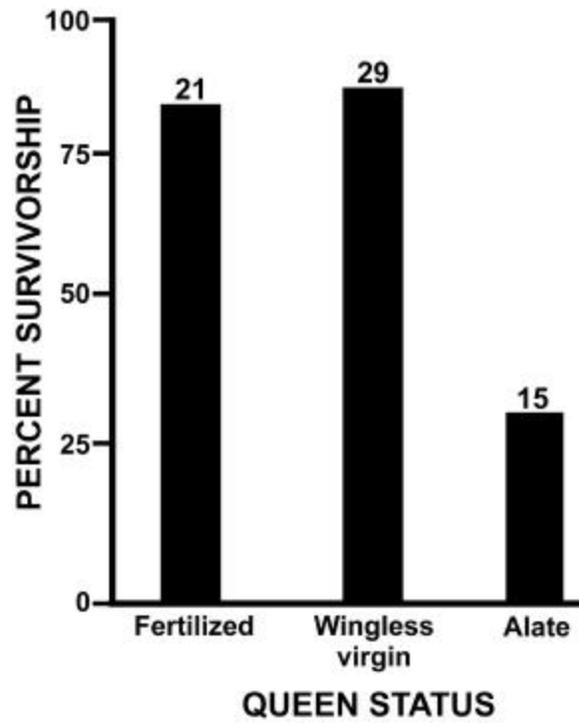


Fig. 10. Percent survivorship of queens introduced to queenless colonies. Colonies were queenless from 0 to 15 days, and introduced queens were either fertilized, wingless virgins or alates. Numbers above bars are sample sizes.

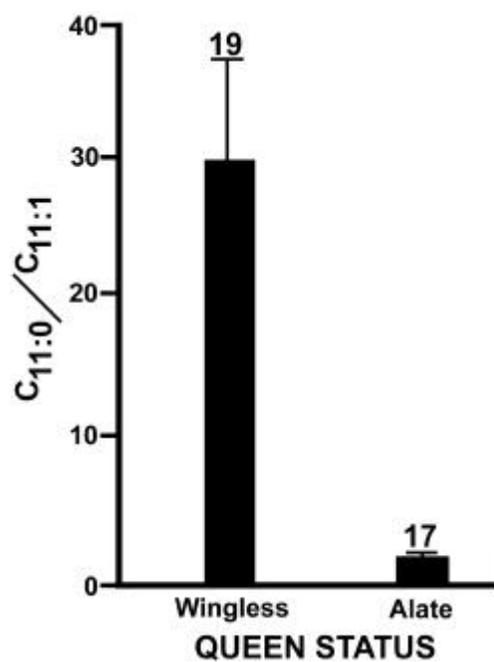


Fig. 11. Ratio of peak area of the main piperidine alkaloids of wingless and alate queens. C_{11:0} denotes a piperidine alkaloid with an 11-carbon chain and no double bond, and C_{11:1} denotes an alkaloid with a 11-carbon chain and one double bond. Sample sizes are indicated by numbers above the standard error bars.

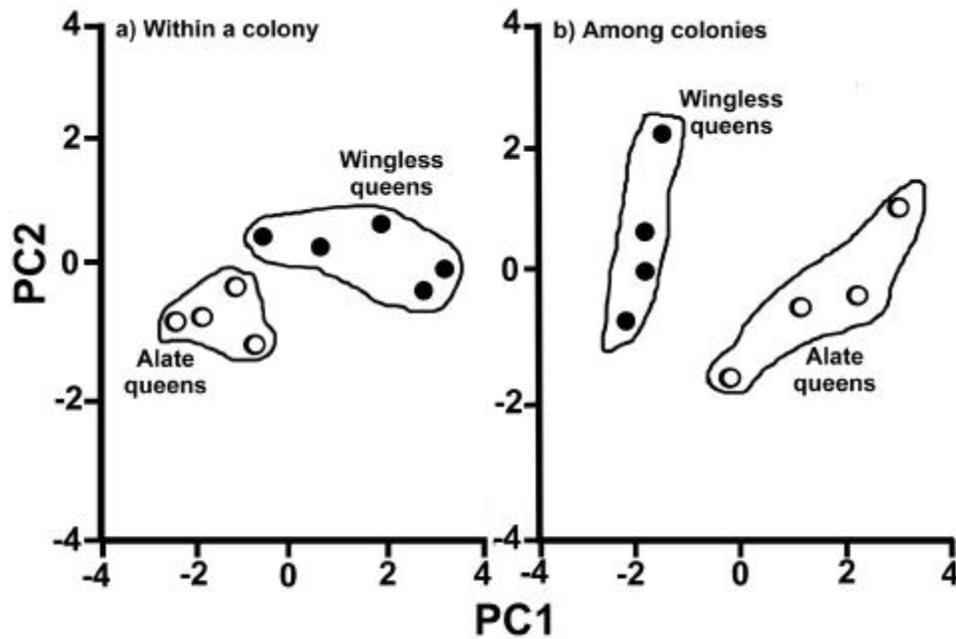


Fig. 12. First and second principle components of an analysis involving the five main cuticular hydrocarbons of wingless and alate queens. Comparisons were made among individuals within a colony (a) and among colonies (b).