Fate of Newly Mated Queens Introduced into Monogyne and Polygyne *Solenopsis invicta* (Hymenoptera: Formicidae) Colonies

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ABSTRACT Nestmate recognition is of prime importance in maintaining ant colony integration and organization. Monogyne red imported fire ant, *Solenopsis invicta* Buren, colonies are highly territorial and aggressive toward non-nestmate conspecific workers. In contrast, workers from polygyne nests in the United States show no aggression toward workers from other conspecific colonies (polygyne or monogyne). Nests within a polygyne population form a 'supercolony,' with free exchange of workers and food between nests. The difference in conspecific nestmate recognition is a major distinguishing feature of the two *S. invicta* forms in the United States. We report here the discovery of an exception to this dichotomy. High levels of worker aggression are released by the introduction of newly mated queens into both polygyne and monogyne colonies. This suggests that nestmate recognition involving female sexuals does not follow the same mechanism used to explain nestmate recognition behavior between workers.

KEY WORDS fire ant, polygyny, aggression, alate, nestmate recognition

THE RED IMPORTED fire ant, *Solenopsis invicta* Buren, was accidentally imported from South America into the United States in the 1930s (Trager 1991). First reported around Mobile, AL, the ant quickly dispersed through human transport in nursery stock throughout the South, initially forming a patchy distribution. From these population nuclei, the ant filled the intervening gaps through additional human transportation and natural spread (Lofgren 1986). The fire ant currently occupies ≈150,000,000 ha in 13 states and Puerto Rico. The combination of a potent sting, aggressive behavior, high population densities, and preference for disturbed habitats causes many medical, agricultural, and environmental problems (Lofgren 1986). These problems have been accentuated with the development of polygyne (multiple queens per nest) populations, which generally doubles or triples worker densities (Porter 1992, Macom and Porter 1996).

Polygyne populations are patchily distributed throughout the range of *S. invicta* in the United States, indicating that they have arisen multiple times (Porter 1992). *Solenopsis invicta* has an extensive range in its South American homeland, spanning Brazil, Bolivia, Uruguay, and part of northern Argentina (Trager 1991). However, in its South American homeland, polygyne populations have been reported only in Argentina (Ross et al. 1996, Porter et al. 1997). The Argentinean polygyne colonies differ from those in the United States. Queens within a polygyne colony in the United States are unrelated (Ross and Fletcher 1985a), whereas polygyne queens in Argentina are closely related (Ross et al. 1996), indicating that either intranidal mating occurs or female alates have mating

flights and return to their natal nest. There may be hundreds of queens per colony in the United States, but polygyne nests in Argentina generally have <10 queens (Ross et al. 1996). Based on these differences it is likely that polygyne colonies in the United States originate by a different mechanism than that of their native South American counterpart.

The evolution of social organization in Hymenoptera is commonly attributed to its haplodiploid basis of sex determination, where colony cooperation is based on a higher relatedness between sisters (workers) than between mother (queen) and daughters (Hamilton 1964). In polygyne colonies the relatedness between workers is decreased, which poses a dilemma for this theory (Keller and Vargo 1993, Heinze and Tsuji 1995). Polygyne colonies are considered an evolutionarily derived condition (Hölldobler and Wilson 1977) in response to unusual ecological factors such as predation pressure, risk of parasitism, and changes in resource levels (Hölldobler and Wilson 1977, Herbers 1993). The ecological factors that may have driven the evolution of polygyny in the native range of *S. invicta* are unknown. In the United States, polygyne colonies were not reported until about 40 yr after its introduction, and proliferation of patchily distributed polygyne populations has only been reported from the 1980s to the present (Lofgren 1986, Porter 1993), which can be explained through rapid "polygyne gene" selection, or an ecological event that released an existing polygyne potential, or a combination of the two. In any case, nestmate recognition must play an important role in the development and maintenance of polygyne *S. invicta* in the United States.

Polygyne colonies tend to have more small workers and fewer large workers (Greenberg et al. 1985, Porter 1992). A major behavioral difference between monogyne and polygyne populations is their aggressive behavior toward conspecific workers (Morel et al. 1990). Monogyne fire ant workers from queenright colonies (colonies with a functional queen) are highly aggressive toward conspecific and heterospecific worker ant intruders (Obin and Vander Meer 1989), whereas polygyne colony workers are aggressive toward heterospecific intruders but not conspecific workers (Morel et al. 1990). Thus, each monogyne colony in a population is an enclave, defending its territory; whereas colonies in a polygyne population exhibit exchange of workers and food (Bhatkar and Vinson 1987) and probably queens and brood (Vargo and Porter 1989).

Mating flights occur about 100 m in the air, after which the newly mated queens land on the surface and attempt to find a suitable place to found a new colony (Markin et al. 1972). During mating flights and colony founding, newly mated queens are vulnerable to predation, especially on the ground from other ants.

Existing polygyne populations must accept new queens to maintain themselves or expand. New polygyne colonies and populations must arise by bringing multiple queens together in some way. Thus, the behavior of polygyne and monogyne workers toward newly mated queens dictates the population dynamics of both monogyne and polygyne fire ants. For example, if worker recognition of newly mated queens fits into the same system as that of worker/worker recognition, then newly mated queens would be readily adopted into polygyne colonies, but not into monogyne colonies. This study was designed to test whether monogyne and polygyne fire ant worker behavior toward female sexuals is analogous to that of conspecific worker/worker behavior.

Materials and Methods

The following experiments were designed to define the behavior of queenright monogyne and polygyne workers toward foreign sexuals, both inseminated and uninseminated. *Solenopsis invicta* populations near Gainesville, Alachua County, FL, consist of both monogyne and polygyne populations. We consider colonies with two or more inseminated queens to be polygyne. Although it is not possible to say with absolute certainty that a colony is monogyne, we based our characterization on the set of criteria listed below. Newly mated queens were collected after mating flights in an area dominated by monogyne colonies; therefore, the newly mated queens are primarily monogyne-derived.

Monogyne Sub-Colonies. Queenless monogyne *S. invicta* sub-colonies were obtained from colonies collected near the USDA Center for Medical, Agricultural, and Veterinary Entomology, in Gainesville, FL. Approximately 500 ml of nest soil containing brood and workers was collected from each nest. The source colonies were considered monogyne if no dealates

were found during excavation or a single physogastric dealate (queen) was found, the colony contained a full size range of workers (Greenberg et al. 1985), and the workers were highly aggressive toward introduced polygyne workers (Morel et al. 1990).

Polygyne Sub-Colonies. Worker ants were collected from two known polygyne populations south of Gainesville, FL, near Micanopy and Williston, FL. Nest sub-colonies were collected by one of the following two methods: (1) Excavated nests were examined at the site. If two or more dealated female sexuals were found in the nest, ≈500 ml of nest soil containing brood and workers was collected. The dealated females associated with each nest were taken back to the laboratory separately. (2) Nests were excavated, brought back to the laboratory, and examined for multiple female dealates. Dealates collected using either method were dissected and examined for the presence of a pearl-white, kidney shaped spermatheca to determine if they had been inseminated. After two dealates from a collected nest were found to have been inseminated, no more dealates were dissected. This was adequate to show functional polygyny, because in fire ants all inseminated queens contribute to colony egg production (Vander Meer et al. 1992).

Installation in the Laboratory. Monogyne and polygyne sub-colonies were set up in the laboratory as follows. Approximately 300 ml of nest soil containing workers (≈300 ants) and some brood was transferred to enamel pans (28 by 15 by 5 cm). The sides of the pans were coated with Fluon (ICI, Wilmington, DE) to prevent escapes. All sexuals were removed from the soil in the test pans and discarded. Several cotton balls were placed in the nest soil and moistened with water. The ants were left undisturbed overnight before bioassays commenced. No food was provided. After <72 h of collection from the field the sub-colonies were discarded. The room temperature was 24–28°C, humidity and lighting were ambient.

Polygyne and Monogyne Queens. The acceptance of established polygyne and monogyne queens was also tested. Established polygyne colony queens were obtained from two field-collected polygyne colonies maintained in the laboratory for a minimum of 6 mo. Monogyne queens were obtained from field-collected monogyne colonies maintained in the laboratory until the queens were needed for introductions. Colony maintenance was as described in Banks et al. (1981).

Newly Mated Queens. Several age-based categories of newly mated/colony founding queens were used for introduction into monogyne and polygyne test colonies, because they may be discovered by established monogyne and polygyne workers at any time during the colony foundation process. Newly mated queens were collected near the USDA, Center for Medical and Veterinary Entomology, Gainesville, FL, laboratory. To ensure that the newly mated queens were not from previous mating flights, we searched and physically cleared the area of queens either the afternoon before or the morning of the expected flight. If this was not possible, only newly mated queens walking on the surface in search of a suitable

Table 1. Definition of the categories of postmating queen treatments added to experimental units

Treatment Category	Definition
Newly mated queens	1–8 h postmating flight
Young founding queens	2–8 d postmating flight
Old founding queens	17–20 d postmating flight
Postclaustral queens	21–30 d postmating flight
Mature polygyne queens	Field collected—age unknown
Mature monogyne queens	Field collected—age unknown
Female alates	Mature (>15 mg); monogyne-derived
Female dealates	Mature; artificially dealated; monogyne-derived

Table 2. Behavioral units and aggression scores used to assess nestmate recognition

Score	Behavioral Response
9	Immediate lunge, grab, and stinging
8	Opponent surrounded and "held" in mandibles; appendages pulled/bitten off; eventual stinging
7	Opponent held (as in no. 8) but released; biting; abdomen-curling (stinging position), but no stinging
6	As in no. 7, but no abdomen-curling
5	Alarm (running, abdomen elevation, and vibration) and recruitment
4	Mandible gaping; rapid antennation; "sidling" (maintaining a lateral orientation to and slowly circling opponent)
3	Rapid antennation with antennae extended for >2 s
2	Antennation for <2 s. If mobile, opponent is followed slowly for several cm; if opponent stationary, worker stops
1	As in no. 2, but opponent does not induce following nor stop

nest site were collected. Newly mated queens were weighed as a group, maintained in trays (28 by 15 by 5 cm). The sides of the pans were coated with Fluon to prevent escapes. The trays were partially covered and water was provided by the addition of moistened cotton balls, which also provided numerous refuges for the queens. The cotton balls were checked periodically and water was added as needed. No food was given to the newly mated queens, because this period represented the claustral colony foundation stage. These newly mated queens were the source of newly mated queens (1–8 h postmating), young founding queens (2–8 d postmating), old founding queens (17–20 d postmating), and postclaustral queens (21–30 d postmating) used for introductions into test colonies or sub-colonies (Table 1). Newly mated queens from one mating flight were weighed individually, maintained in a tray as above and sampled for their weights 3, 6, 9, and 20 d after mating. The weight of queens during the claustral colony foundation stage is indicative of the physiological changes and development of the first workers that occur during this phase of colony foundation (Vander Meer et al. 1982).

Aggression Bioassay. Nestmate recognition between resident colonies and introduced female sexuals was assessed by a bioassay that measured aggressivity (Obin 1986). Individual female sexuals, designated as "intruders" were introduced into a pan containing a sub-nest of "resident" ants. The response of each resident worker coming in contact with the intruder was scored on a scale (1–9) of increasing aggressive behavior (Table 2). To guarantee independent sampling, only the most aggressive response elicited by the first 20 interactions of the intruder with resident ants was recorded. After 20 interactions, or less if the highest possible aggressive response was obtained early, the intruder was removed and placed in a vial for later determination of its weight and examination for the presence or absence of a spermatheca. Intruders from like treatments (see Fig. 2 and Table 1 for treatment categories) were placed in the same vial. All worker/worker intra-nest control bioassays consisted of the removal and reintroduction of a worker into its own colony. Inter-nest controls consisted of the introduction of monogyne or polygyne non-nestmate worker intruders into like social form monogyne or polygyne resident test colonies or colony fragments. Bioassays were carried out in the

same room in which the colony fragments were being maintained, and were completed within 72 h of their laboratory installation. Colony fragments were never tested in two successive bioassays. Comparisons were made using Wilcoxon signed rank test.

Olfactometer Bioassay. The Y-tube olfactometer design has been described elsewhere (Vander Meer et al. 1988). Compressed air (breathing air quality) was passed into the two choice arms at a regulated 0.2 liters/min for a total flow of 0.4 liters/min. Treatments were dissected poison sacs from monogyne alates, newly mated queens (<8 h postmating), young founding queens (3–5 d postmating), old founding queens (17–20 d postmating), and polygyne queens (Table 1). Poison sacs were dissected from each of the above categories and macerated in hexane. The volume of hexane extract was adjusted to give a concentration of 0.33 queen equivalents per 10 μl of solvent. Treatment samples and the solvent blank were applied (10 μl) to filter paper strips (0.3 by 2.5 cm, Whatman No. 1, Whatman, Hillsboro, OR), air dried for 1–2 min, and each was placed into one of the olfactometer choice arms. Worker ants were introduced into the entrance stem (Vander Meer et al. 1988) and the initial choice of the first 20 workers was recorded. After the first choice test the olfactometer was rinsed with acetone, dried, and set up exactly as before. The sample was retested with workers from the same colony, but with the sample and control in reverse positions. This procedure reduced bias inherent in the apparatus. A complete replicate was the sum of the results from the two tests. A minimum of three replicates was obtained for each treatment. Data were analyzed by chi-squared tests and comparisons between means were made with t-tests.

Monogyne Female Alates. Alates were collected from monogyne (defined above) colonies found adjacent to the site where newly mated queens were collected. The nests were excavated and female sexuals collected and transferred to "sub-colony" trays

along with colony soil and workers. These sub-colonies were maintained in the laboratory in the same manner as the other sub-colonies. Alates were separated from the sub-nest, weighed, and those that weighed >15 mg (mature alates) were randomly introduced into monogyne and polygyne sub-colonies and the worker aggression scores recorded.

Monogyne Female Dealates. To determine if the loss of wings (this normally occurs directly after mating and landing on the ground) process influenced worker recognition/aggression, alates were dealated (de-winged) mechanically under a microscope. Dealation triggers the physiological changes associated with mating, but without insemination (Glancey et al. 1981). Monogyne field colony alates were separated from two colonies (see above) and brought back to the laboratory. They were weighed and alates weighing >15 mg were placed in a container with a moistened cotton ball. The other alates were discarded. Approximately 20 alates from each of the two colonies were used. Dealation was accomplished by cooling the alates in a refrigerator, then breaking off their wings with forceps. Artificial dealates were randomly introduced into monogyne and polygyne sub-colonies approximately eight hours after dealation and worker aggression scores recorded. Mature alates and functional polygyne queens were similarly introduced and aggression scores recorded for comparison.

Introduction of Newly Mated Queens into Queenright Monogyne Colonies. Five mature field collected queenright monogyne colonies were maintained in the laboratory for a minimum of 5 mo before the introduction of newly mated queens. The monogyne queens were readily identifiable because their abdomens were distended and they were covered with phoretic mites. Five newly mated queens (<2 h postmating flight) were added to the foraging arena of each monogyne colony. The colonies were checked within 24 h for queen mortality.

Introduction of Alates and Several Queen Types into Queenright Polygyne Colonies. Nine polygyne colonies were set up in the laboratory with four queens each. The following sequential introductions were made to each polygyne colony over a 35-d period: (1) five newly mated queens (<2 h postmating flight); (2) five founding queens (8 d postmating flight); (3) five polygyne queens from unrelated source colonies; (4) five female alates, each from a different source colony (n = 9), were added on the same day they were removed from the source colony; (5) five postclaustral monogyne queens (20–30 d after mating); and (7) one physogastric monogyne queen was introduced into each of six test polygyne colonies. Each category of queen was marked by clipping different tarsal claws on the feet (Tschinkel and Howard 1978) several hours before use. Initial aggression levels were recorded. The polygyne test colonies were checked daily for dead female sexuals.
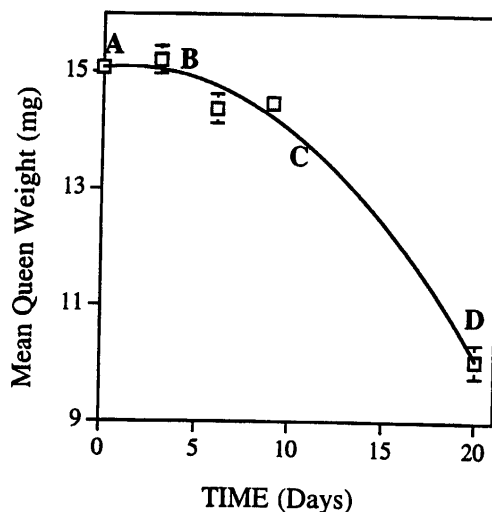

Fig. 1. Second order polynomial plot of the mean weight loss (±SE) of newly mated queens (n = 20) over time and under claustral conditions ($r^2 = 0.987$). Letters indicate the position on the regression line corresponding to the post-mating age of queens used in bioassays. A, newly mated queens = 1–8 h postmating; B, young founding queens = 2–8 d old; C, worker attractant pheromones commence production by day 10 (Glancey et al. 1981); D, old founding queens = 17–20 d postmating.

Results

Newly Mated Queens. Newly mated queens were generally of high weight (mean ± SD, 14.8 ± 0.58 mg; 94.6 ± 27.9 queens per group; n = 5 groups). Another group of newly mated queens collected from the same location were weighed individually (15.08 ± 0.88 mg; n = 120). These newly mated queens were sampled 3, 6, 9, and 20 d after mating. The decrease in their weight as they aged is shown in Fig. 1. Founding queens weighed only 66% of their original weight by 20 d after mating (10.05 ± 0.92 mg). The mean initial weight of the newly mated queens suggested that most of the newly mated queens were derived from monogyne parental nests (Porter and Tschinkel 1986, Porter et al. 1988).

Aggression Toward Newly Mated Queens and Established Polygyne Queens. The introduction of newly mated queens, young founding queens, or old founding queens into either monogyne or polygyne sub-colonies elicited high levels of aggression in all cases (Fig. 2). Attacks on the queens were often not immediate, especially when the intruder responded to contact by remaining motionless. However, eventual movement yielded immediate attacks, accompanied by alarm, recruitment (worker attraction to intruder), and gaster flagging (Obin and Vander Meer 1985). Young founding queens, weighed an average of 14.8 mg (n = 27). Old founding queens weighed an average of 11.4 mg, which fits the weight loss observed in Fig. 1. The 6- to 8-h-old newly mated queens were not weighed, but previously collected newly mated
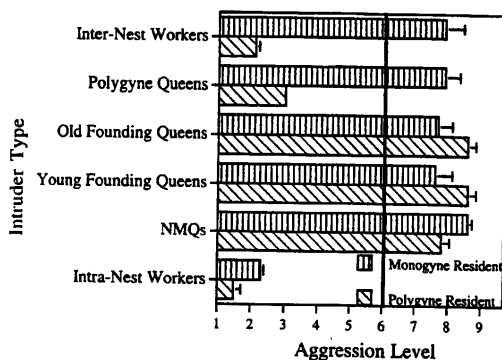
Fig. 2. Mean aggression levels ($n = 16$; ±SE) are shown for the indicated intruder introductions to monogyne and polygyne residents. Intra-nest control: workers were removed from their mother colony and returned in the same manner as intruders. Inter-nest control: worker intruders from non-nestmate colonies were introduced into test colony units of like social form. The vertical line indicates the aggression level that usually leads to mortality.

queens from the same location weighed an average of 15 mg. The reaction of monogyne and polygyne residents to functioning polygyne queen intruders differed (Fig. 2). Polygyne resident workers were highly attracted to the "intruding" polygyne queens and quickly formed an entourage around the queens. The queens walked freely around the bioassay tray with no apparent signs of distress. In contrast, monogyne residents were highly aggressive to introduced polygyne queens; however, worker attack was not immediate if the initial response of the queen was to become motionless. Polygyne queens mutually inhibit each other's egg-laying rate, which is reflected in their ovariole development and weight. The introduced polygyne queens had an average weight of 9.0 mg. Intra-nest control removal and reintroduction of monogyne and polygyne workers back into their parent nest fragment elicited only investigative responses (Fig. 2). Inter-nest controls elicited the expected high aggression in monogyne colonies and investigative behavior in polygyne colonies (Morel et al. 1990). Three samples each of the previously introduced young founding queens, old founding queens, and polygyne queens were randomly selected and checked for insemination. All queens were inseminated.

Olfactometer Bioassays. The olfactometer results are shown in Fig. 3. Neither newly mated queen (poison sacs dissected <8 h after mating flight) nor monogyne-derived alate poison sacs were attractive to workers. However, poison sac extracts from young founding queens, old founding queens, and functional polygyne queens were significantly attractive when analyzed by chi-square with a null hypothesis that equal numbers of workers will go to the two Y-tube choice arms. Poison sacs from young founding queens had the greatest standard error, probably because this is a transitory period where the queens are just beginning to produce the queen attractant pheromone (Glancey et al. 1981).
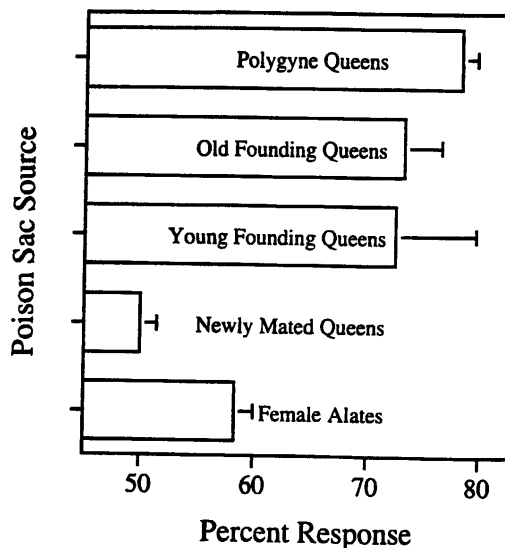

Fig. 3. The mean percent response ($n = 3$; ±SE) are shown for the indicated poison sac extract treatments in an olfactometer bioassay (Vander Meer et al. 1988). A percent response above 65% is significantly attractive (chi-squared test; $\chi^2 > 3.85$, $P < 0.05$, df = 2). Female alates were mature and monogyne-derived. The other queen treatment categories, newly mated, young founding, old founding queens, are defined in Table 2. Mature colony queens were from laboratory maintained polygyne colonies.

Effect of Dealation on Worker Aggression. The effect of mechanical dealation on monogyne and polygyne worker aggression was assessed with mature monogyne alates that had been artificially dealated. Polygyne queens were used as the positive controls. The results are shown in Fig. 4. There were no statistical differences in the response of polygyne residents
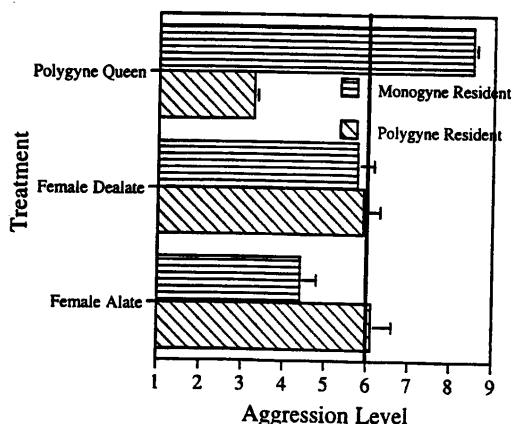

Fig. 4. Mean aggressive response ($n = 16$; ±SE) is shown of monogyne and polygyne residents toward mature female alates, mature female alates that had been artificially dealated, and mature polygyne queens. The vertical line indicates the aggression level that usually leads to mortality.
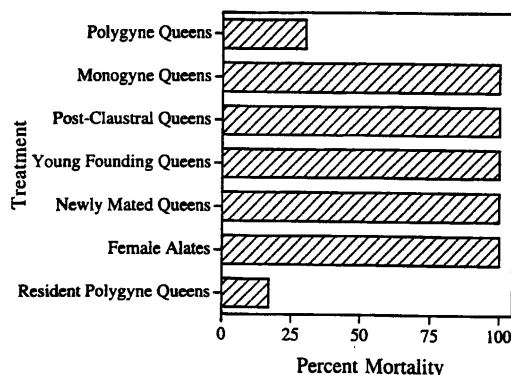
Fig. 5. Percent of the indicated female sexual treatments executed by workers in queenright polygyne colonies ($n = 9$). Female alates are mature. Newly mated queens were introduced less than 2 h after their mating flight; Founding queens were introduced 8 d after their mating flight. Post-claustral queens were introduced 21–30 d after mating. Six highly physogastric monogyne queens were introduced. See methods section for further details.

to alates or dealates (Wilcoxon sign rank test: $Z = -0.392, P = 0.695, n = 16$). However, monogyne workers were significantly more aggressive toward female sexual dealates than they were to the corresponding female alates (Wilcoxon signed rank test: $Z = -2.825$, $P = 0.005, n = 16$). Polygyne residents were tolerant of polygyne queens, with aggression scores reaching only the investigative level (3.25 ± 0.1; see Fig. 4). However, monogyne residents were significantly more aggressive toward polygyne queens than dealates or alates (comparison of treatments with the closest means, polygyne queens versus dealates: $Z = -3.233, P = 0.0012, n = 16$).

Fate of Newly Mated Queens in Queenright Monogyne Colonies. Monogyne resident workers were observed to be immediately aggressive toward introduced newly mated queens, although some newly mated queens did not have contact with workers immediately, because they stayed in the vial cap used for the introduction. After <24 h, five female sexual corpses were recovered from each of the five replicates. The presence of phoretic mites and their state of gaster distension identified the remaining queen in each replicate as the original queen.

Fate of Alates and Several Queen Types Introduced into Queenright Polygyne Colonies. The percent rejection of mature female alates and several developmental categories of postmating queens after introduction into queenright polygyne colonies is shown in Fig. 5. All alates were executed within nine hours of introduction. Similarly, 100% of newly mated queens and 100% of the young founding queens were killed by polygyne colony workers within 18 h of their introduction. All postclaustral queens and physogastric monogyne colony queens were attacked and killed by the polygyne colony workers. The only female sexual group to have any success in surviving introduction into the polygyne colonies were established polygyne

queens from other polygyne colonies; 72% were accepted (34/47) into the polygyne colonies into which they were introduced. High levels of aggression were observed for all intruders except when mature polygyne queens were introduced (Fig. 5). After the 5-wk period required for the serial introduction of the various treatments, 86% of the original colony queens (4 queens × 9 colonies) survived.

Discussion

Nestmate recognition is of prime importance in maintaining colony integration and organization (Wilson 1971, Hölldobler and Michener 1980) and represents a major difference between monogyne and polygyne *S. invicta* (Morel et al. 1990). Nestmate recognition cues consist of odor signals that originate either intrinsically (genetically controlled) or extrinsically (environmentally derived). Environmentally derived cues are acquired from food, nest material, and the general environment; whereas, genetically determined odor signals (discriminators) may originate from either workers, queens, or both (Hölldobler and Michener 1980, Breed and Bennett 1987). These odor cues are present on the cuticle of individuals, because recognition responses are elicited following the sweep of the antennae over the surface of another individual (Wilson 1971). In large colony species, such as *S. invicta*, the individual cue discriminators are distributed to other colony members through grooming and trophallaxis. The cues, heritable and environmentally derived, change over time; therefore, each worker continuously updates its learned cue template (Vander Meer et al. 1989, Provost et al. 1993). If an individual's template does not match the cues of an intruder, then aggression occurs.

Workers from *S. invicta* polygyne nests in the United States show no aggression toward workers from neighboring or even distant conspecific colonies (polygyne or monogyne), although they are highly aggressive toward interspecific intruders (Morel et al. 1990, Vander Meer et al. 1990). Free exchange of workers, food (Bhatkar and Vinson 1987), and probably queens occurs between polygyne *S. invicta* nests within a population, resulting in the formation of one large "supercolony" (Wilson 1971). Both monogyne and polygyne queens are monoandrous (Ross and Fletcher 1985a). The lack of intraspecific aggression between fire ant workers from already established polygyne populations is attributed to the presence of multiple matriline and patriline discriminators, and the distribution of a wide variety of environmentally derived cues that produce a broader worker neural template compared with that expected for the territorial monogyne form (Vander Meer et al. 1990). The difference in conspecific nestmate recognition is a major distinguishing feature of the two *S. invicta* forms in the United States (Morel et al. 1990). We report here an exception to this dichotomy. High levels of aggression are released by newly mated queens from both polygyne and monogyne resident colony work-

ers. These results have interesting implications for our understanding of fire ant nestmate recognition.

The lack of polygyne worker intraspecific aggression has been explained by the broad array of heritable and environmental cues experienced by these workers. Polygyne workers are relatively nonaggressive toward highly aggressive monogyne workers, and what aggression is observed is initiated by the monogyne intruder (Morel et al. 1990). This is consistent with the broad polygyne template hypothesis and suggests that nonaggressive newly mated queens should be readily accepted into polygyne colonies but our results show that they are not. Female alates from both monogyne and polygyne forms are maintained and tolerated in their parental colonies before mating flights. However, unlike the acceptance of monogyne workers introduced into polygyne colonies (Morel et al. 1990), monogyne-derived alates elicited aggressive behavior from workers and were invariably executed. After mating flights, newly mated queens elicited high levels of aggression from monogyne and polygyne worker types and were executed when introduced into monogyne or polygyne colonies. Production of queen-related pheromones in the newly mated queen categories (Table 1) did not moderate the high levels of aggression from workers (Glancey et al. 1981). This is in sharp contrast to polygyne worker/worker interactions. The evidence suggests that female sexuals, most importantly newly mated queens, do not fall into the cue/template scenario developed for worker/worker interactions. Are our behavioral results concordant with what is known about monogyne/polygyne fire ant genetics?

Current genetic evidence derived from a United States polygyne population is complex and suggests the following: (1) queens within a polygyne nest are unrelated (Ross and Fletcher 1985a), (2) >90% of polygyne males are diploid and sterile (Ross and Fletcher 1985b), (3) at least 80% of all polygyne queens mate with monogyne males (Ross 1997), (4) the frequency of polygyne males mating with monogyne females is zero (Shoemaker and Ross 1996), and (5) polygyne fire ant populations are usually derived from an existing polygyne population (Ross and Shoemaker 1997). These data help to define the genetic makeup of existing polygyne populations and their relationship with monogyne populations.

Ross and Keller (1998) found that the protein-encoding gene, Gp-9, could be used as a predictor of queen acceptance. Monogyne queens were invariably of the BB genotype, whereas in polygyne colonies, queens were always genotype Bb. The bb genotype is lethal with potential workers and female alates not surviving to adulthood. The workers and female alates from monogyne colonies are always BB. Worker and female alate progeny from polygyne colonies are Bb, or BB, and Bb, depending on the genotype of the haploid male with which each queen mated. It is expected that female alates and workers from polygyne field colonies will be a mixture of BB and Bb genotypes, because polygyne colonies potentially contain 10s of queens, the population as a whole acts as a "supercolony" (Morel et al. 1990, Wilson 1971), and ≈80% of all polygyne queens (Bb) have mated with monogyne males (B; Ross 1997).

Previous work on nestmate recognition (Morel et al. 1990) established that polygyne workers tolerate each other (genotypes BB and Bb) and do not display aggressive behavior toward monogyne workers (BB). In contrast, monogyne-derived workers (BB) are highly aggressive toward all non-nestmates. Thus, application of Gp-9 genotype information to worker nestmate recognition does not explain the behavioral dichotomy and we fall back to our previous working hypothesis involving differences between the two social forms in their experience-based nestmate recognition template.

Female alates from monogyne and polygyne populations participate in the same mating flights and land together wherever environmental and other factors may dictate. The newly mated queens have to run the predator gauntlet, most important of which are other ant species (Porter et al. 1988), especially workers from established monogyne and polygyne S. invicta colonies. In laboratory experiments using polygyne colonies (Ross and Keller 1998), monogyne- or polygyne-derived newly mated queens of the Gp-9 BB genotype were all executed, and approximately half of the Bb genotype polygyne-derived newly mated queens were executed within 3 d of introduction. Our best estimate of newly mated queen survival rates in polygyne populations comes from field release/recapture experiments where survival has been ≈1% (Glancey and Lofgren 1988, Porter 1991; newly mated queens collected from a polygyne area, S.D.P., unpublished data). There is not enough information on polygyne queen turnover to determine if a 1% newly mated queen survival rate is enough to maintain polygyne populations. In contrast with workers, newly mated queens suffer high rates of intraspecific worker execution regardless of social form or Gp-9 genotype. This can be explained in terms of the drive for existing queens to limit reproductive competition.

Queen-produced primer pheromones of both fire ant social forms generally target the suppression of reproductive/resource competition (Vargo 1998). This takes several forms with S. invicta. One primer pheromone acts on workers and female alates to inhibit ovary development and dealation in female alates (Fletcher and Blum 1981). Another queen-primer pheromone inhibits the production of new female sexuals through an effect on caste determination (Vargo and Fletcher 1986a, 1986b; Vargo 1988). In polygyne colonies, queens mutually suppress each other's egg-laying rate (Obin et al. 1988, Morel et al. 1990, Vargo 1992). Once the female alates leave the nest on mating flights they are released from the influence of the colony queen(s), and the resulting newly mated queens falling into either population type now represent yet another form of competition for the existing colony queen(s).

Whether investigated through nestmate recognition or via genetic correlates, the dichotomy in worker/worker interactions between monogyne and poly-

gyne populations exists as does the exception we highlight here when newly mated queens are considered. Mechanisms for the initiation and the maintenance of polygyne colonies require newly mated queen adoption. We demonstrate in a subsequent article (R.K.V.M., unpublished data) the powerful effect of monogyne and polygyne fire ant queens on conspecific aggression. This provides an important behavioral link to the genetic selection of newly mated queens and offers plausible alternative mechanisms for initiation and maintenance of polygyne fire ant populations.

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