Behavioral Ecology Vol. 10 No. 4: 428–435

Queen recruitment in a multiple-queen population of the fire ant *Solenopsis invicta*

Michael A. D. Goodisman[a] and Kenneth G. Ross[b]
[a]Department of Genetics and [b]Department of Entomology, University of Georgia, Athens, GA 30602-7223, USA

We assessed patterns of new queen recruitment in a polygyne (multiple queens per nest) population of the fire ant *Solenopsis invicta* in its introduced range. Newly recruited queens were identified using four physiological markers, and genotypic data from nuclear and mitochondrial markers were used to estimate relatedness of new nest mate queens to each other and to the older nest mate queens. In total, 1.7% of the queens collected in late spring and early summer were deemed to be new recruits. The relatedness of these queens to each other and to the older queens within nests was not significantly different from zero, suggesting that newly recruited queens represent a random sample of potential reproductive queens in the population. Moreover, the number of new queens recruited within nests was not correlated with the number of older queens present and did not differ significantly from a Poisson distribution. Thus, queen recruitment in this population of *S. invicta* appears to occur at random with respect to the number of older queens present within nests. *Key words:* fire ants, polygyny, relatedness, social insects, *Solenopsis invicta*. *[Behav Ecol 10:428–435 (1999)]*

Multiple reproductive queens frequently coexist within single social insect colonies (Bourke and Franks, 1995; Crozier and Pamilo, 1996; Hölldobler and Wilson, 1990; Keller, 1993; Ross and Carpenter, 1991). These multiple-queen (polygyne) colonies differ substantially from conspecific colonies headed by a single reproductive queen (monogyne colonies). In ants, for example, queen fecundity, dispersal, and longevity, as well as nest mate relatedness, generally are reduced in polygyne colonies (Bourke and Franks, 1995; Crozier and Pamilo, 1996; Hölldobler and Wilson, 1990). Knowledge of the proximate mechanisms by which multiple queens come to inhabit a single nest is integral to understanding the factors that lead to these important fitness-related differences between the two types of colonies.

Most studies investigating new queen recruitment have used queen introductions under laboratory conditions (e.g., Fletcher and Blum, 1983; Keller and Ross, 1993a; Stuart et al., 1993; Sundström, 1997). These studies revealed that factors such as queen mating status and nest of origin can be important in determining queen acceptance. However, laboratory conditions do not necessarily mimic those found in the field and thus may not always accurately represent natural processes. Field analyses of queen recruitment, by circumventing this problem, offer a rarely explored but valuable avenue of investigation into the dynamics of polygyne colony sociogenesis.

The polygyne form of the introduced fire ant *Solenopsis invicta* is an excellent candidate for assessing patterns of queen recruitment under natural conditions. The social biology of *S. invicta* has been well studied (Hölldobler and Wilson, 1990; Ross, 1993; Ross and Keller, 1995a; Vinson, 1997), facilitating the design and interpretation of meaningful field studies. Moreover, the high colony queen number observed in introduced polygyne populations (Goodisman and Ross, 1997; Ross, 1993; Vargo and Fletcher, 1987) implies that queen recruitment occurs at a fairly high rate and thus newly recruited queens may be detectable under natural conditions (e.g., Glancey and Lofgren, 1988).

This study had three main goals. The first was to estimate the frequency of new queen recruitment in an introduced, polygyne population of *S. invicta*. This information is critical to understanding the patterns of polygyne colony growth and may be used to estimate the life span of polygyne queens. This second variable is particularly important to the study of queen number in ants because the recruitment of multiple queens is often associated with a decrease in queen longevity (Bourke and Franks, 1995; Keller and Genoud, 1997; Hölldobler and Wilson, 1990). Estimates of queen longevity in monogyne *S. invicta* populations already exist (Tschinkel, 1987), and a corresponding estimate for polygyne queens would be of considerable interest.

The second goal of this study was to estimate the relatedness of newly recruited queens to each other and to the older queens within nests. Previous studies of nest mate relatedness in introduced polygyne populations of *S. invicta* revealed that queen relatedness was statistically indistinguishable from zero (Goodisman and Ross, 1997, 1998; Ross, 1993; Ross and Fletcher, 1985). Nevertheless, the relatedness of newly recruited queens may differ from that of older queens. For instance, elevated relatedness of newly recruited queens would signal that they were not drawn at random from the pool of potential reproductives in the population but were the offspring of one or a few older queens in the colony. Moreover, this result would suggest that differential queen mortality based on relatedness occurs during recruitment. This study attempts to detect such features by comparing the relatedness of newly recruited queens to that of older queens within the same colonies.

The final objective of this investigation was to describe colony-level patterns of new queen recruitment. The relationship between the number of new queens and the number of older queens within nests, as well as the distribution of newly accepted queens across nests, may yield insights into the mechanisms governing the recruitment process (Crozier and Pamilo, 1996; Nonacs, 1988; Pamilo, 1991). This study explicitly examines these important patterns under natural conditions.

Address correspondence to M. A. D. Goodisman, who is now at the Department of Genetics, La Trobe University, Bundoora, VIC, 3083, Australia. E-mail:madgood@gen.latrobe.edu.au.

Received 11 May 1998; revised 16 December 1998; accepted 6 January 1999.

© 1999 International Society for Behavioral Ecology

METHODS

Development of markers

To detect newly recruited queens (NRQs) within nests, it was first necessary to identify markers that distinguish NRQs from older queens. To find such markers, we compared 114 alate (winged), prereproductive queens with 45 dealate (wingless), egg-laying queens (wing shedding is associated with the onset of reproduction; Vargo and Laurel, 1994). These ants originated from seven previously collected polygyne nests that had been kept in the laboratory for at least 1 week and were not used in other parts of this study. Because queens undergo substantial physiological changes as they become reproductively active (Hölldobler and Wilson, 1990; Toom et al., 1976a,b,c; Vander Meer et al., 1982), we reasoned that NRQs would have characteristics intermediate between alate and dealate queens. In total, we identified four markers useful in identifying NRQs.

Activity at glycerol-3-phosphate dehydrogenase-1 *locus*
The enzyme product of the locus *glycerol-3-phosphate dehydrogenase-1* (*G3pdh–1*) supports flight in *S. invicta,* and its activity diminishes after alate queens shed their wings (Hung et al., 1979). We assayed the activity of this enzyme visually, using protein electrophoresis and standard staining techniques on samples of thoracic tissue from queens (Shoemaker et al., 1992). The banding patterns of alates became discernible approximately 30 s after adding the requisite stain ingredients, and genotypes could be scored readily 5–10 min later. The enzyme activity of dealate queens was not nearly as strong. Bands could first be seen 15 min after staining and were just strong enough to score reliably after 45 min. The intensity of staining for *G3pdh–1* in dealates never reached the level of that in alates, even after the gels were allowed to stain for more than 2 h. Thus, we expected *G3pdh–1* band intensity in NRQs to be substantially stronger than in older, dealate queens.

Banding pattern from general protein stain
Protein electrophoresis on thoracic tissue revealed another marker that differentiated alate and dealate queens. Using a nonspecific general protein stain (Shoemaker et al., 1992), we identified a single band, hereafter referred to as the ''alate protein band,'' that consistently appeared in the banding profiles of alate queens but never in those of older dealate queens. We expected the banding profile of NRQs to contain this band, although its intensity may be reduced relative to that seen in alate queens that have not flown or initiated oogenesis.

Condition of wing muscles
Ant queens histolyze their flight muscles to provide a source of energy when initiating reproduction (Hölldobler and Wilson, 1990). The result of this process can be seen in *S. invicta* by inspecting the thoracic cavity of queens (Glancey et al., 1980; Markin et al., 1972). The wing muscles of alate queens comprise visible red fibrous strands. In contrast, the thoraces of dealate queens contain a puffy, whitish substance, with little evidence of muscle fibers. We expected NRQs to show slightly histolyzed wing muscles.

Fat content of gasters
Ant queens accumulate fat after they eclose. This fat serves as an energy source and is metabolized by the queens as they initiate reproduction (Keller and Passera, 1989). We used standard methods (Keller and Passera, 1988, 1989; Keller and Ross, 1993b) to measure the mass of stored fat in the gasters (abdomen minus propodeum) of alate and dealate *S. invicta* queens. Gasters were dried for 24 h and weighed. Fat was then extracted from the gasters with 4–5 ml of petroleum ether (boiling point 40–60°C), and the gasters were dried and weighed again. The difference between the weights before and after ether extraction was taken to be the mass of stored fat in the gaster.

Alate queen gasters contained 1.20 ± 0.05 ($\bar{x}$ ± SEM) mg of fat. [This value is lower than previously reported for *S. invicta* alate queens (Keller and Ross, 1993b), perhaps because the individuals used here had not matured fully.] In contrast, fat accounted for only 0.29±0.03 mg in dealate queen gasters. We found that a weight-standardized measure of gaster fat content, defined as (mass of gaster before extraction − mass of gaster after extraction)/(mass of gaster before extraction), yielded substantial power in distinguishing the two classes of queens. After standardization, the means for alate and dealate queen gaster fat content transform to 0.49 ± 0.01 and 0.16 ± 0.01, respectively, and differ significantly from one another ($F_{1,158} = 295.61$, $p < .0001$). We anticipated that NRQs would show transformed fat content values slightly below those of alate queens but still substantially greater than older, dealate queens.

Time course of change in NRQ markers

We wished to assess the time span over which NRQs could be identified within nests. That is, how long would these individuals bear the above markings and be distinguishable from the older queens? To do this, we first collected 155 polygyne dealate queens after mating flights (DeHeer et al., 1999) and put them into culture in the laboratory (as in Bernasconi and Keller, 1996). Queens were isolated in groups of about 10, and provided with food, water, and approximately 30 worker pupae. We recognize that this scenario may differ from the context in which polygyne queens normally initiate reproduction (Glancey and Lofgren, 1988; Porter, 1991; Vargo and Porter, 1989), however, introduction of queens directly into polygyne nests was not an option because the majority of queens are eliminated by workers under laboratory conditions (Keller and Ross, 1995). Queens were harvested over the course of the next 15 days and scored for the 4 characteristics described above to monitor the change of the markers over time. Two queens died over the course of the study and were not included in the analysis.

Collection of samples

We collected 92 *S. invicta* nests on 5 different days from 6 sites separated by no more than 2 km in Walton County, Georgia, USA. To maximize the probability of discovering NRQs, nests were collected in the late spring and early summer, which apparently represents the major period of mating activity and queen recruitment in introduced *S. invicta* (Glancey and Lofgren, 1988; Vinson and Greenberg, 1986). To capture the majority of queens within nests, collecting took place on warm, sunny days that were preceded by rainfall. Nests were placed in buckets, and the inhabitants were separated from the soil by flooding (Jouvenaz et al., 1977) and placed into large trays. Within 48 h, all dealate queens were collected by searching through the ants in the trays. Nests were considered to be polygyne if they contained two or more dealate queens.

Analysis of queens

A large proportion of queens remains permanently unmated in introduced polygyne *S. invicta* populations (Ross, 1993; Ross and Keller, 1995b). Therefore, we determined the mating status of queens by examining queen spermathecae.

Sperm in mated queens was evident as an opaque, whitish mass. All queens were scored for activity at *G3pdh–1,* presence/absence of the alate protein band, evidence of flight muscles, and standardized fat content, as described above. Queens that matched expectations derived from the alate/dealate comparisons and time course experiments for these markers were considered to be NRQs.

We determined the multilocus genotypes of queens electrophoretically at three polymorphic nuclear loci (*Aat-2, Gp-9,* and *Pgm-1*) in addition to *G3pdh–1* (Ross, 1997; Ross and Shoemaker, 1997; Shoemaker et al., 1992). Whereas the loci *Aat-2, Pgm-1,* and *G3pdh–1* presumably are neutral (Shoemaker et al., 1992), *Gp-9* (or a locus closely linked to it) apparently is under strong selection in the polygyne form of *S. invicta* (Ross, 1997). Alate queens and workers display all three possible genotypes at this diallelic locus, but reproductive queens are virtually always (99.9%) heterozygous (Ross, 1997). Therefore, any putative NRQs that were homozygous at *Gp-9* were excluded from analysis.

Queen genotypes also were assayed at a 4-kb region of the mitochondrial DNA (mtDNA) (Ross and Shoemaker, 1997). The mtDNA haplotype was obtained by amplification with polymerase chain reaction followed by digestion with the enzyme *Hin*fI, which differentiates among the three haplotypes (two common and one rare) found in this polygyne population (Ross and Shoemaker, 1997; Shoemaker and Ross, 1996).

Genotypic data from the three neutral nuclear loci (*Aat-2, Pgm-1,* and *G3pdh–1*) and the mtDNA were used in conjunction with the program Relatedness 4.2 (Queller and Goodnight, 1989) to calculate the relatedness of nest mate queens. Genetic differentiation among sites was accounted for by using the "deme" function of the program. We used all captured queens to calculate the population allele frequencies (P^*), and groups (nests) were always weighted equally. Standard errors for the relatedness estimates were obtained by jackknifing over nests; a relatedness value was considered to be statistically different from zero if the 95% confidence intervals of the estimate ($r \pm 1.96 \times$ SEM) did not overlap zero.

Within-nest correlations between the number of NRQs and the number of older queens were calculated using Spearman's correlation coefficient (r_S). Significance of differences in number of NRQs among sites and among days was assessed with a G test of independence. A Kolmogorov-Smirnov goodness-of-fit test was used to determine if the numbers of new queens accepted into nests differed from a Poisson distribution.

RESULTS

Time course of change in NRQ markers

Activity of the product of *G3pdh–1* in our experimental queens was noticeably greater than that of egg-laying, dealate controls for the first 7 days after mating. These results are consistent with previous studies of this enzyme in *S. invicta* (Hung et al., 1979). The alate protein band showed a similar decay pattern and was visible in queens through day 6 of isolation. The wing muscles of experimental queens were notably different from those of mature, dealate queens for approximately 5 days after the mating flight. Subsequently, the contents of the thoracic cavity varied and became difficult to consistently distinguish from dealate controls. The rate of histolysis in this study apparently was slightly greater than that reported in earlier studies (Glancey et al., 1980; Markin et al., 1972). This difference may be attributable to the fact that polygyne queens were used in this experiment, whereas monogyne queens were used in the earlier analyses.

Standardized fat content decreased linearly during the time-course experiment ($r^2 = .20$, $F_{1, 151} = 38.56$, $p < .0001$), with the relationship between fat content and time described by the equation: fat content $= -0.013 \times$ day $+ 0.507$. An average experimental queen reached the upper 95% limit ($\bar{x} + 1.96 \times \text{SD} = 0.32$) for the approximately normal distribution of fat content in older dealate queens in about 14 days. However, if the linear trend continued, the mean of the experimental queens would not equal that of the dealate controls (0.16) for approximately 27 days.

We found no noticeable differences in the rate of decay of the four markers between mated and unmated queens (data not shown). When considering all the markers together, we conclude that NRQs can be differentiated from mature queens for roughly 1 week after shedding their wings and initiating reproduction.

Table 1
Numbers of nests, total numbers of queens, and numbers of newly recruited queens (NRQs) collected from six sites in a single polygyne population of *S. invicta* in northern Georgia

Site	Date collected (1996)	Nests	Queens	
			Total	NRQs (proportion of total)
1	May 29	16	395	4 (0.010)
2	May 29	15	595	18 (0.030)
3	May 30	11	397	2 (0.005)
4	May 30, June 12	23	431	3 (0.007)
5	July 16	10	165	9 (0.055)
6	July 18	10	614	7 (0.011)
All sites		85	2597	43 (0.017)

Distribution and characteristics of nests and queens

Of the 92 nests collected, 85 were considered to be polygyne, whereas the remaining 7 contained either 1 or no queens and thus were not included in other analyses. Table 1 shows the sampling dates, number of nests, and number of queens collected from each site and the entire population. Queen number per nest varied widely (mean = 30.55, SD = 41.46, range 2–257), although it did not differ significantly among sites ($F_{5, 79} = 2.08$, $p > .05$). Overall, the frequency of mated queens (0.488) was relatively low, but close to that observed at a nearby site in a previous study (Ross and Keller, 1995b).

Distribution and characteristics of NRQs

In total, 45 queens from 25 of the nests showed characteristics consistent with being NRQs. However, two of these individuals were homozygous at the locus *Gp-9* and thus would not have become permanent reproductives (Ross, 1997); therefore, they were eliminated from further study, leaving a total of 43 NRQs from 24 nests.

NRQs accounted for 1.7% of all queens collected. Only 5 of the 43 NRQs (11.6%) could be confirmed as having mated, a proportion that is significantly lower than for the older queens in the population ($z = 63.49$, $p < .0001$). Another notable point is that NRQs were significantly lighter than the mature queens in our sample (two-way ANOVA: colony, $F_{84, 2487} = 16.39$, $p < .0001$; queen status, $F_{1, 2487} = 36.79$, $p < .0001$; interaction, $F_{23, 2487} = 1.36$, $p > .1$). This result suggests that the fecundity of mature, reproductive queens increases after the recruitment period or that lighter NRQs undergo higher rates of mortality after initial acceptance.

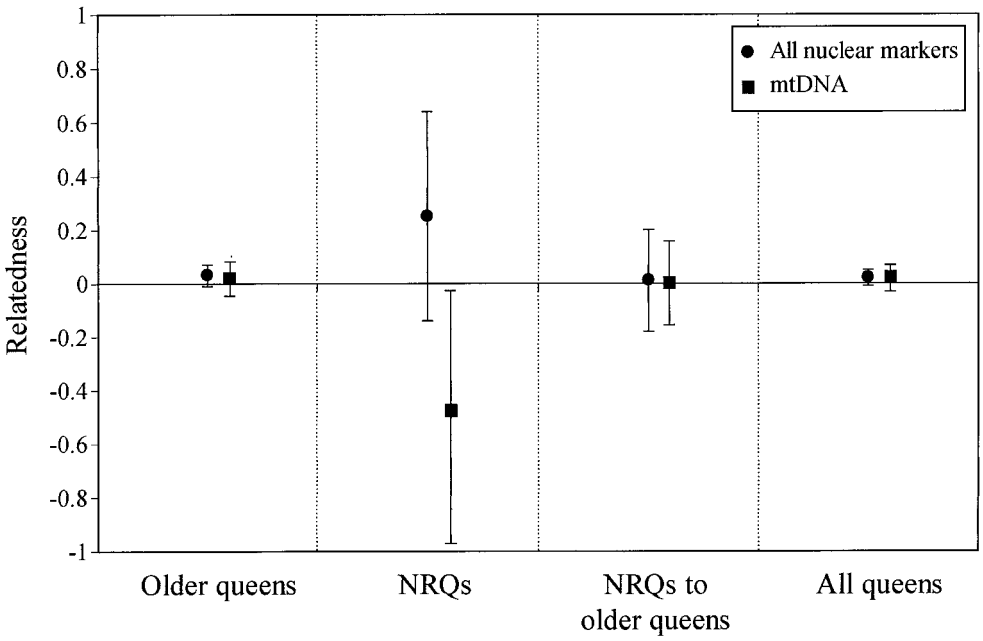

Figure 1
Nest mate queen relatedness calculated with nuclear and mitochondrial markers. Estimates are for older queens to each other, newly recruited queens (NRQs) to each other, NRQs to older queens, and all queens combined. The error bars delimit the 95% confidence intervals around the relatedness estimates.

Relatedness of nest mate queens
In accord with previous results (Goodisman and Ross, 1997; Ross, 1993; Ross and Fletcher, 1985), the relatedness of the older nest mate queens was indistinguishable from zero (95% confidence intervals overlap with zero) when either nuclear or mitochondrial markers are considered (Figure 1). Of specific interest for this study, however, are the relatedness estimates of the NRQs to each other and to the older queens. Except for the marginally significant, negative mitochondrial estimate for NRQs, these values do not differ significantly from zero.

Patterns of new queen recruitment
The proportions of NRQs differed among the six sites (G test of independence, $G_5 = 24.53$, $p < .001$) and among the 5 days on which samples were collected (G test of independence, $G_4 = 23.82$, $p < .001$). Because nests were collected from most of the sites on only 1 day, the effects of site and day are confounded and cannot be distinguished from one another. However, there is no obvious trend between proportion of NRQs and day collected (Table 1).

Spearman's correlation coefficient was used to investigate the relationship between the number of older queens and number of NRQs within nests (Figure 2). The correlation between the two variables is not significant if all nests are included in the analysis ($r_S = .004$, $n = 85$, $p > .5$) nor if only nests that recruited new queens are considered ($r_S = -.125$, $n = 24$, $p > .5$). Thus, our data cannot reject the hypothesis that NRQs are recruited into nests at random with respect to the number of older queens present. We also tested whether the distribution of NRQs per colony differed from that expected under a Poisson distribution. A total of 61, 11, 10, 2, 0, 0, and 1 nests accepted 0, 1, 2, 3, 4, 5, and 6 NRQs, respectively. This distribution does not differ significantly from a Poisson distribution with a mean of 0.51 (Kolmogorov-Smirnov goodness of fit test, $z = 1.06$, $p > .2$).

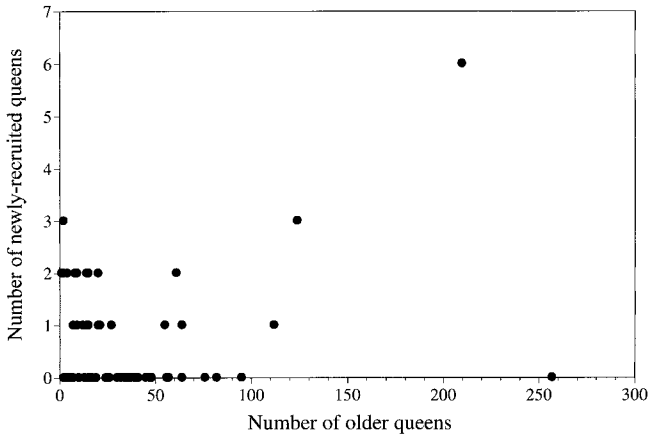

Figure 2
Relationship between number of older queens and number of newly recruited queens (NRQs) in 85 nests from a single polygyne population of *S. invicta*. The correlation between the two variables does not differ significantly from zero ($r_S = .004$, $p > .5$).

Confirmation of NRQ status

The markers developed to identify NRQs would likely misidentify as legitimate NRQs prereproductive queens that had lost their wings accidentally due to the trauma of the collection process. These "traumatic dealates," if present in substantial numbers, could substantially alter our conclusions regarding queen recruitment. Three tests can be conducted to evaluate the importance of this potential problem.

Standardized fat content of gasters
If the sample of NRQs includes a large proportion of traumatic dealates, then the standardized fat content of the sample should approach that of alate controls. However, if the putative NRQs actually are new reproductive queens, then the standardized fat content of their gasters should be significantly less than that of the alate controls. We found that the mean standardized fat content of the NRQs (0.38 ± 0.01) is significantly below that of alate controls (0.49 ± 0.01, $t_{155} = 5.69$, $p < .0001$) and significantly above that of dealate controls (0.16 ± 0.01, $t_{87} = 55.88$, $p < .0001$). This intermediate fat content is consistent with the NRQ designation.

Relatedness of NRQs

Nest mate alates in introduced polygyne *S. invicta* populations exhibit significant positive relatedness to one another (Ross, 1993). Therefore, if a large fraction of putative NRQs in our sample were traumatic dealates, estimates of relatedness between NRQs would be expected to be greater than zero. Moreover, significant positive relatedness of traumatic dealates to the older, nest mate queens that would have produced them might also be expected. In contrast, if the individuals we identified as NRQs are indeed new queens, then their relatedness to each other and to the older queens would be expected to be indistinguishable from zero, as has repeatedly been found to be the case for reproductive queens in this population (Goodisman and Ross, 1997, 1998; Ross, 1993; Ross and Fletcher, 1985). From Figure 1 we see that none of the nestmate queen relatedness estimates involving NRQs are significantly greater than zero. Thus, these relatedness estimates are consistent with the NRQ designation.

Frequency of wing breakage

To assess the probability of traumatic dealation during the collecting process, 583 alate queens that were incidentally collected from 8 of the nests for this study were examined to see if one or more of their 4 wings were missing. If traumatic dealation occurred frequently, then many of these alates would have fewer than four wings.

Inspection of the alates revealed that 571, 10, 2, and 0 had lost 0, 1, 2, and 3 of their wings, respectively. Using maximum likelihood methods, we found that the distribution of wing breakage matches a binomial distribution (exact test, $p > .3$) where the probability of losing any single wing is 0.006. Thus, the probability of a queen losing all four of her wings through traumatic dealation is $(0.006)^4 = 1.3\times10^{-9}$. It therefore is unlikely that any of the queens designated as NRQs were traumatic dealates.

DISCUSSION

We studied the process of queen recruitment in a wild population of introduced polygyne *S. invicta*. Newly recruited queens constituted 1.7% of the total number of queens sampled during late spring and early summer. Moreover, the relatedness of the NRQs to one another and to the older queens in their nests was statistically indistinguishable from zero when both nuclear and mitochondrial markers were considered. We found no significant correlation between the number of NRQs and the number of older queens within nests, and the distribution of new queens within nests fit a Poisson distribution. Thus, we conclude that NRQs are accepted into nests at random with respect to existing queen number.

Frequency of recruitment and queen longevity

The frequency of NRQs observed in this study can be used to estimate queen longevity within natural polygyne populations. Such calculations require simplifying assumptions and therefore should be taken as providing only rough estimates. We first suppose that our study population is at equilibrium with respect to recruitment rate and queen number. We next assume that all NRQs replace queens that died and that mortality follows an exponential distribution (Lee, 1992). From our laboratory studies, NRQs could be identified for approximately 1 week, and so 1.7% of the queens within this population die per week. If this rate is constant throughout the year, then the mean life span of a polygyne *S. invicta* queen is 58.8 (1/0.017) weeks, or approximately 1 year. However, if recruitment only occurs during the period of substantial alate production, which lasts from May through September in this population (Vargo and Fletcher, 1987), then the expected life span increases to almost 3 years. Our results are consistent with a previous laboratory study of polygyne queens in this species. Ross (1988) found that approximately 40% of polygyne queens established in laboratory colonies died in less than 1 year. If mortality occurred at a constant rate, then the life expectancy of polygyne *S. invicta* queens from this study is $1/0.4 = 2.5$ years.

Tschinkel (1987) estimated the typical life span of successful colony-founding monogyne *S. invicta* queens as ranging from 5 to 7 years. The lower estimated life span of polygyne queens in this species conforms to previous results from other species (Keller and Genoud, 1997; Keller and Passera, 1990) and expectations under life-history theory; polygyne queens likely produce sexual offspring at an earlier age than monogyne queens, and a shorter life span is generally associated with earlier reproduction (Bourke and Franks, 1995, Keller and Genoud, 1997).

Relatedness of NRQs

Our relatedness estimates for NRQs indicate that they are unrelated to each other and to the older queens in the nest. These results are consistent with previous studies in polygyne *S. invicta* in the introduced range that showed that nest mate queens are no more closely related to one another than they are to queens in other nests (Goodisman and Ross, 1997, 1998; Ross, 1993; Ross and Fletcher, 1985; Ross et al., 1996). Thus, the NRQs appear to represent a random sample of the potential reproductive queens in the population.

A laboratory study of *Formica truncorum* (Sundström, 1997) and a field study of *F. lugubris* (Fortelius et al., 1993) reported that polygyne workers do not discriminate between nest mate and non–nest-mate females that are introduced into colonies. However, polygyne queens of *F. truncorum* are related (Sundström, 1993), indicating that such discrimination may occur under natural conditions or that queen movement between nests is substantially restricted. A similar experiment in polygyne *Leptothorax curvispinosus* found that queens were more likely to be adopted if they were introduced into their natal nest (Stuart et al., 1993). Like *F. truncorum,* nest mate queens in this species tend to be related (Stuart et al., 1993), and thus the recruitment process likely displays substantial differences from that in the introduced fire ant.

Patterns of recruitment

The patterns of queen recruitment observed in this study may be used to test hypotheses concerning the development of polygyne nests. For instance, if queen number within each nest remains approximately the same over time, then we would expect a negative relationship between the number of NRQs and number of older queens within nests. This correlation should arise because all nests would only be replacing queens that had died, and nests with many queens would need to replace more queens than those with fewer queens. However, our data do not reflect this pattern. Rather, our results are consistent with nests accepting NRQs at random with respect to the number of older queens present. Thus, queen number within nests appears to be subject to variation over time.

It is somewhat surprising that the number of older queens within nests does not influence new queen recruitment. Levels of queen pheromones within polygyne *S. invicta* nests, which appear to be related to queen number, play otherwise important roles in the regulation of reproduction. For example, queen-derived pheromones suppress the production of sexuals, the initiation of reproduction by virgin queens, and

queen fecundity (Vargo, 1992; Vargo and Fletcher, 1986a,b, 1987, 1989; Vargo and Laurel, 1994). Therefore, it might be expected that the high levels of pheromone associated with many queens would induce workers to reject additional queens. If recruitment in introduced polygyne *S. invicta* nests occurs at random, then queen number within nests potentially can increase without bound, explaining the high queen numbers observed in some nests in older polygyne populations (Goodisman and Ross, 1997, this study; Ross, 1993; Ross et al., 1996).

The fraction of NRQs within our study nests did not show any consistent change over the course of the reproductive season (Table 1). This finding differs from that of Fortelius et al. (1993), who noted that queen recruitment in *F. lugubris* decreased through the flight season. It is possible that the recruitment biology of *S. invicta* differs from *F. lugubris*. Also, this study may have detected too few NRQs or extended over an insufficient period of time to discern any seasonal pattern.

Matedness in NRQs

A notable feature of our data set is that older queens were mated significantly more often than were NRQs. One possible explanation for this result is that unmated queens are more successful at entering polygyne nests than mated queens. Indeed, two studies of polygyne *Formica* ants reported such a result (Fortelius et al., 1993; Sundström, 1997). However, if polygyne *S. invicta* nests accepted and retained unmated and mated queens at the ratio observed in the new recruits, then the proportion of older mated queens in the population should be substantially lower than is observed.

It is possible that unmated NRQs mate intranidally after entering nests. However, the proportion of mated queens collected on the ground immediately after mating flights in this study population is similar to the proportion of mated, older queens within nests, suggesting that most mating occurs during the flight and not after queens enter an established colony (Goodisman M, DeHeer C, Ross K, unpublished data).

Another explanation proposes that workers eliminate mated and unmated queens at different rates (most queens attempting to enter polygyne *S. invicta* nests are killed; Keller and Ross, 1993a). That is, many of the mated queens that attempt to enter nests are rapidly eliminated, and many of the unmated queens initially observed also would be killed over time. A related hypothesis is that the markers we used to detect NRQs decay more slowly in unmated queens than in mated queens. Thus, newly recruited mated queens become indistinguishable from older queens sooner than their unmated counterparts. Such hypotheses are supported by studies that show that mating helps trigger physiological and behavioral changes that mark the onset of oogenesis (Cupp et al., 1973; Toom et al., 1976b). However, our experimental assessment of the rate of decay of markers did not reveal any notable differences between mated and unmated queens (data not shown).

Comparison of recruitment patterns to those found in a previous study

The results of this study differ substantially from those of a related study in a different introduced population of polygyne *S. invicta*. Glancey and Lofgren (1988) found that 10 of 16 confirmed polygyne nests recruited new queens, and 51.0% of the total queens showed characteristics consistent with being NRQs. This proportion of NRQs is significantly greater than that found in our study (1.7%, $z = 30.19$, $p < .0001$). The contrasting results are not likely due to variation in the reliability of markers for new queens in the two studies because a common marker was used, nor are they likely due to differences in season of sampling because collections were made in the late spring in both cases. Rather, the contrasting proportions of NRQs detected may be due to differences in such demographic features as the density or age of nests in the two populations.

Despite the difference in the proportion of NRQs observed, this previous study displays important qualitative consistencies with some results from the present investigation. For instance, the correlation between the number of older queens present in polygyne nests and the number of NRQs does not differ significantly from zero ($r_S = -.022$, $n = 16$, $p > .9$) for the Glancey and Lofgren data. Moreover, although the distribution of NRQs among nests in the Glancey and Lofgren study differs significantly from a Poisson distribution (Kolmogorov-Smirnov $z = 1.96$, $p < .001$), it does not differ significantly from a normal distribution ($z = 1.27$, $p > .05$) with estimated mean and standard deviation of 6.19 and 12.76, respectively. The fit of number of NRQs in nests to random distributions (the Poisson or the normal) in the two studies is consistent with the hypothesis that queens in both populations may be entering nests at random and without regard to the number of older queens present.

Conclusions

Three hypotheses have been put forward to explain the acceptance of multiple queens into established social insect colonies: mutualism, kin selection, and parasitism (Crozier and Pamilo, 1996; Nonacs, 1988; Pamilo, 1991). Under the mutualistic hypothesis, cooperation leads to an increase in the personal fitness of the NRQs and the older queens within nests. Although our data cannot formally reject this hypothesis, mutualism probably cannot explain queen recruitment in polygyne colonies in introduced *S. invicta* because increasing queen number decreases both queen reproduction (Vargo, 1992; Vargo and Fletcher, 1986b, 1987, 1989) and queen longevity (Bourke and Franks, 1995; Keller and Genoud, 1997; Tschinkel, 1987; this study). Kin selection hypotheses also are unlikely to explain the acceptance of multiple queens in introduced populations. In contrast to native South American populations (Ross et al., 1996), queens within introduced polygyne *S. invicta* nests are statistically unrelated to one another (Goodisman and Ross, 1997, 1998; Ross and Fletcher, 1985; Ross, 1993), and so existing queens likely gain no fitness benefits by allowing new queens to enter. In addition, workers in introduced populations do not appear to favor related sexuals in their nest (DeHeer and Ross, 1997), so that nepotism does not seem to rescue inclusive fitness benefits for polygyne workers.

Parasitism of preexisting nests by unrelated queens appears to be the best explanation for the acceptance of multiple queens in introduced polygyne populations of *S. invicta*. The density of nests in North American populations greatly exceeds that in native South American populations (Porter et al., 1992, 1997). This change may have led to the occupation of most available *S. invicta* nesting sites, which would in turn create selective pressure on prereproductive queens to enter established nests (Crozier and Pamilo, 1996; Elmes, 1973; Herbers, 1986, 1993; Nonacs, 1988; Pamilo, 1991; Rosengren et al., 1993; Ross et al., 1996). The number of queens attempting to enter nests may be so high that recognition cues become confused and workers are unable to prevent many queens from joining the colony. Therefore, in this species, a proximate environmental change appears to have dramatically altered the causes for the acceptance of multiple reproductive queens.

We thank M. Chapuisat, C. DeHeer, J. Evans, M. Mescher, D. Promislow, and two anonymous reviewers for helpful discussion and comments on an earlier draft, and D. Promislow for use of the analytical balance. This research was supported in part by Environmental Protection Agency Graduate Fellowship U-914967-01-0 (to M.A.D.G.), the Georgia Agricultural Experiment Stations of the University of Georgia, and the National Geographic Society.

REFERENCES

Bernasconi G, Keller L, 1996. Reproductive conflicts in cooperative associations of fire ant queens (*Solenopsis invicta*). Proc R Soc Lond B 263:509–513.

Bourke AFG, Franks NR, 1995. Social evolution in ants. Princeton, New Jersey: Princeton University Press.

Crozier RH, Pamilo P, 1996. Evolution of social insect colonies: sex allocation and kin selection. Oxford: Oxford University Press.

Cupp EW, O'Neal J, Kearney G, Markin GP, 1973. Forced copulation of imported fire ant reproductives. Ann Entomol Soc Am 66:743–745.

DeHeer CJ, Goodisman MAD, Ross KG, 1999. Simple genetic basis for a dispersal polymorphism in polygyne *Solenopsis invicta*. Am Nat (in press).

DeHeer CJ, Ross KG, 1997. Lack of detectable nepotism in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 40:27–33.

Elmes GW, 1973. Observations on the density of queens in natural colonies of *Myrmica rubra* L. (Hymenoptera: Formicidae). J Anim Ecol 42:761–771.

Fletcher DJC, Blum M, 1983. Regulation of queen number by workers in colonies of social insects. Science 219:312–314.

Fortelius W, Rosengren R, Cherix D, Chautems D, 1993. Queen recruitment in a highly polygynous supercolony of *Formica lugubris* (Hymenoptera, Formicidae). Oikos 67:193–200.

Glancey BM, Glover A, Lofgren CS, 1980. The thoracic crop in *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Sociobiology 5: 272–278.

Glancey BM, Lofgren CS, 1988. Adoption of newly-mated queens: a mechanism for proliferation and perpetuation of polygynous red imported fire ants, *Solenopsis invicta* Buren. Fla Entomol 71:581–587.

Goodisman MAD, Ross KG, 1997. Relationship of queen number and queen relatedness in multiple-queen colonies of the fire ant *Solenopsis invicta*. Ecol Entomol 22:150–157.

Goodisman MAD, Ross KG, 1998. A test of queen recruitment models using nuclear and mitochondrial markers in the fire ant *Solenopsis invicta*. Evolution 52:1416–1422.

Herbers JM, 1986. Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. Behav Ecol Sociobiol 19:115–122.

Herbers JM, 1993. Ecological determinants of queen number in ants. In: Queen number and sociality in insects (Keller L, ed). Oxford: Oxford University Press; 262–293.

Hölldobler B, Wilson EO, 1990. The ants. Cambridge, Massachusetts: Belknap Press.

Hung ACF, Dowler MG, Vinson SB, 1979. Electrophoretic variants of α-glycerophosphate dehydrogenase in the fire ant *Solenopsis invicta*. Can J Genet Cytol 21:537–542.

Jouvenaz DP, Allen GE, Banks WA, Wojcik DP, 1977. A survey for pathogens of fire ants, *Solenopsis* spp., in the southeastern United States. Fla Entomol 60:275–279.

Keller L, 1993. Queen number and sociality in insects. Oxford: Oxford University Press.

Keller L, Genoud M, 1997. Extraordinary lifespans in ants: a test of evolutionary theories of ageing. Nature 389:958–960.

Keller L, Passera L, 1988. Energy investment in gynes of the Argentine ant *Iridomyrmex humilis* (Mayr) in relation to the mode of colony founding in ants (Hymenoptera:Formicidae). Intl J Invert Reprod Dev 13:31–38.

Keller L, Passera L, 1989. Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). Oecologia 80:236–240.

Keller L, Passera L, 1990. Fecundity of ant queens in relation to their age and the mode of colony founding. Insect Soc 37:116–130.

Keller L, Ross KG, 1993a. Phenotypic basis of reproductive success in a social insect: genetic and social determinants. Science 260:1107–1110.

Keller L, Ross KG, 1993b. Phenotypic plasticity and ''cultural transmission'' of alternative social organizations in the fire ant *Solenopsis invicta*. Behav Ecol Sociobiol 33:121–129.

Keller L, Ross KG, 1995. Gene by environment interaction: effects of a single gene and social environment on reproductive phenotypes of fire ant queens. Funct Ecol 9:667–676.

Lee ET, 1992. Statistical methods for survival data analysis. New York: John Wiley and Sons.

Markin GP, Collins HL, Dillier JH, 1972. Colony founding by queens of the red imported fire ant, *Solenopsis invicta*. Ann Entomol Soc Am 65:1053–1058.

Nonacs P, 1988. Queen number in colonies of social Hymenoptera as a kin-selected adaptation. Evolution 42:566–580.

Pamilo P, 1991. Evolution of colony characteristics in social insects. II. Number of reproductive individuals. Am Nat 138:412–433.

Porter SD, 1991. Origins of new queens in polygyne red imported fire ant colonies (Hymenoptera: Formicidae). J Entomol Sci 26:474–478.

Porter SD, Fowler HG, Mackay WP, 1992. Fire ant mound densities in the United States and Brazil (Hymenoptera: Formicidae). J Econ Entomol 85:1154–1161.

Porter SD, Williams DF, Patterson RS, Fowler HG, 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): escape from natural enemies? Environ Entomol 26:373–384.

Queller DC, Goodnight KF, 1989. Estimating relatedness using genetic markers. Evolution 43:258–275.

Rosengren R, Sundström L, Fortelius W, 1993. Monogyny and polygyny in *Formica* ants: the result of alternative dispersal tactics. In: Queen number and sociality in insects (Keller L, ed). Oxford: Oxford University Press; 308–333.

Ross KG, 1988. Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta (*Hymenoptera: Formicidae). Behav Ecol Sociobiol 23:341–355.

Ross KG, 1993. The breeding system of the fire ant *Solenopsis invicta*: effects on colony genetic structure. Am Nat 141:554–576.

Ross KG, 1997. Multilocus evolution in fire ants: Effects of selection, gene flow and recombination. Genetics 145:961–974.

Ross KG, Carpenter JM, 1991. Phylogenetic analysis and the evolution of queen number in eusocial Hymenoptera. J Evol Biol 4:117–130.

Ross KG, Fletcher DJC, 1985. Comparative study of genetic and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 17:349–356.

Ross KG, Keller L, 1995a. Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. Annu Rev Ecol Syst 26:631–656.

Ross KG, Keller L, 1995b. Joint influence of gene flow and selection on a reproductively important genetic polymorphism in the fire ant *Solenopsis invicta*. Am Nat 146:325–348.

Ross KG, Shoemaker DD, 1997. Nuclear and mitochondrial genetic structure in two social forms of the fire ant *Solenopsis invicta*: insights into transitions to an alternate social organization. Heredity 78:590–602.

Ross KG, Vargo EL, Keller L, 1996. Social evolution in a new environment: the case of introduced fire ants. Proc Natl Acad Sci USA 93: 3021–3025.

Shoemaker DD, Costa JT, Ross KG, 1992. Estimates of heterozygosity in two social insects using a large number of electrophoretic markers. Heredity 69:573–582.

Shoemaker DD, Ross KG, 1996. Effects of social organization on gene flow in the fire ant *Solenopsis invicta*. Nature 383:613–616.

Stuart RJ, Gresham-Bissett L, Alloway TM, 1993. Queen adoption in the polygynous and polydomous ant, *Leptothorax curvispinosus*. Behav Ecol 4:276–281.

Sundström L, 1993. Genetic population structure and sociogenetic organisation in *Formica truncorum* (Hymenoptera; Formicidae). Behav Ecol Sociobiol 33:345–354.

Sundström L, 1997. Queen acceptance and nestmate recognition in monogyne and polygyne colonies of the ant *Formica truncorum*. Anim Behav 53:499–510.

Toom PM, Cupp EW Johnson CP, 1976a. Amino acid changes in newly inseminated queens of *Solenopsis invicta*. Insect Biochem 6:327–331.

Toom PM, Cupp E, Johnson CP Griffin I, 1976b. Utilization of body reserves for minim brood development by queens of the imported fire ant, *Solenopsis invicta*. J Insect Physiol 22:217–220.

Toom PM, Johnson CP, Cupp EW, 1976c. Utilization of body reserves during preoviposition activity by *Solenopsis invicta*. Ann Entomol Soc Am 69:145–148.

Tschinkel WR, 1987. Fire ant queen longevity and age: Estimation by sperm depletion. Ann Entomol Soc Am 80:263–266.

Vander Meer RK, Glancey BM, Lofgren CS, 1982. Biochemical changes in the crop, oesophagus and postpharyngeal gland of colony-founding red imported fire ant queens (*Solenopsis invicta*). Insect Biochem 12:123–127.

Vargo EL, 1992. Mutual pheromonal inhibition among queens in polygyne colonies of the fire ant *Solenopsis invicta*. Behav Ecol Sociobiol 31:205–210.

Vargo EL, Fletcher DJC, 1986a. 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, 1986b. Queen number and the production of sexuals in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Behav Ecol Sociobiol 19:41–47.

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.

Vargo EL, Fletcher DJC, 1989. On the relationship between queen number and fecundity in polygyne colonies of the fire ant *Solenopsis invicta*. Physiol Entomol 14:223–232.

Vargo EL, Laurel M, 1994. Studies on the mode of action of a queen primer pheromone of the fire ant *Solenopsis invicta*. J Insect Physiol 40:601–610.

Vargo EL, Porter SD, 1989. Colony reproduction by budding in the polygyne form of *Solenopsis invicta* (Hymenoptera: Formicidae). Ann Entomol Soc Am 82:307–313.

Vinson SB, 1997. Invasion of the red imported fire ant (Hymenoptera: Formicidae). Am Entomol 43:23–39.

Vinson SB, Greenberg L, 1986. The biology, physiology, and ecology of imported fire ants. In: Economic impact and control of social insects (Vinson SB ed). New York: Praeger; 193–226.