

A TEST OF QUEEN RECRUITMENT MODELS USING NUCLEAR AND MITOCHONDRIAL MARKERS IN THE FIRE ANT *SOLENOPSIS INVICTA*

MICHAEL A. D. GOODISMAN^{1,2} AND KENNETH G. ROSS³

¹Department of Genetics, University of Georgia, Athens, Georgia 30602-7223

E-mail: madgood@gen.latrobe.edu.au

³Department of Entomology, University of Georgia, Athens, Georgia 30602-2603

Abstract.—We assess nestmate queen relatedness and the genetic similarity of neighboring nests in the polygyne (multiple-queen) social form of the introduced fire ant *Solenopsis invicta* using both nuclear and mitochondrial markers. We find that estimates of queen relatedness calculated with both types of markers do not differ statistically from zero. Furthermore, there is no significant relationship between the genetic similarity and geographic proximity of nests in each of six study sites. In contrast to these findings, sites show strong mitochondrial, but no nuclear, genetic differentiation. Our results suggest that nonnestmate queen recruitment occurs at a high frequency in introduced populations of this species. Moreover, queens within nests seem to represent a random sample of the queens within the site in which they reside. Therefore, kin selection models that rely on the recruitment of only nestmate queens to explain the persistence of polygyny in ants do not apply to polygyne *S. invicta* in its introduced range.

Key words.—Evolution, fire ants, introduced species, monogyny, mtDNA, polygyny, relatedness, social insects, *Solenopsis invicta*.

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The evolution of a sterile caste in the eusocial insects potentially poses a problem for traditional natural selection theory, because it is difficult to explain how genes for sterility can be passed on to future generations. This dilemma was essentially resolved by Hamilton's theory of kin selection, whereby nonreproductive individuals increase their inclusive fitness by raising relatives rather than reproducing directly (Hamilton 1964a,b). However, the existence of multiple-queen (polygyne) social insect colonies may pose an additional burden for kin selection theory, because the relatedness between members of the sterile caste and the individuals they help rear generally is reduced in such colonies relative to the relatedness in single-queen (monogyny) colonies (Hamilton 1964b; Keller 1995).

Polygyne colonies are common in many ant species (Hölldobler and Wilson 1977, 1990; Keller 1993; Bourke and Franks 1995). The existence of these colonies generally is explained by strong selection acting on new queens to enter previously established colonies when the probability is low that a new queen can successfully found her own colony (Herbers 1986, 1993; Nonacs 1988; Pamilo 1991). In these cases, new queens are expected to be recruited into their natal nests, thereby maintaining relatively high queen relatedness. Therefore, the workers still raise relatives and the essential tenets of kin selection theory may not be violated (Bourke and Franks 1995). Accordingly, many studies that have measured the relatedness of queens in polygyne nests using nuclear markers have found these queens to be close kin. However, in some ant species, queen relatedness has been found to be very low or even statistically indistinguishable from zero (reviewed in Hölldobler and Wilson 1990; Herbers 1993; Rosengren et al. 1993; Bourke and Franks 1995; Ross and Keller 1995a; Crozier and Pamilo 1996). In such cases, colonies appear to accept foreign queens as reproductives, with the result that workers are likely to raise mostly nonrelatives.

This social organization may be evolutionarily unstable, because the altruistic behavior of the sterile caste is vulnerable to the invasion of selfish or nepotistic alleles (Crozier 1979; Pamilo 1991; Bourke and Franks 1995).

Recent theories have been developed to explain the existence of such polygyne ant societies. These theories first propose that the societies are stable because the worker ants are necessarily sterile, so that under no circumstances can they increase their inclusive fitness by direct reproduction. Therefore, the only possible means for workers to increase their fitness is by helping to raise the few relatives present in the colony (Keller 1995). Second, the theories suggest that the queens within these colonies are, in fact, distant relatives (Nonacs 1988; Pamilo 1991; Keller 1995). These models also suggest a specific method of queen recruitment. Polygyne colonies begin with just a few closely related queens and, as the colony ages, additional reproductives are adopted. The newly recruited queens originate from within the nest, but they mate with nonnestmate males. Therefore, queen relatedness decays over several generations even though queens are always adopted into their natal nests.

A strong test of this proposed method of queen recruitment is to estimate queen relatedness using a maternally inherited marker, such as DNA from the mitochondrial genome (mtDNA). A simple expectation from such a study is that, regardless of the estimates obtained from nuclear markers, the relatedness value obtained using mtDNA will be equal to one because all queens within a nest are members of a single matriline. Previous studies have examined the distribution of mtDNA within and among polygyne ant nests and found that in most cases nestmates did, in fact, segregate for only a single mtDNA haplotype (Stille and Stille 1992, 1993; Carew et al. 1997; Tay et al. 1997). However, queens were assayed in only one of these studies (Stille and Stille 1992) and, in this case, the estimated nuclear relatedness had already been found to be quite high (Stille et al. 1991), indicating that the multigenerational mechanism of queen ac-

² Corresponding author.

ceptance probably does not occur. Moreover, in none of these studies were the *same* individuals genotyped at both nuclear and mitochondrial markers. Therefore, a direct comparison between the relatedness estimates derived from the two types of markers could not be performed.

The primary objective of this study is to obtain both nuclear and mitochondrial estimates of queen relatedness for polygyne colonies of the introduced fire ant *Solenopsis invicta*. Previous estimates of queen relatedness using nuclear markers were statistically indistinguishable from zero (Ross and Fletcher 1985; Ross 1993; Goodisman and Ross 1997), making this species a particularly attractive candidate for testing the proposed mechanism of recruitment of outbred nestmate queens. If the hypothesis is correct, then the estimates obtained from the two types of markers should yield dramatically different results. Although queen relatedness calculated with nuclear markers will be close to zero, the corresponding estimates from a mitochondrial marker should be substantially greater than zero.

A secondary goal of this investigation is to use the two classes of markers to examine the genetic similarity of neighboring nests (population viscosity) in introduced, polygyne *S. invicta*. Consideration of such genetic structure is particularly relevant for this study because it can inflate estimates of queen relatedness (Pamilo 1984, 1989) and may influence the strength and scope of kin selection (Kelly 1994). In general, such structure arises through the limited dispersal of individuals (Hamilton 1964b) and may be expected in polygyne ants if queens are recruited into their natal or neighboring nests and if new nests originate as buds of old nests (Chapuisat et al. 1997), as they do in *S. invicta* (Vargo and Porter 1989). Importantly, the use of both nuclear and mitochondrial markers may allow us to detect differential gene flow mediated by the two sexes. For example, a finding of high mitochondrial genetic structure in conjunction with low nuclear genetic structure would be the signature of low female-mediated but high male-mediated gene flow, as may be expected if the proposed mechanism of recruitment of outbred nestmate queens occurs. Population viscosity has been documented in many polygyne ants (Pamilo 1981, 1983; Crozier et al. 1984; Pamilo and Rosengren 1984; Boomsma et al. 1990; Stille and Stille 1993; Sundström 1993; Herbers and Grieco 1994; Seppä and Pamilo 1995; Banschbach and Herbers 1996; Chapuisat et al. 1997; Ross et al. 1997), including introduced *S. invicta* (Shoemaker and Ross 1996; Ross and Shoemaker 1997). However, the previous population genetic studies of the introduced form of this species examined structure on the scale of kilometers. Here, we search for extremely fine-scale, microgeographic structure by considering the genetic relationships of individual nests often separated by only a few meters.

MATERIALS AND METHODS

Ninety-two *S. invicta* nests were collected from six closely spaced sites (all within 2 km of one another) in Walton County, Georgia (Fig. 1). To maximize the probability of capturing the majority of resident queens, nests were collected on warm, sunny days that were preceded by rainfall. Prior to collection, the distances between nests within sites were re-

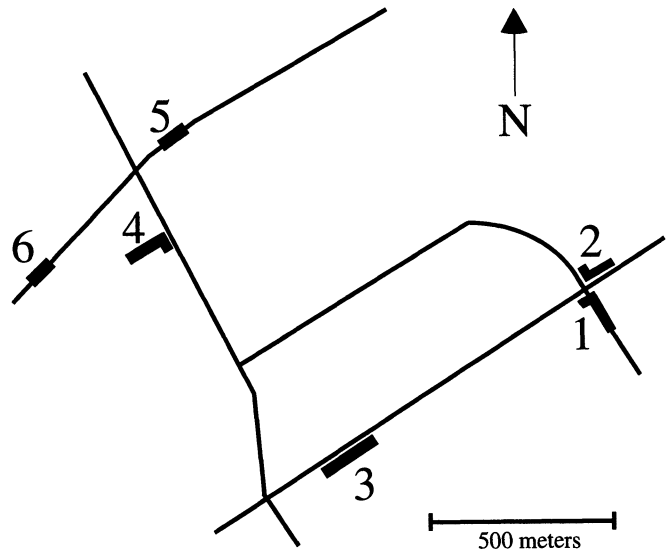


FIG. 1. Locations and sizes of six sampling sites from which polygyne queens of *Solenopsis invicta* were collected. Lines indicate roads.

corded. Nests were then excavated and placed in buckets, and the inhabitants were separated from the soil by flooding (Jouvenaz et al. 1977) and placed into large trays in a rearing room. Within 48 h, reproductive, dealate (wingless) queens were collected by systematically searching through the ants in the trays. Nests were considered to be polygyne if they contained two or more dealate queens. To determine whether the queens had mated, their spermathecae were examined following dissection. Sperm in mated queens was evident as an opaque, whitish mass.

We used previously described methods of allozyme electrophoresis (Shoemaker et al. 1992; Ross and Shoemaker 1997) to determine the genotypes of queens at three polymorphic nuclear loci (*Aat-2*, *G3pdh-1*, and *Pgm-1*). Each queen's haplotype also was assayed at a PCR-amplified mtDNA fragment. Methods are as described in Ross and Shoemaker (1997), with the exceptions that the PCRs were carried out in a final volume of 15 μ l, and the resulting PCR product was digested with a single enzyme, *HinfI*. Digestion with this single enzyme distinguishes the three haplotypes (two common and one rare) found within this polygyne population (Shoemaker and Ross 1996; Ross and Shoemaker 1997).

The relatedness of nestmate queens was calculated using the program Relatedness 4.2 (Queller and Goodnight 1989). Calculations were conducted using all queens, and site-level structure was incorporated by using the "deme" function of the program. All individuals were used to calculate the background population allele frequencies (P^*) that are central to estimating relatedness with this algorithm, with groups (nests) 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 ($r \pm 1.96$ [SEM]) did not overlap zero.

Genetic differentiation among sites was estimated for the nuclear markers by calculating values of the fixation index,

TABLE 1. Number of nests sampled, queen number per nest, and proportion of queens mated at six sites in a single polygyne population of *Solenopsis invicta* in northern Georgia.

Site	Nests	Queens		
		Mean	Range	Proportion mated
1	16	24.69	2–76	0.454
2	15	39.67	2–216	0.370
3	11	36.09	5–82	0.555
4	23	18.74	2–76	0.468
5	10	16.50	3–47	0.469
6	10	61.40	6–257	0.600
All sites	85	30.56	2–257	0.488

F_{ST} , using the program Relatedness 4.2 (Queller and Goodnight 1989). Standard errors of these values were calculated by jackknifing over sites, and values of F_{ST} were considered to be statistically different from zero if the 95% confidence intervals of the estimate ($F_{ST} \pm 1.96 [SEM]$) did not overlap zero. Genetic differentiation among sites for the mtDNA marker was estimated by the statistic ϕ_{ST} , as calculated by the program WINAMOVA (Excoffier et al. 1992). The value of ϕ_{ST} was considered to be significantly different from zero ($P < 0.05$) if fewer than 5% of 1000 estimates obtained by random permutations of individuals among sites were greater than the observed measure.

The relationship between geographic distance and genetic similarity between pairs of nests also was used to measure population viscosity within and among the sampled sites. Nei's standard genetic distance (D ; Nei 1987) was calculated for all pairs of nests and compared to the geographic distances using correlation coefficients (Ott 1988). Significance of the correlations was determined by a Mantel test (Dietz 1983; Smouse et al. 1986); the correlations were considered to be different from zero ($P < 0.05$) if fewer than 2.5% of 1000 estimates obtained by random permutations of the genetic and geographic distances were more extreme than the observed measure.

RESULTS

Eighty-five of the 92 nests collected were considered to be polygyne. The remaining seven nests contained either one or no queens, and thus were not included in further analyses. Table 1 shows the distributions of nests and queens per nest, as well as the proportions of mated queens, at the six sites and in the entire study population. As is typical in this population, queen number varied greatly from nest to nest (cf.

Vargo and Fletcher 1987; Ross 1993; Goodisman and Ross 1997), although it did not differ significantly among sites ($F_{5,79} = 2.08$, $P > 0.05$). The frequency of mated queens was relatively low (Vargo and Fletcher 1989; Ross and Keller 1995a,b; Goodisman and Ross 1997), and differed significantly from site to site (chi-square test of independence, $\chi^2_5 = 73.24$, $P < 0.0001$). All genetic calculations were conducted using all queens, mated queens only, and unmated queens only. Because the results for these three classes do not vary qualitatively, only the results obtained using all queens are presented.

We first investigate genetic differentiation among the six sites in the population. As in previous studies, the nuclear and mitochondrial markers were essentially biallelic (Shoemaker et al. 1992; Shoemaker and Ross 1996; Goodisman and Ross 1997; Ross and Shoemaker 1997). We find that the value of F_{ST} for all three nuclear markers combined is not significantly different from zero ($F_{ST} = -0.001$), as judged by the fact that the 95% confidence interval (-0.004 – 0.003) overlaps zero. In contrast, the ϕ_{ST} statistic for the mtDNA marker shows a highly significant deviation from zero ($\phi_{ST} = 0.136$, $P < 0.001$). Thus, our results show heterogeneity in mitochondrial but not nuclear gene frequencies across the study sites.

We next examine genetic structure at the level of nests within sites. Our primary interest lies in the values of nest-mate queen relatedness, which are given in Table 2 for all nuclear markers combined and for the mitochondrial marker. As expected, estimates of nuclear relatedness are statistically indistinguishable from zero (Ross and Fletcher 1985; Ross 1993; Goodisman and Ross 1997). What is more important, however, is that all estimates of mitochondrial relatedness also are not significantly different from zero. The mitochondrial values are not always positive or greater than the nuclear values, and they do not differ significantly from the corresponding nuclear values (as judged by the fact that none of the mitochondrial relatedness estimates lie outside the 95% confidence intervals of the corresponding nuclear estimate). Moreover, 74 of the 85 nests possessed both of the common mtDNA haplotypes, indicating that multiple matrilineages were represented in the pool of reproductives in these nests. Therefore, we conclude that queen relatedness in polygyne *S. invicta* nests, calculated with reference to the local site, does not differ from zero when estimated with either nuclear or mitochondrial markers. We note, however, that if the significant differentiation in mitochondrial haplotype frequencies across sites is not accounted for in our calculations, we obtain a value of mitochondrial queen relatedness that is signifi-

TABLE 2. Relatedness of nestmate queens calculated for three nuclear markers combined and for a single mitochondrial marker in six sites of a polygyne population of introduced *Solenopsis invicta*. Ninety-five-percent confidence intervals are given in parentheses.

	Site						
	1	2	3	4	5	6	Average across all sites
All nuclear markers	0.04 (−0.02, 0.10)	0.10 (−0.01, 0.20)	−0.01 (−0.05, 0.03)	−0.02 (−0.10, 0.05)	0.02 (−0.09, 0.13)	0.04 (−0.02, 0.10)	0.02 (−0.01, 0.05)
mtDNA	−0.01 (−0.21, 0.20)	0.06 (−0.05, 0.17)	0.01 (−0.05, 0.08)	0.05 (−0.03, 0.13)	−0.04 (−0.20, 0.10)	0.02 (−0.07, 0.10)	0.02 (−0.04, 0.07)

TABLE 3. Correlation between Nei's genetic distance (D) and geographic distance for pairs of nests calculated for three nuclear markers combined and for a single mitochondrial marker in six sites of a polygyne population of introduced *Solenopsis invicta*. Ninety-five-percent confidence intervals for randomly generated correlations are given in parentheses, and values in bold differ significantly from zero ($P < 0.05$).

	Site						
	1	2	3	4	5	6	Sites pooled
All nuclear markers	0.08	0.14	−0.17	0.04	−0.13	−0.01	−0.02
	(−0.16, 0.18)	(−0.22, 0.24)	(−0.25, 0.27)	(−0.12, 0.12)	(−0.28, 0.28)	(−0.25, 0.30)	(−0.03, 0.03)
	0.24	−0.08	−0.14	0.08	−0.02	0.07	0.24
	(− 0.18 , 0.18)	(−0.21, 0.23)	(−0.26, 0.28)	(−0.13, 0.12)	(−0.27, 0.29)	(−0.27, 0.31)	(− 0.03 , 0.03)

cantly different from zero ($r = 0.15 \pm 0.04$). This result emphasizes the importance of the scale of reference when calculating relatedness.

Finally, we assess the relationship between geographic distance between nests and their genetic similarity. Table 3 shows the correlations between genetic distance (D) and geographic distance for nests in our sample. Only one of the 12 within-site, independent correlations is significantly different from zero. Moreover, the within-site correlations obtained with the mitochondrial marker are not always greater than those obtained from the corresponding nuclear markers, nor are the mtDNA correlations necessarily positive. When nests from all sites are pooled the correlation between genetic and geographic distance between nests is positive and highly significant for the mtDNA marker, presumably due to the strong genetic differentiation in mitochondrial haplotype frequencies among sites combined with the clinal pattern in these frequencies previously found in this area (Shoemaker and Ross 1996; Ross and Shoemaker 1997). This finding is reinforced when the correlations are calculated using the genetic distance between the six sites in the population rather than the individual-nest distances. In this case, the correlation for the mtDNA marker is 0.25, whereas those for the nuclear markers are -0.31, 0.09, and 0.07 for *Aat-2*, *G3pdh-1*, and *Pgm-1*, respectively. Although none of these values differ significantly from zero (randomly generated confidence intervals around zero range from about -0.50 to 0.50), due in part to the small number of sites, the high positive correlation for the mitochondrial marker is consistent with the calculations for the individual nests. Thus, there does not appear to be a relationship between spatial proximity and genetic similarity of nests within sites, and the existence of such a relationship over the entire population for the mitochondrial marker can be explained by among-site differentiation.

DISCUSSION

High relatedness between interacting individuals within ant colonies is believed to be important in promoting and maintaining the altruistic behavior of the sterile worker caste (Hamilton 1964a,b; Crozier and Pamilo 1996). Therefore, the presence of polygyne ants in which colonies contain many unrelated queens is difficult to explain under kin selection theory, because the workers in such colonies raise mostly nonrelatives and thus obtain very low indirect fitness returns (Hölldobler and Wilson 1990; Bourke and Franks 1995; Keller 1995; Crozier and Pamilo 1996). To address this problem, queen recruitment models have been formulated that combine

elements of kin selection theory with traits particular to these problematic ants. Specifically, it has been proposed that queens in such colonies normally mate with nonnestmate males but then reenter their natal nests to reproduce. Over the course of several generations, this method of queen recruitment leads to a deterioration of nuclear relatedness. Nevertheless, the workers are related to the sexuals they raise, even if in some cases the relationships are very distant and the numbers of relatives raised are few (Nonacs 1988; Pamilo 1991; Keller 1995). This pattern of queen recruitment can be distinguished from recruitment of nonnestmate queens if both maternally inherited (mitochondrial) and biparentally inherited (nuclear) markers are considered. Under the above model, we expect polygyne ants that display low levels of nuclear relatedness to still display high levels of mitochondrial relatedness.

The main finding of this study is that queens in polygyne nests of the introduced fire ant *S. invicta* show nuclear and mitochondrial relatedness levels that are statistically indistinguishable from zero. Thus, we conclude that nonnestmate queens frequently are accepted into such nests, and a mechanism involving recruitment of only nestmate queens does not seem to apply in this ant. Acceptance of foreign queens must occur with some regularity, because if it did not and if queen mortality rates were fairly high (as is likely in natural populations of polygyne ants; Hölldobler and Wilson 1990; Seppä 1994; Bourke and Franks 1995; Evans 1996), then genetic drift would lead to the rapid fixation of mitochondrial haplotypes within nests and thus to high mitochondrial relatedness estimates. Moreover, frequent foreign queen recruitment may be necessary to account for the high queen number observed in some of the nests described in this (Table 1) and other studies (Ross 1993; Ross and Keller 1995a). Such recruitment of foreign queens into polygyne nests of introduced *S. invicta* may occur by several means. Nonnestmate queens may enter nests singly after mating flights, as has been documented through mark-recapture techniques (Glancey and Lofgren 1988). Alternatively, the fusion of two large polygyne nests or of a large nest with one or more foundress groups would constitute recruitment of multiple nonnestmate queens simultaneously.

A second important result of our study is that there is no relationship between geographic and genetic distances between nests within sites. This result may seem surprising given that polygyne nests in this species frequently originate as buds of preexisting nests (Vargo and Porter 1989). However, the effectively zero relatedness of nestmate queens es-

essentially precludes the possibility of any other genetic relationships among nests within sites. Because queens within nests are unrelated, queens within new buds also will be unrelated (assuming they are a random sample of the queens within the parental nest); therefore, no genetic associations between nests and their buds are possible. The lack of any type of detectable genetic structure within sites indicates that nestmate queens represent an effectively random sample of the queens in the site in which they reside.

In contrast to these results, the sites that we studied do exhibit heterogeneity with respect to haplotype frequencies at the mtDNA (but not the nuclear markers) over a scale of hundreds of meters. These results are consistent with previous studies of polygyne *S. invicta* that have found low nuclear but high mitochondrial genetic differentiation among sites separated by several kilometers in an introduced population (Shoemaker and Ross 1996; Ross and Shoemaker 1997). In general, greater differentiation at mitochondrial than nuclear markers may be expected, because mtDNA has a smaller effective population size than nuclear DNA and thus is more sensitive to genetic drift (Avice 1994). However, the lack of significant microgeographic mtDNA structure seen in introduced, monogyne *S. invicta* (Ross and Shoemaker 1997), the ~ 100 -fold greater value of ϕ_{ST} over F_{ST} found in our study, and the differentiation in proportion of mated queens among sites indicate that other factors probably are responsible for the significant among-site mtDNA structure. Specifically, female-mediated gene flow is likely to be lower than male-mediated gene flow in the polygyne social form at scales of hundreds of meters.

The presence of microgeographic genetic structure above the nest level has been detected in many other ant species using nuclear or mitochondrial markers (Pamilo 1981, 1983; Crozier et al. 1984; Pamilo and Rosengren 1984; Crozier and Pamilo 1986; Boomsma et al. 1990; Stille and Stille 1993; Sundström 1993; Herbers and Grieco 1994; Seppä and Pamilo 1995; Banschbach and Herbers 1996; Gadau et al. 1996; Chapuisat et al. 1997; Tay et al. 1997). This structure can potentially confound estimates of nestmate relatedness (Pamilo 1984, 1989; Chapuisat et al. 1997). Indeed, in this introduced polygyne population, mitochondrial genetic structure leads to a significantly positive estimate of mitochondrial relatedness for nestmate queens if the haplotype frequency differences among sites are not taken into account. Higher-level genetic structure also may enhance the effects of kin selection in some situations (Kelly 1994). However, this potential complication does not appear to apply to introduced polygyne *S. invicta*. Nuclear genetic structure is apparently very limited at any microgeographic scale and therefore is not likely to be a significant factor in promoting kin selection.

Using the site and population-level results described here, we propose a synthetic framework to explain the proliferation of the polygyne form of this species in its introduced range. New polygyne populations may originate very rarely from preexisting monogyne populations (Ross and Keller 1995a; Shoemaker and Ross 1996; Ross and Shoemaker 1997). Once established, the vast majority of new polygyne nests within a site are created by budding of old nests (Vargo and Porter 1989), and the new queens that are recruited into nests come mostly from other nests dispersed throughout the site. These

queens participate in localized nuptial flights and mate with polygyne males that disperse across sites, as well as widely dispersing males from nearby monogyne populations (Ross and Keller 1995b; M. Goodisman, C. DeHeer, and K. Ross, unpubl. data). Thus, as in many other polygyne ants (reviewed in Bourke and Franks 1995), female-mediated gene flow may be low relative to that of males at scales greater than a few hundred meters.

The presence of unrelated queens within nests is not predicted under kin selection theory (Bourke and Franks 1995). Resident queens in nests should not allow foreign queens to enter and reproduce, because the founding of a nest and the production of the worker force represent a significant energetic investment (Hölldobler and Wilson 1990). Furthermore, workers in nests containing multiple unrelated queens likely obtain relatively low inclusive fitness benefits because they raise mostly nonrelatives. Somewhat surprisingly, given this situation, polygyne worker fire ants do not appear to gain any inclusive fitness benefits by other means. For example, it might be expected that the reproductive skew observed among nestmate queens in the laboratory (Ross 1988) would increase the relatedness between workers and the sexual brood that they rear over that predicted from queen relatedness alone. However, relatedness between workers and nestmate sexual brood is indistinguishable from zero in polygyne populations in the field (Ross et al. 1996). Another way that workers could obtain higher inclusive fitness returns would be if they favor sexuals to which they are most closely related (Carlin et al. 1993; Snyder 1993). However, no evidence exists that worker fire ants in polygyne nests behave nepotistically with respect to their investment in young sexuals (DeHeer and Ross 1997).

Although polygyne *S. invicta* workers in the introduced range are mostly unrelated to the brood they rear, polygyne populations apparently have not suffered from the invasion of selfish alleles for nepotism (DeHeer and Ross 1997) or worker reproduction, as might be expected (Crozier 1979; Pamilo 1991; Carlin et al. 1993; Snyder 1993). This latter fact may be related to the phylogenetically conserved inability of *Solenopsis* workers to lay eggs (Hölldobler and Wilson 1990), a worker trait that apparently is important in the maintenance of polygyny in other ants in which nestmate queens display very low relatedness (Keller 1995). In this case, a worker may have little choice but to obtain the low inclusive fitness benefits from raising a very small fraction of relatives.

Introduced polygyne *S. invicta* has been considered to be unicolonial, a social system which is, in part, defined by extensive intermixing of individuals among nests (Bhatkar and Vinson 1987; Bourke and Franks 1995). Surprisingly, despite the apparent frequent recruitment of nonnestmate queens, evidence exists that polygyne nests do not exchange individuals continuously. Rather, correlations between queen number and worker relatedness (Ross 1993), and between queen number and worker mass (Goodisman and Ross 1996), suggest that polygyne nests are discrete, closed societies for at least part of their existence, because extensive movement of either queens or workers among nests would be expected to erase these relationships.

Local genetic structure in introduced polygyne *S. invicta*

populations differs in some respects from that of native polygyne populations in South America (Ross and Keller 1995a). For example, queen number is significantly lower in the native populations and nestmate queens show nuclear relatedness levels that are substantially greater than zero (Ross et al. 1996). Therefore, nests in native populations appear to recruit related queens almost exclusively as new reproductives. Furthermore, native polygyne populations exhibit microgeographic genetic structure above the level of the nest when either nuclear or mitochondrial markers are considered (Ross et al. 1997). These genetic changes, which reflect differences in reproductive patterns between introduced and native polygyne *S. invicta*, are likely the result of ecological differences in the two ranges. For instance, the recruitment of nonnestmate queens may be rooted in the high density of fire ant nests found in introduced populations (Ross and Keller 1995a). *Solenopsis invicta* was introduced to North America without many of its natural enemies (Jouvenaz et al. 1977; Orr et al. 1995), and this seems to be an important factor responsible for the higher nest densities in the introduced range than in the native range (Porter et al. 1992, 1997). This change may have led to the saturation of fire ant nesting sites, which, in turn, would place strong selective pressure on prereproductive queens to enter already established nests (Herbers 1986, 1993; Nonacs 1988; Pamilo 1991; Rosengren et al. 1993; Bourke and Heinze 1994; Ross and Keller 1995a). After mating flights, the number of queens attempting to gain access to nests may be so high that workers are overwhelmed and unable to prevent all foreign queens from entering. Thus, proximate, ecological factors appear to have led to radical changes in the social organization and local genetic structure of polygyne *S. invicta*.

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