

Social evolution in a new environment: The case of introduced fire ants

(queen number/relatedness/mating system/male diploidy/*Solenopsis invicta*)

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ABSTRACT The inadvertent introduction of the fire ant *Solenopsis invicta* to the United States from South America provides the opportunity to study recent social evolution by comparing social organization in native and introduced populations. We report that several important elements of social organization in multiple-queen nests differ consistently and dramatically between ants in Argentina and the United States. Colonies in Argentina contain relatively few queens and they are close relatives, whereas colonies in the United States contain high numbers of unrelated queens. A corollary of these differences is that workers in the native populations are significantly related to the new queens that they rear in contrast to the zero relatedness between workers and new queens in the introduced populations. The observed differences in queen number and relatedness signal a shift in the breeding biology of the introduced ants that is predicted on the basis of the high population densities in the new range. An additional difference in social organization that we observed, greater proportions of permanently unmated queens in introduced than in native populations, is predicted from the loss of alleles at the sex-determining locus and consequent skewing of operational sex ratios in the colonizing ants. Thus, significant recent social evolution in fire ants is consistent with theoretical expectations based on the altered ecology and population genetics of the introduced populations.

Social organization—the number, relationships, and reproductive output of members of a group—constitutes the defining features of social life (1). The ecological and genetic factors causing evolution of social organization in ants are of great interest to evolutionary biologists, because knowledge of these factors can illuminate the mechanisms by which diverse forms of cooperative breeding originate and are elaborated (1–7). In eusocial insects, ecological factors are widely believed to be important in shaping such basic features of social organization as colony queen number and queen reproductive roles (4–10), but demonstration of the effects of such factors remains elusive (5, 11). Specific genetic factors influencing reproductive roles and the form of social organization have seldom been documented (1, 12–16).

The inadvertent introduction of the fire ant *Solenopsis invicta* to the United States from South America provides a unique opportunity to study recent social evolution by comparing social organization in the native and newly colonized ranges. Such comparative studies are especially pertinent because of important advances in our knowledge of the ecology and genetics of the ants in both areas. Recent ecological studies have revealed that introduced fire ants attain colony sizes and densities far in excess of those that occur in

the native range (17), presumably due to ecological release from the effects of natural enemies that normally limit the survival and growth of established nests. High population densities in the USA are likely to reduce the availability of new nesting sites as the open, disturbed habitats required by this species for nesting (18) become increasingly saturated. Increased habitat saturation is hypothesized to influence colony queen number and related elements of social organization in ants by favoring the adoption of new queens into existing nests and disfavoring solitary nest foundation by dispersing queens (1, 5, 7, 11, 19, 20). Moreover, habitat saturation may promote incidental mixing of nonnestmates, potentially eroding the discrimination abilities of workers involved in the recruitment of new queens and the regulation of colony queen number (21–24). Both mechanisms predict a positive association between degree of habitat saturation and colony queen number.

Recent genetic studies have revealed that genetic variation has been lost in introduced fire ants because of a population bottleneck during founding of the USA populations (25). Loss of variation at the major sex-determining locus is especially evident, as it is associated with a large increase in the frequency of sterile (diploid) males in introduced populations (25). Such an increase in male sterility may affect another basic feature of social organization, the frequency of egg-laying queens that remain permanently unmated, by altering operational sex ratios and disrupting the mating system (26). Thus, colonization of the USA by *S. invicta* has been accompanied by well-documented ecological and genetic changes predicted to have significant consequences for fundamental properties of social organization such as colony queen number, nestmate relatedness, and queen mating success.

In this report we examine differences in these properties in multiple-queen (polygyne) colonies of *S. invicta* from the native and introduced ranges. The results indicate great similarity in social organization between localities within each range but pronounced differences between the native and introduced ants. The significant evolution of social organization inferred to have occurred in introduced populations of the polygyne form is consistent with changes predicted on the basis of the altered ecology and population genetics in the new range. Of special interest is the transition from nests with few closely related queens to nests with many unrelated queens, because the consequent rearing of unrelated sexuals by workers in the introduced populations poses an apparent paradox for inclusive fitness theory (7, 27).

MATERIALS AND METHODS

The fire ant *S. invicta* was introduced into the USA from South America in the 1930s (28) and has since expanded its new range rapidly. Two social forms of *S. invicta* exist in the native and

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introduced ranges, one in which nests contain a single egg-laying queen (monogyne form) and one in which nests contain multiple queens (polygyne form). In the monogyne form, colony founding is independent: newly mated queens start colonies without workers after dispersing some distance from the parent colony (29). In the polygyne form, the subject of this study, colony founding usually is dependent: queens and workers from an existing colony emigrate as a group to form a new colony near the parent colony (30). Colony founding in the polygyne form may occur independently as well in some cases, suggesting polymorphism in queen reproductive strategies. Queens of both forms mate with a single male (25, 31), presumably only (monogyne form) or mainly (polygyne form) during extensive swarming flights. The monogyne form is presumed to be ancestral in the introduced range (1), with local enclaves of polygyne arising secondarily in established monogyne populations.

Ants were collected from polygyne nests excavated at two localities in Argentina (Corrientes, Formosa) and two in the USA (Georgia, Texas). The Argentine localities are separated by a major biogeographic boundary (the Río Paraná) and are genetically distinct (32); the USA localities are at opposite ends of the introduced range. All excavated nests were recognized as containing multiple egg-laying queens because nestmate workers possessed genotype arrays at several electrophoretic loci incompatible with their being the offspring of a single queen (31, 33).

The average numbers of mated egg-laying queens per nest were estimated indirectly from the genetic variation within nests and directly from the counts of collected queens. The indirect estimate is the effective queen number, estimated from the genetic relatedness of daughters of single queens (r_s), the relatedness of nestmate workers (r_w), and the relatedness of mated nestmate queens (r_q) (33, 34). The value of r_s is fixed at 0.75 because *S. invicta* queens mate once (25, 31). Values of r_w and r_q were estimated from electrophoretic genotypic data from a large number of polymorphic loci in each population (see legend to Table 1). Only loci exhibiting mendelian patterns of inheritance and showing no evidence of being under selection (15, 25, 33, 35) were used for estimating relatedness (36). Direct estimates of the numbers of egg-laying queens are the arithmetic and harmonic means of the counts made following nest excavations. The harmonic mean queen number is of interest because it is expected to equal the effective number (indirect estimate) under simple assumptions (33, 34). Variances for the relatedness estimates and direct estimates of queen number, obtained by jackknifing over nests, were used to generate 95% confidence intervals by assuming the *t* distribution.

Mating status was determined by dissecting queens and examining the spermatheca (sperm storage organ). Proportions of mated queens were estimated by using a resampling procedure to avoid colony-level effects. A single queen was drawn at random from each nest 1000 times (with replacement), with the arithmetic mean of the 1000 resampled population proportions taken as the population estimate. The 95% confidence intervals about these mean values were obtained by dropping the 25 highest and the 25 lowest of the 1000 resampled proportions.

Sample sizes for estimating colony queen number, relatedness, and proportions of mated queens are given in the legends to Tables 1 and 2.

Local population genetic structure was assessed in Argentina by calculating Wright's inbreeding coefficient (F_{IS}) as well as the relatedness between nonnestmates in neighboring nests. Estimates of F_{IS} were obtained from allele and genotype frequencies generated by resampling single (independent) genotypes 1000 times (with replacement) from each nest included within a 5-km diameter deme (33, 37). Estimates were based on eight loci (15 alleles) surveyed in 516 ants from five

demes in Corrientes and on eight loci (14 alleles) surveyed in 408 ants from five demes in Formosa. Estimates of relatedness between nests located within clusters of diameter < 10 m were based on eight loci surveyed in 276 ants from 10 clusters in Corrientes and on seven loci surveyed in 162 ants from 7 clusters in Formosa.

RESULTS

Introduced populations of polygyne *S. invicta* differ dramatically from native populations of this form in the number of egg-laying queens per nest and the relatedness of these queens (Table 1). The number of mated queens per nest estimated indirectly from the genetic data is over twice as high in introduced populations as native populations, a disparity reflected also in the direct counts of queens collected from the excavated nests. The increase in queen number in introduced populations is accompanied by a pronounced drop in the average relatedness of nestmate queens. Relatedness values in Argentina are those expected if queens often are sisters or mother-offspring pairs, whereas values in the USA are statistically indistinguishable from zero. As expected, the increased number and decreased relatedness of egg-laying queens has led to a significant drop in relatedness between workers and the new queens they rear in the introduced ants compared to the native ants (Table 1); indeed, workers in the USA are on average no more closely related to the young winged (non-egg-laying) queens from their own nest than they are to those from other nests in the same population.

These differences in queen number and relatedness signal a significant shift in the breeding system of the polygyne form in the introduced range. Nests in Argentina must recruit new egg layers almost entirely from queens that are reared in (and mate in or near) the same nest, because other mechanisms that conceivably could generate the observed high levels of queen relatedness can be discounted. For instance, high queen relatedness does not result from recruitment of queens from highly inbred local demes, because inbreeding is insignificant ($F_{IS} \pm SE = 0.011 \pm 0.014$ in Corrientes and 0.006 ± 0.027 in Formosa) and neighboring nests are not closely related (between-nest $r \pm SE = 0.055 \pm 0.053$ in Corrientes and 0.111 ± 0.039 in Formosa). The low relatedness of nestmate queens in the USA is consistent with data from mark-recapture studies (38, 39), indicating that at least some (and perhaps most) new queens are recruited from foreign nests after having participated in mating flights.

The native and introduced populations differ also in the proportions of egg-laying queens that remain permanently unmated, with such queens occurring at least twice as frequently in introduced as in native populations (Table 2). This difference means that the disparity in number of egg-laying queens per nest between native and introduced populations is even greater than indicated by the data in Table 1, because both the indirect and direct estimates concern only mated queens.

DISCUSSION

The differences in colony queen number and relatedness observed between native and introduced *S. invicta* of the polygyne form are consistent with predictions based on the different population densities in the two ranges. Constraints on independent founding in the introduced range are expected to generate increased selection on queens to seek adoption into existing colonies and on members of established nests to accept such queens (5, 11, 20, 40). High population densities in the USA suggest that appropriate nesting habitats are more often saturated, and opportunities for independent founding by dispersing queens are correspondingly more limited, than in the native range (19). Thus, queens attempting either to

Table 1. Number of mated egg-laying queens and nestmate relatedness in polygyne nests in native and introduced populations of *S. invicta*

	Native populations		Introduced populations			
	Corrientes	Formosa	Georgia		Texas	
Number of mated egg-laying queens per nest						
Indirect estimate	6.12	4.02	1984 13.1	1990 13.2	—	
Direct estimates						
Harmonic mean*	3.63 (2.34–4.92)	2.60 (1.60–3.60)	1985 8.74 (4.79–12.7)	1990 9.54 (5.68–13.4)	1987 5.67 (2.60–8.73)	1995 10.4 (3.23–17.7)
Arithmetic mean	6.46 (4.39–8.53)	4.58 (3.45–5.72)	33.8 (25.7–42.0)	28.3 (17.9–38.8)	20.0 (12.3–27.7)	35.4 (16.9–53.9)
Relatedness of mated egg-laying queens	0.45 (0.30–0.60)	0.46 (0.25–0.67)	1984 0.01 (–0.03–0.06)	1990 0.05 (0.00–0.10)	1995 0.02 (–0.03–0.07)	
Relatedness between workers and young winged (non-egg-laying) queens	0.24 (0.12–0.36)	0.15 (0.08–0.23)	1990 0.00 (–0.03–0.03)		—	

The 95% confidence intervals for direct estimates of queen number and relatedness are in parentheses. Values for relatedness of mated queens (r_q) were based on four loci in Corrientes, seven in Formosa, two in Georgia in 1984, six in Georgia in 1990, and six in Texas. Relatedness between workers and young winged (non-egg-laying) queens was estimated from five loci in Corrientes, six in Formosa, and seven in Georgia. Values for the relatedness of nestmate workers (r_w), used to obtain indirect estimates of queen number, were based on eight loci in Corrientes, seven in Formosa, two in Georgia in 1984, and eight in Georgia in 1990. All relatedness estimates for Formosa were corrected for the low but significant relatedness observed between neighboring nests. Sample sizes for estimating relatedness are as follows: Corrientes, 528 workers from 44 nests, 181 queens from 26 nests, 170 winged queens from 20 nests; Formosa, 399 workers from 34 nests, 108 queens from 20 nests, 165 winged queens from 22 nests; Georgia (1984), 826 workers from 20 nests, 1261 queens from 26 nests; Georgia (1990), 895 workers from 27 nests, 765 queens from 27 nests, 1662 winged queens from 27 nests; Texas (1995), 310 queens from 30 nests. Sample sizes for direct estimates of queen number are as reported above for Corrientes, Formosa, and Georgia (1990). Sizes of the remaining samples are as follows: Georgia (1985), 2502 queens from 74 nests; Texas (1987), 660 queens from 33 nests; Texas (1995), 708 queens from 20 nests.

*Harmonic means of the direct counts of queens are expected to equal the effective queen numbers obtained indirectly (33, 34); the fact that they consistently are 60–70% lower suggests that similar proportions of egg-laying queens eluded capture during the excavations in each population.

remain in their natal nest or to enter another nest to become egg layers may have higher average reproductive success than queens attempting to found nests independently, even though queens using the former strategies have reduced fecundity compared to independently founding queens (41). Workers initially are expected to accept only new queens originating from their own nest, but their nestmate discrimination abilities may weaken as queen number increases (21–24), possibly leading to a runaway process of acceptance of both nestmate and foreign queens (7, 42). Incidental mixing of nonnestmates in dense populations may accelerate this breakdown in discrimination abilities. A major consequence of this increase in queen number is a decrease in relatedness among colony members, with the effect that workers in the USA typically rear unrelated sexuals. Polygyne ants generally can be characterized by the alternative syndromes of few closely related queens or many distantly related queens per nest (7, 20), suggesting that the transition from one syndrome to the other in *S. invicta* reflects a fundamental ecologically driven shift in social organization in ants.

The difference in proportions of unmated queens observed between Argentina and the USA supports the proposal that frequent male sterility caused by a loss of genetic variation at

the sex-determining locus has led to the elevated abundance of such queens in the introduced range (26). Loss of alleles, documented in introduced *S. invicta* for a large number of genes (25), is the expected consequence of a population bottleneck such as that which presumably accompanied initial establishment of the ants in the USA (43). Such a loss is likely to have a pronounced effect on sex determination and sex ratios in social Hymenoptera such as fire ants because of their unique genetic mode of sex determination. Sex is determined via a system of genetic complementarity whereby diploids heterozygous at the sex-determining locus become females, and haploids (hemizygotes) or homozygous diploids become males (25, 44). A reduction in sex-allele diversity leads to increased homozygosity at this locus and consequent elevated frequencies of males that are diploid; thus, such males are common in the USA but uncommon in Argentina (25). Because diploid males are sterile (45) and appear to be produced at the expense of haploid males (1), the frequent occurrence of diploid males in the USA creates a highly female-biased operational sex ratio (estimated at 6.2 queens per fertile male in the Georgia polygyne population; ref. 46). The apparent consequence of this female-biased sex ratio in an organism in which both sexes mate singly (31, 33) is that a

Table 2. Proportion of egg-laying queens that are permanently unmated in polygyne nests in native and introduced populations of *S. invicta*

Native populations		Introduced populations			
Corrientes	Formosa	Georgia		Texas	
		1985	1993	1987	1995
0.01 (0.00–0.07)	0.12 (0.08–0.19)	0.26 (0.16–0.37)	0.27 (0.23–0.31)	0.26 (0.15–0.39)	0.29 (0.16–0.40)

The 95% confidence intervals about the values are shown in parentheses. Sample sizes for estimating the proportions are as follows: Corrientes, 185 queens from 28 nests; Formosa, 121 queens from 26 nests; Georgia (1985), 3595 queens from 74 nests; Georgia (1993), 1156 queens from 450 nests; Texas (1987), 942 queens from 33 nests; Texas (1995), 1440 queens from 50 nests.

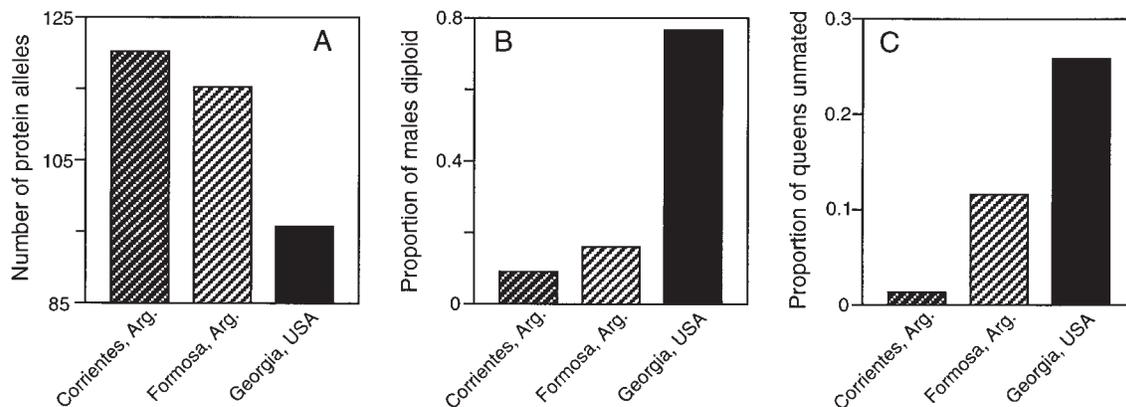


FIG. 1. Number of alleles, proportion of males diploid, and proportion of queens remaining permanently unmated in native (Argentina) and introduced (USA) populations of the polygyne form of *S. invicta*. The loss of alleles at protein markers in the USA (A) is paralleled by a loss of alleles at the sex-determining locus, which leads to increased homozygosity at this locus and elevated proportions of males that are sterile diploids (B). This increased frequency of male sterility creates strongly female-biased operational sex ratios responsible for increased proportions of queens failing to acquire mates in the USA (C; also Table 2). The relationship among the three depicted parameters predicted by this scenario holds not only between the native and introduced ants but also between the two native populations. Protein alleles are those present at 76 electrophoretic loci in samples of 30–36 nests from each population (25). Proportions of diploid males are maximum-likelihood estimates based on individual banding phenotypes at polymorphic electrophoretic loci [Corrientes, 161 males from 17 nests (9 loci); Formosa, 326 males from 26 nests (10 loci); Georgia, 1182 males from 25 nests (two loci)] (25). Data for number of alleles and diploid males are not available for the introduced population from Texas.

substantial proportion of queens are unable to obtain mates despite being reproductively active (1, 47). In Argentina, where diploid males are rare, significantly lower proportions of unmated queens are expected than in the USA, as we have found. Parallel patterns in abundance of marker alleles, frequency of diploid males, and frequency of unmated queens exist across several native and introduced populations (Fig. 1), providing strong support for this hypothesized effect of loss of genetic variation on mating success of polygyne queens.

This study documents a unique historical example of significant social evolution that has occurred in association with well defined ecological and genetic changes. An important conclusion is that high population density and intense intraspecific competition can promote the evolution of a social organization in which workers rear unrelated sexuals, implicating such ecological factors in the evolution of other ant social systems not based on close kinship. Also important is the conclusion that loss of genetic variation can mediate evolution of social organization by means of the effects of reduced sex-allele diversity on sex ratios and mating systems.

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