

Effects of social organization on gene flow in the fire ant *Solenopsis invicta*

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A CONTROVERSIAL model of speciation proposes that the development of alternative social organizations within populations of group-living animals may drive the inception of reproductive isolation¹⁻³. The alternative social behaviours, which are selectively favoured in some social or ecological contexts, may be correlated with distinctive reproductive traits such that significant barriers to interbreeding emerge between coexisting social variants. Evidence for this mode of speciation is almost non-existent³⁻⁸, but it provides one of the most compelling mechanisms for sympatric speciation^{3,8} and could conceivably explain many species origins. Here we examine variation in mitochondrial DNA and two unique nuclear genes to demonstrate that gene flow between sympatric social forms of the fire ant *Solenopsis invicta* is restricted to only one of four possible routes. The loss of the other routes results from incompatibilities in the social systems of the two forms, demonstrating the potential for social selection to generate significant barriers to gene flow and to initiate reproductive isolation.

The fire ant *Solenopsis invicta* includes two distinctive social forms that differ in the number of egg-laying queens per nest; nests of the monogyne (M) form contain only a single queen, whereas those of the polygyne (P) form may contain 200 or more queens⁸. The two forms occur sympatrically in many parts of the native range in South America⁹ as well as in the introduced range

in the United States⁸, where polygyny is thought to have arisen repeatedly from monogyny under conditions of high local population density⁸. The social forms differ in several features of their breeding biology, which are associated with colony queen number and are expected to influence gene flow between the forms. The differences include the site of mating, extent of dispersal and mode of colony founding^{8,10}. We use markers of the mitochondrial and nuclear genomes to infer how these differences affect gene flow between the social forms where they occur in sympatry in the introduced range.

Fire ants were sampled from an area of northern Georgia, USA, where the two forms co-occur (Fig. 1). Restriction-fragment length polymorphism analysis of a maternally inherited 4-kilobase mitochondrial DNA (mtDNA) fragment led to the detection of four composite haplotypes (designated A, B, C and D). An analysis of the haplotype variance¹¹ reveals that only a relatively small amount (<14%) occurs among sampling sites within each form, with such among-site differentiation significant only in the P form (Table 1). This confinement of among-site differentiation to the P form accords with the suggestion that queens of the P form are less vagile than queens of the M form (ref. 8 and K.G.R. and D.D.S., manuscript submitted). The majority of haplotype variation (>50%) occurs between the social forms, with this interform differentiation being statistically significant using either of two genetic distance metrics (Table 1). Thus, queen-mediated gene flow is greatly restricted between the social forms of *S. invicta*.

A site-by-site comparison of the haplotype frequencies (Table 2) reveals the magnitude of queen-mediated interform gene flow occurring via different routes. One common haplotype in the P form (C) is lacking in the M form, even at the Eastville site immediately downwind of the main area of polygyny, a pattern consistent with the complete absence of female migration from the P form to the M form. Two haplotypes with moderate pooled frequencies in the M form (B and D) are virtually absent in the P form. Only two of 230 P queens, both from the Walton Academy site at the windward boundary of the main P population with the M form, possess either of these haplotypes (Table 2). Their complete absence from the other Georgia P sites, as well as

TABLE 1 Hierarchical analysis of molecular variance for mtDNA haplotypes of *S. invicta*

Variance component	Equidistant metric			Euclidean metric		
	% of total variance	Φ -statistics	<i>P</i>	% of total variance	Φ -statistics	<i>P</i>
All samples						
Between social forms	51.4	$\Phi_{FT} = 0.514$	0.020	55.9	$\Phi_{FT} = 0.559$	0.030
Among sites						
Within forms	13.6	$\Phi_{SF} = 0.280$	<0.001	13.4	$\Phi_{SF} = 0.303$	<0.001
All sites	(65.0)	$\Phi_{ST} = 0.650$	<0.001	(69.3)	$\Phi_{ST} = 0.693$	<0.001
Within sites	35.0			30.7		
Monogyne (M) samples						
Among sites	0	$\Phi_{SF} = 0$	0.444	0.5	$\Phi_{SF} = 0.005$	0.294
Within sites	100			99.5		
Polygyne (P) samples						
Among sites	36.1	$\Phi_{SF} = 0.361$	<0.001	35.8	$\Phi_{SF} = 0.358$	<0.001
Within sites	63.9			64.2		

The equidistant metric assumes that all four haplotypes are equally divergent from one another, whereas the euclidean metric is a distance measure that equals the number of restriction site differences between haplotypes. The total haplotypic variance is partitioned among three levels in the upper part of the table: between forms, among sites and within sites. The proportion of variance among all sites (in parentheses) is the sum of the variance between forms and the variance among sites within forms. The statistic Φ_{FT} measures the extent of differentiation between forms, Φ_{SF} measures differentiation among sites within each form, and Φ_{ST} measures differentiation among all sites. The probabilities (*P*) that the estimates of the Φ -statistics differ from zero (no differentiation) were determined by permutation analysis using 1,000 randomly permuted data matrices¹¹.

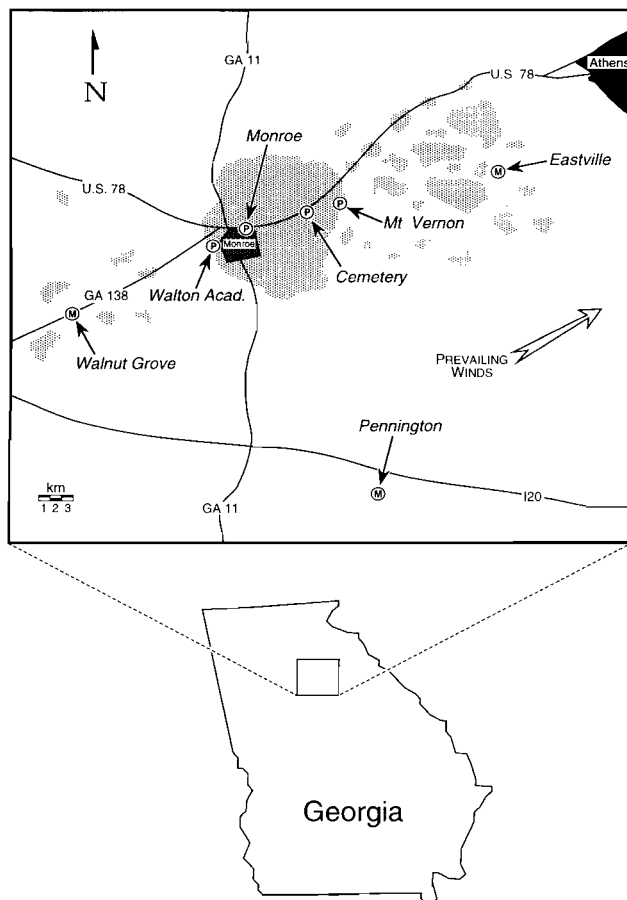


FIG. 1 Locations of seven sampling sites for monogyne (M) and polygyne (P) *S. invicta* in northern Georgia, USA. Areas in which only P nests occur are indicated by stippling; other areas contain mostly or only M nests. All 149 nests sampled at the three M sites were assigned to the monogyne form on the basis of diagnostic nestmate genotype arrays at five polymorphic allozyme loci^{19,24,28}. All 230 nests sampled at the four P sites were assigned to the polygyne form on the basis of the discovery of more than one egg-laying (wingless) queen in each. The mean direction of the prevailing winds during the main mating flights is shown¹⁵.

from a P site in Mississippi (K.G.R. and D.D.S., manuscript submitted), indicates that haplotype B occurs at the Walton Academy site because of a small amount of mtDNA leakage from the M to the P form at their interface. The pronounced mtDNA differentiation observed between the forms at our Georgia study locality is significant because nests at many of the sites are within a single dispersal distance of nests of the alternative form¹².

The importance of interform gene flow that occurs by means of dispersing P males mating with M queens was examined by studying allele frequencies at two nuclear loci under different selection regimes in the two forms. The alleles *Pgm-3^b* and *Gp-9^b* are maintained at moderate to high frequency in the P form because of strong directional selection or overdominance; these alleles are much less common (*Pgm-3^b*) or absent (*Gp-9^b*) in the M form, where such selection pressures are absent (refs 13, 14 and K.G.R., manuscript submitted). Frequencies of *Pgm-3^b* in the inferred mates of M queens are virtually identical to the frequencies in the queens and in wild-caught males sampled from the same M sites, but differ strongly from the frequencies in queens and wild-caught males from P nests (Fig. 2). Moreover, *Gp-9^b* was inferred to be completely absent in the mates of M queens, even though 50% of males from P nests are expected to carry it (K.G.R., manuscript submitted). Thus, allele frequencies in the mates of M queens at these two nuclear loci are those expected if such males derive exclusively from M nests. This conclusion again is particularly significant for the M site directly downwind of the main area of polygyny (Eastville).

These data demonstrate that three of four potential routes of gene flow do not lead to significant movement of genes between the social forms of *S. invicta*. That the final route is important in this respect is indicated by previous results showing that P queens commonly mate with and use the sperm of M males. For instance, *Pgm-3^b* and *Gp-9^b* frequencies inferred for the mates of P queens are those expected if M males predominate among these mates (ref. 10 and K.G.R., manuscript submitted), and the estimated proportion of such interform matings drops off with increasing distance from M nests¹⁵. Substantial nuclear gene flow occurring via this route also explains the fact that P populations throughout the introduced range in the US closely resemble adjacent M populations in their allele frequencies at a diversity of polymorphic nuclear loci^{10,16}.

The two social forms of *S. invicta* are thus linked by an unusual

TABLE 2 Frequencies of four mtDNA haplotypes in the monogyne and polygyne forms of *S. invicta*

	Haplotypes			
	A	B	C	D
Monogyne (M) form				
Walnut Grove	0.837	0.163	0	0
<i>N</i> = 43	(0.720–0.930)	(0.070–0.280)		
Pennington	0.894	0.053	0	0.053
<i>N</i> = 38	(0.868–1.0)	(0–0.132)		(0–0.132)
Eastville	0.867	0.118	0	0.015
<i>N</i> = 68	(0.779–0.941)	(0.044–0.191)		(0–0.044)
All Sites	0.866	0.114	0	0.020
<i>N</i> = 149	(0.805–0.919)	(0.060–0.168)		(0–0.047)
Polygyne (P) form				
Walton Academy	0.159	0.032	0.809	0
<i>N</i> = 63	(0.079–0.254)	(0–0.079)	(0.746–0.921)	
Monroe	0.167	0	0.833	0
<i>N</i> = 54	(0.074–0.259)		(0.741–0.926)	
Cemetery	0.057	0	0.943	0
<i>N</i> = 53	(0–0.132)		(0.868–1.0)	
Mt Vernon	0.700	0	0.300	0
<i>N</i> = 60	(0.583–0.800)		(0.200–0.417)	
All Sites	0.280	0.007	0.713	0
<i>N</i> = 230	(0.222–0.335)	(0–0.022)	(0.652–0.770)	

The 95% confidence intervals about the estimates (in parentheses) were obtained by drawing 1,000 bootstrap replicates from the original data sets and eliminating the 25 extreme high and 25 extreme low frequencies derived from these replicates. *N* is the number of nests (= individuals) sampled.

pattern of gene flow in which one sex mediates gene flow in only one direction. This pattern can be explained on the basis of the contrasting breeding systems and reproductive phenotypes distinguishing the two forms^{8,10,15}. M queens disperse widely during mating flights and found new colonies independently (without workers), relying on extensive nutrient reserves acquired during adult maturation to accomplish this task¹⁷. In contrast, P queens mate in their natal nest, where they attempt to become egg layers^{18,19}, or they participate in mating flights and seek adoption into other P nests^{18,20}. In either case, they initiate egg laying in an existing colony, so that large nutrient reserves are not necessary. Indeed, excessive reserves are disadvantageous to queens attempting to become egg layers in P nests, because workers of this form are intolerant of queens with 'high-reserves' phenotypes¹⁴. Thus, queens are not expected to be important agents of interform gene flow because M queens possess an inappropriate phenotype for securing adoption into P nests and the colonies they found independently invariably become monogyne^{14,21}, and because P queens generally do not have sufficient reserves to start monogyne colonies independently and they are not accepted into established M colonies^{22,23}. Males of the P form also are not expected to mediate significant interform gene flow (by mating with M queens during mating flights), because most P males (>90%) are sterile²⁴ and fertile P males may specialize in within-nest mating⁸. The remaining route of gene flow, M males mating with P queens, is expected to be important, as has been found, because operational sex ratios are highly female biased in the P form but slightly male biased in the M form²⁵. Thus, M males that disperse into P populations should achieve high mating success because of the continual availability of receptive P queens.

Despite considerable gene flow by a single route, the two social forms of *S. invicta* may be vulnerable to eventual cessation of all genetic exchange, as might occur for instance if mating flights were completely abandoned by P queens in favour of within-nest mating (loss of the mating flight coupled with adoption of within-nest mating seems to be a common evolutionary trend in polygyne ants^{8,26}). From this perspective, the loss of most routes of gene flow between the fire ant social forms may illustrate important steps in the development of complete reproductive isolation between conspecific populations that have diverged in social organization^{1,28}. These ants thus provide the most direct evidence

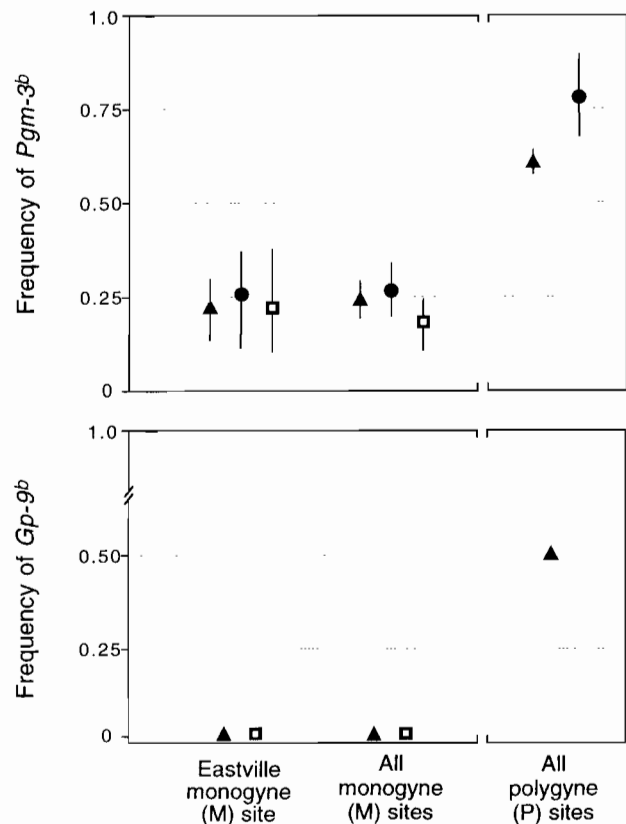


FIG. 2 Frequencies of the alleles *Pgm-3^b* and *Gp-9^b* in egg-laying queens (filled triangles) of the two social forms and in the inferred mates of monogyne (M) queens (open squares) of *S. invicta*. Also shown for comparison are *Pgm-3^b* frequencies in wild-caught males of the two forms (filled circles). *Gp-9* is not expressed in males (K.G.R., manuscript submitted), so data for this gene are not available from wild-caught males. However, because virtually all egg-laying polygyne (P) queens are *Gp-9^b* heterozygotes (K.G.R., manuscript submitted), 50% of the fertile males produced in P nests are expected to bear allele *Gp-9^b* in the hemizygous condition. Bars represent 95% confidence intervals about the frequency estimates derived from 1,000 bootstrap replicates (see Table 2).

to date for the feasibility of sympatric speciation driven by social selection. □

Methods

Mitochondrial DNA analyses. A 4-kb portion of the mtDNA (including the A + T-rich noncoding region) was amplified by polymerase chain reaction (PCR) from a single queen per nest and digested with the restriction enzymes *Bam*HI, *Dpn*II, *Eco*RV, *Eco*RI, *Hae*III, *Hha*I, *Hind*III, *Hinf*I, *Kpn*I, *Msp*I, *Rsa*I, *Taq*I and *Xba*I (K.G.R. and D.D.S., manuscript submitted). Digestion products were separated electrophoretically in 1.5% agarose gels, stained with ethidium bromide, and visualized using ultraviolet light. To confirm that the desired region of the mtDNA molecule was amplified, one end of the PCR product was sequenced and aligned with a previously published sequence from the honey bee²⁷. Maternal inheritance of the 4-kb fragment was demonstrated by examining the variable digestion products from the enzyme *Taq*I in nestmates from ten monogyne colonies. The presence or absence of restriction sites inferred using complete and partial digestion procedures defined the composite haplotypes.

Estimation of *Pgm-3^b* and *Gp-9^b* frequencies. The genotypes of M queens and their single mates were inferred by inspecting genotype arrays for female and male offspring from single M nests. Such reconstruction of parental genotypes is possible because a queen's sons arise from her unfertilized eggs and her daughters arise from eggs fertilized by the sperm of a single haploid male^{24,26}. For P queens and wild-caught males, allele frequencies were estimated using a resampling procedure when more than one individual was collected per nest¹⁵. Wild-caught males from P nests were confirmed to be fertile haploids on the basis of their size and their banding patterns at four allozyme loci¹³. Methods for scoring genotypes at *Pgm-3* and *Gp-9* and evidence for Mendelian inheritance of the products of these genes are presented elsewhere (refs 13, 28 and K.G.R., manuscript submitted). Sample sizes for estimating *Pgm-3^b* and *Gp-9^b* frequencies are as follows. Eastville M site: *Pgm-3^b*, egg-laying queens and their male mates from 41 nests, 149 wild-caught males from 19 nests; *Gp-9^b*, egg-laying queens and their male mates from 67 nests. All M sites: *Pgm-3^b*, egg-laying queens and their male mates from 118 nests, 497 wild-caught males from 62 nests; *Gp-9^b*, egg-laying queens and their male mates from 149 nests. All P sites: *Pgm-3^b*, egg-laying queens from

427 nests, 140 wild-caught males from 28 nests; *Gp-9^b*, egg-laying queens from 427 nests.

Received 16 July; accepted 27 August 1996.

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ACKNOWLEDGEMENTS. We thank J. Avise, C. DeHeer, J. Evans, M. Goodisman, L. Keller, M. A. Moran and E. Vargo for comments on the manuscript. This research was funded by grants from the National Geographic Society and the Georgia Agricultural Experiment Stations, University of Georgia. D.D.S. was supported by a grant from the US Department of Agriculture to J. Jaenike.

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