Nuclear and mitochondrial genetic structure in two social forms of the fire ant *Solenopsis invicta*: insights into transitions to an alternate social organization

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We describe genetic structure at microgeographical scales in two social forms of the introduced fire ant *Solenopsis invicta* using nuclear and mitochondrial markers. Fire ant populations in northern Georgia, U.S.A. are structured in fundamentally different ways at the two genomes. Virtually all nuclear genetic variation resides within sampling sites; no differentiation was apparent between the social forms and only minimal differentiation was found among sites in only one social form (the polygyne form, which has nests with numerous queens). In contrast, substantial mtDNA variation exists at all levels; the forms are significantly differentiated and strong divergence was detected among sites in the polygyne form. No mtDNA differentiation was found among sites in the monogyne form (which has nests with a single queen). These results are predicted on the basis of the social biology of the ants. Monogyne queens and males of both forms presumably disperse widely, and polygyne queens commonly mate with monogyne males, thus explaining the uniformity of nuclear allele frequencies across sampling sites and between the forms. Polygyne queens display habits consistent with limited dispersal, and queens of both forms seem unable to establish themselves as reproductives in nests of the alternate form, thus explaining the strong microgeographical structure within the polygyne form and differentiation between the forms found using mtDNA markers. The mtDNA haplotypes are distributed among sites in a manner that suggests a mechanism for the spread of polygyny in introduced fire ants. Monogyne queens apparently mate occasionally with polygyne males and acquire a Mendelian nuclear factor allowing the expression of polygyny in their sexual daughters.

Keywords: allozymes, fire ants, genetic structure, mtDNA, social organization, *Solenopsis invicta*.

Introduction

Many issues in evolutionary biology revolve around the existence of genetic structure in wild populations (Barton & Clark, 1990). Thus, evolutionary geneticists seek to document patterns of structure at various scales and to understand how organismal attributes generate these patterns (Avisé, 1994). This task is increasingly accomplished by combining knowledge of an organism's natural history with data from diverse molecular markers, including markers with contrasting modes of inheritance that can illuminate the roles of the different sexes and life stages

in mediating gene flow and generating structure (Avisé, 1994; Dong & Wagner, 1994; Hoelzer *et al.*, 1994).

Social organisms are appealing subjects for the study of genetic structure because their social habits can strongly limit gene flow in one or both sexes, leaving a distinctive record of structure detectable with appropriate markers (Chepko-Sade & Halpin, 1987; Shoemaker & Ross, 1996; van Staaden *et al.*, 1996). Thus, for instance, more pronounced structure may be detected with mitochondrial DNA (mtDNA) markers than markers of the nuclear genome in social animals with philopatric females and dispersing males (Hoelzer *et al.*, 1994). Moreover, the form of social organization can vary

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substantially within social species (Ross & Keller, 1995), providing useful comparative contexts for discerning how changes in social behaviour affect genetic structure.

Ants are especially good models for studying the impact of social behaviour on genetic structure for two reasons. First, there is extensive intraspecific variation in social organization, and this variation is often correlated with distinctive reproductive and dispersal behaviours (Bourke & Franks, 1995; Ross & Keller, 1995). Thus, knowledge of the social biology of local populations can be used to make predictions about genetic structure. Secondly, the evolution of social behaviour is itself expected to be influenced by local structure, most obviously because such structure reflects patterns of relatedness between interactants (Barton & Clark, 1990).

The fire ant *Solenopsis invicta*, an introduced pest in the U.S.A., displays important variation in its social organization. Two distinctive social forms exist in this species, the monogyne (**M**) form, in which a single egg-laying queen resides in each colony, and the polygyne (**P**) form, in which numerous such queens reside in each colony. The two forms differ in many features of their social biology expected to affect gene flow patterns within and between them (Ross & Keller, 1995). For instance, **M** queens disperse widely during mating flights and found new colonies without workers (independently), relying on extensive nutrient reserves acquired during adult maturation (Toom *et al.*, 1976). In contrast, **P** queens mate in the natal nest, where they attempt to become egg layers, or they participate in mating flights and seek adoption into other **P** nests (Glancey & Lofgren, 1988; Porter, 1991), and they do not acquire extensive reserves (Porter *et al.*, 1988; Keller & Ross, 1993a). The actual process of colony multiplication in the **P** form, termed budding, involves the emigration of queens and workers from the parent nest to establish a nearby daughter nest (Vargo & Porter, 1989). Queens thus appear to be much less vagile in the **P** form than the **M** form. Furthermore, the differences in phenotype between young queens of each form (e.g. in amount of nutrient reserves), coupled with a strong preference of **P** workers for the **P**-queen phenotype, make it unlikely that queens often become egg layers in nests of the alternate form (Ross & Shoemaker, 1993; Shoemaker & Ross, 1996). This suggests that gene flow between the forms can be mediated by males only. Indeed, several studies indicate that interform gene flow commonly occurs by means of **P** queens mating with migrant **M** males (Ross & Shoemaker, 1993; Ross, 1997).

The differences in social organization and its correlates between the two social forms of *S. invicta* lead to the following predictions concerning genetic structure in sympatric populations: (i) strong micro-geographical structure in mtDNA variation exists in the **P** form but is absent in the **M** form; (ii) micro-geographical structure in nuclear gene variation is absent in both forms; (iii) strong mtDNA differentiation exists between the forms; and (iv) nuclear gene differentiation is absent between the forms. We tested these predictions by surveying the hierarchical distribution of variation at nuclear and mtDNA markers in an area of sympatry in the introduced range. The patterns of genetic structure observed not only match the patterns predicted, they also yield potential insights into the manner by which polygyny spreads.

Materials and methods

Sample collection

Most samples of *S. invicta* were collected from northern Georgia, U.S.A. (Clarke, Morgan, Oconee, and Walton Counties; Fig. 1). Polygyny was first detected in this area in the late 1970s just east of the town of Monroe and was conspicuous at the time because all nearby populations were monogyne (Fletcher, 1983). Polygyny gradually expanded outward in all directions from its original locus, creating a large core **P** population in which monogyne is absent. It also spread more rapidly in a saltational manner towards the east, the direction of the prevailing winds during major mating flights, with the result that a mosaic distribution of the two forms has been created between Mt. Vernon and Eastville. The approximate chronological sequence of this eastward spread of the **P** form is indicated in Fig. 2.

Nests of the **P** form were sampled from six sites lying along a transect parallel to the prevailing winds and spanning an area from the western boundary of the core **P** population to the most easterly, recently established **P** sites outside of Athens (Fig. 1). Polygyny was confirmed in each **P** nest by finding two or more wingless queens. Nests of the **M** form were sampled from three sites, one each at the upwind and downwind ends of the **P** transect and one 30 km south of it (Fig. 1). Monogyne was confirmed in each **M** nest by examining the genotypes of at least eight nest-mates at five polymorphic allozyme loci to ensure the presence of genotype distributions expected in colonies headed by a single monandrous queen (Shoemaker *et al.*, 1992). Wingless queens from **P** nests and winged queens from **M** nests were

Fig. 1 Distributions of the monogyne (M) and polygyne (P) social forms of *Solenopsis invicta* in northern Georgia, U.S.A., and locations of nine sampling sites for this study. Areas in which only P nests occur are indicated by stippling; other areas are known or presumed to contain mostly M nests. The location where polygyny was first detected in this area (Fletcher, 1983) is indicated by the darkest shading in the centre of the core P population. The current distributions were mapped by visiting several hundred sites during 1995 and 1996 and inferring the social organization of 6–10 nests per site using informative field criteria (Greenberg *et al.*, 1985). The mean direction of the prevailing winds during major mating flights is shown.

the sources of protein and DNA used to score allozyme genotypes and mtDNA haplotypes. The mtDNA haplotypes of only a single queen per nest were scored, whereas genotypes at the nuclear loci were scored from up to 10 queens per nest.

Additional samples of polygyne *S. invicta* were collected outside of Georgia for comparative purposes. A total of 453 wingless queens collected from 30 nests in Travis Co., TX, were used to generate allozyme data, and 33 wingless queens collected from as many nests in Holmes Co., MS, were used to generate mtDNA data.

Assessment of allozyme variation

Genotypes at seven polymorphic allozyme loci (Table 1) were determined by means of starch gel electrophoresis. Each locus is effectively biallelic in the U.S.A., and progeny studies have confirmed Mendelian inheritance of their products (Shoemaker *et al.*, 1992). Data from *Pgm-3* were not included in analyses of the P sites or comparisons between the social forms because this locus is affected by selection in polygyne *S. invicta* (Ross, 1992).

Assessment of mtDNA variation

A 4-kb portion of the mtDNA (including the A+T-rich noncoding region) was PCR-amplified using the primer SR-J-14612 (5'-AGGGTATCTAA-TCCTAGTTT-3'; Simon *et al.*, 1994) and a primer we designed (DDS-COII4; 5'-TAAGATGGTAAT-GAAGAGTAG-3'). One end of the 4-kb product was sequenced and aligned with honey bee sequence (Crozier & Crozier, 1993) to confirm that the target region of the mtDNA molecule was amplified. Maternal inheritance of the fragment was demonstrated by examining the variable digestion products obtained from the restriction enzyme *TaqI* in nest-mates from 10 M colonies. PCR reactions were performed in 100 µL volumes containing 10× buffer, 0.1 mM each of dATP, dCTP, dTTP and dGTP, 3.5 mM of MgCl₂, 25 pmol of each primer, 100 ng of template DNA, and 5 U of *Taq* DNA polymerase. Amplifications were carried out in a thermal cycler programmed as follows: 1 min at 94°C for one cycle; 30 s at 94°C, 1 min at 50°C, and 4.5 min at 68°C for 30 cycles; 5 min at 72°C for one terminal cycle. The amplified PCR products were

Fig. 2 Chronological sequence of the eastward spread of the polygyne (P) form of *Solenopsis invicta* from its original locus of establishment in northern Georgia (area of darkest shading), and frequency of mtDNA haplotype C at six P sites spanning the study area. Bars indicate the 95 per cent confidence intervals about the haplotype frequencies. Haplotype C has not been found in the monogyne (M) form in Georgia.

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 (5–8 μ L per reaction). Digestion products were electrophoresed in 1.5 per cent agarose gels, stained with ethidium bromide, and visualized under UV light. The presence or absence of restriction sites inferred using complete and partial digestion procedures defined the composite haplotypes.

Genetic data analyses

Nuclear (allozyme) markers Allele frequencies at sites from which a single ant per nest was collected were computed from genotype frequencies using all individuals. The 95 per cent confidence intervals (CIs) about the allele frequency estimates for the pooled Georgia P sites were obtained by drawing 1000 bootstrap samples from the original pooled

data sets and dropping the 25 highest and 25 lowest allele frequency estimates. Genotype frequencies at each site were tested for correspondence to Hardy–Weinberg equilibrium (HWE) using χ^2 -tests with small-sample and continuity corrections (Lessios, 1992).

Estimates of allele frequencies and tests for correspondence to HWE are more complicated for the sites at which several ants per nest were genotyped because the potential nonindependence of nest-mate genotypes must be taken into account. A resampling procedure was employed in which a single genotype was drawn at random from each nest at a site, yielding a distribution of independent genotypes. Allele frequencies were estimated as the arithmetic mean frequencies for 1000 such resampled genotype distributions per site. The 95 per cent CIs about the allele frequency estimates were obtained by dropping the 50 extreme values derived from the 1000

Table 1 Frequencies of the 'a' allele at seven polymorphic allozyme loci in the monogyne and polygyne forms of *Solenopsis invicta* sampled from nine sites in northern Georgia and a single (polygyne) site in Texas

Site	Locus						
	<i>Aat-2</i>	<i>Acoh-1</i>	<i>Acoh-5</i>	<i>Est-4</i>	<i>G3pdh-1</i>	<i>Pgm-1</i>	<i>Pgm-3</i>
GEORGIA							
Monogyne (M) form							
Walnut Grove	0.931	0.795	0.712	0.658	0.761	0.798	0.806
<i>N</i> = 42, <i>n</i> = 196							
Pennington	0.864	0.887	0.743	0.613	0.613	0.754	0.716
<i>N</i> = 40, <i>n</i> = 167							
Eastville	0.901	0.793	0.720	0.584	0.694	0.819	0.743
<i>N</i> = 70, <i>n</i> = 485							
All Georgia M sites	0.899	0.819	0.724	0.611	0.691	0.796	0.753
<i>N</i> = 152, <i>n</i> = 848	(0.855–0.928)	(0.780–0.859)	(0.658–0.760)	(0.546–0.666)	(0.633–0.745)	(0.762–0.848)	(0.712–0.811)
Polygyne (P) form							
Walton Academy	0.931	0.900	0.715	0.565	0.738	0.831	—
<i>N</i> = 65, <i>n</i> = 65							
Monroe	0.936	0.909	0.722	0.629	0.692	0.797	—
<i>N</i> = 86, <i>n</i> = 86							
Cemetery	0.909	0.809	0.782	0.647	0.588	0.900	—
<i>N</i> = 85, <i>n</i> = 85							
Mt. Vernon	0.876	0.905	0.748	0.685	0.731	0.838	—
<i>N</i> = 121, <i>n</i> = 121							
Snow's Mill	0.962	0.892	0.754	0.662	0.738	0.815	—
<i>N</i> = 65, <i>n</i> = 65							
Tree Farm	0.885	0.902	0.828	0.642	0.713	0.828	—
<i>N</i> = 61, <i>n</i> = 61							
All Georgia P sites	0.911	0.890	0.757	0.639	0.694	0.836	—
<i>N</i> = 483, <i>n</i> = 483	(0.891–0.930)	(0.870–0.910)	(0.729–0.785)	(0.608–0.672)	(0.664–0.724)	(0.810–0.861)	
TEXAS (P form)							
<i>N</i> = 30, <i>n</i> = 453	0.949	0.948	0.813	0.490	0.524	0.908	—
	(0.900–1.0)	(0.883–1.0)	(0.717–0.900)	(0.367–0.600)	(0.400–0.631)	(0.833–0.967)	

The 'a' allele is defined as the most common allele at each locus in Georgia. The locus *Pgm-3* was not scored in the P form in Georgia or in Texas (where only P nests were sampled) because it is affected by selection in this form (Ross, 1992). *N* indicates the number of nests and *n* the number of individuals sampled. The 95 per cent confidence intervals about the estimates are shown in parentheses for each form in Georgia and for the single Texas site.

resampled genotype distributions. Genotype frequencies at each site were tested for correspondence to HWE by applying χ^2 -tests to each of the 1000 resampled genotype distributions and determining the proportions of tests that yielded values of χ^2 greater than the critical value for $\alpha = 0.05$.

Genetic differentiation among sites and between the forms in Georgia was assessed in several ways. (i) Fisher's exact probabilities of allelic differentiation among sites (combined over loci) were computed using the program GENEPOP (Raymond & Rousset, 1995). (ii) Genetic distances (Nei's D) were estimated for all pairs of sites using the program of Sattler & Hilburn (1985). Estimates of D were based on the six polymorphic allozyme loci as well as 72 other protein-encoding loci previously shown to be effectively monomorphic in *S. invicta* in the U.S.A. (Ross *et al.*, 1993). Mean pairwise distances and their variances were generated for particular classes of paired sites by jackknifing over the relevant pairs, and 95 per cent CIs about the means were estimated by assuming the t distribution. Also, the D -values were employed in an explicit test for differentiation between the social forms in Georgia by calculating the U -statistic of Mueller & Ayala (1982). (iii) Values of the fixation index F_{ST} were estimated at a single level (sites) and at two hierarchical levels (sites within forms) using the methods of Weir & Cockerham (1984). Bias-corrected estimates of these F -statistics and their variances were obtained by jackknifing over loci (95 per cent CIs were generated by assuming the t distribution). (iv) Values of the coefficient of gene differentiation, G_{ST} , were estimated at the same levels using the methods of Nei (1987). Estimates of these G -statistics and their variances were obtained by jackknifing over loci.

Values of F_{ST} estimated for all pairs of Georgia sites were used to compute genetically effective migration rates ($\bar{M}$) using eqn (6) of Slatkin (1993). Values of $\log_{10}(\bar{M})$ were regressed on values of $\log_{10}(d)$, where d is the geographical distance between sites, to search for any signal of isolation by distance.

mtDNA markers Haplotype frequencies at each site were computed using all sampled individuals (one per nest), with the 95 per cent CIs obtained by drawing 1000 bootstrap samples from the original data sets and dropping the 50 extreme values. Haplotype differentiation was studied using the analysis of molecular variance (AMOVA) approach of Excoffier *et al.* (1992), as implemented in the program WINAMOVA. This method partitions haplotype diversity within and between different levels of

hierarchical population subdivision, yielding analogues to F -statistics designated as Φ -statistics. The probabilities that the values of Φ_{ST} at various levels are significantly positive (indicating differentiation) were determined by permutation analysis using 1000 randomly permuted distance matrices.

Relationships among sites based on mtDNA haplotype composition

A tree (phenogram) describing the hierarchical genetic relationships of sites was constructed from the mtDNA data using the method of Holsinger & Mason-Gamer (1996), as implemented in the program NUCLEODIV. The statistical significance of each node was assessed by permutation analysis using 10000 randomly permuted haplotype distributions.

Results

Nuclear genetic structure

Only one of 36 tests (2.8 per cent) for HWE at the Georgia P sites indicated a significant departure of the observed genotype frequencies from those expected. Genotype frequencies pooled over these sites accorded with frequencies expected under HWE at all six surveyed loci. Only one of 21 tests (4.8 per cent) indicated a significant departure from HWE at the Georgia M sites, and genotype frequencies pooled over these sites accorded with HWE at all seven surveyed loci. Thus, genotype frequencies within each social form of *S. invicta* in Georgia generally match Hardy–Weinberg expectations.

Allele frequencies at the allozyme loci are shown for each site and for the sites pooled within each form in Georgia in Table 1. The two forms share identical alleles at the six loci scored in both, and each of these alleles occurs at similar frequencies at the various sampling sites. The 95 per cent CIs for the frequencies of the most common alleles overlap broadly between the forms for all but one locus. These data suggest that little nuclear genetic differentiation exists among sites or between the forms in the Georgia study area. On the other hand, there is evidence for more extensive differentiation between the Georgia and Texas ants, as the 95 per cent CIs for allele frequencies in Georgia do not overlap with those for Texas in one-third of the comparisons.

Results of the formal hierarchical analyses confirm that nuclear gene differentiation among sites and between forms is minimal in Georgia (Table 2). The great preponderance of the total nuclear diversity (around 99 per cent) resides within

Table 2 Hierarchical analysis of nuclear genetic diversity for variable allozyme loci in the monogyne and polygyne forms of *Solenopsis invicta* sampled from nine sites in northern Georgia

	Per cent of total diversity	G-statistics	F-statistics
All samples			
Between social forms	0.5	$G_{FT} = 0.005$ (−0.001–0.010)	$F_{FT} = 0.002$ (−0.003–0.007)
Among sites			
Within forms	0.7	$G_{SF} = 0.007$ (−0.007–0.020)	$F_{SF} = 0.005$ (−0.001–0.012)
All sites	(1.2)	$G_{ST} = 0.012$ (−0.002–0.025)	$F_{ST} = 0.004$ (−0.001–0.009)
Within sites	98.8		
Monogyne (M) form			
Among sites	0.3	$G_{SF} = 0.003$ (−0.013–0.018)	$F_{SF} = 0.003$ (−0.003–0.009)
Within sites	99.7		
Polygyne (P) form			
Among sites	0.8	$G_{SF} = 0.008$ (−0.015–0.031)	$F_{SF} = 0.004$ (0.000–0.009)
Within sites	99.2		

The total genetic diversity is partitioned among three levels in the upper part of the table (based on estimates of the G-statistics): between forms, among sites, and within sites. The proportion of diversity among all sites (in parentheses) is the sum of the diversity between forms and the diversity among sites within forms. The statistics G_{FT} and F_{FT} measure the extent of differentiation between forms, G_{SF} and F_{SF} measure differentiation among sites within each form, and G_{ST} and F_{ST} measure differentiation among all sites. The 95 per cent confidence intervals about the estimates of differentiation are shown in parentheses.

sites, with somewhat more of the remaining small amount apportioned among sites than between the forms. Accordingly, values of all G-statistics and F-statistics for the site and form levels are small, with the 95 per cent CIs invariably overlapping zero. Considering the two forms separately, a slightly greater proportion of the diversity occurs among sites in the P form than in the M form, and the 95 per cent CI for the value of the F-statistic measuring among-site differentiation within the P form (F_{SF}) just overlaps zero (Table 2). Thus, there is a hint of slight nuclear differentiation among sites in the P form but not the M form.

Exact tests reveal that significant allele frequency differentiation exists among all Georgia sites ($\chi^2_{12} = 25.8$, $P = 0.012$), but considering the forms separately, P sites exhibit significant differentiation ($\chi^2_{12} = 23.1$, $P = 0.027$) whereas M sites do not ($\chi^2_{14} = 12.5$, $P = 0.569$). These results support the suggestion from the estimates of F_{SF} that slight but significant microgeographical nuclear structure exists in the P form but not the M form.

Nuclear genetic structure was explored further by examining values of Nei's D estimated for all pairs of sites. Within Georgia, the average genetic distance between M sites is lower than that between P sites, although the 95 per cent CIs for the mean distance within each form overlap. The CI for the M

form overlaps zero, whereas that for the P form does not. These results again suggest that the P form but not the M form exhibits slight microgeographical nuclear structure. Average D between sites of the alternate forms is intermediate to the average D within each form and, as expected given this pattern, application of the Mueller/Ayala test shows that the between-form D -values do not differ significantly from the within-form values. This result supports the hierarchical analyses of structure showing no meaningful nuclear differentiation between the forms.

Values of D between the Georgia and Texas sites significantly exceed the values between the Georgia sites, as indicated by the fact that the 95 per cent CI for the mean D between the states does not overlap with the CIs for mean D within Georgia. This result demonstrates nuclear differentiation between ants from the two states that surpasses any microgeographical differentiation occurring within the Georgia study area.

No evidence for an effect of isolation by distance on nuclear differentiation exists for the sites within Georgia. Geographical distance (d) is a poor predictor of genetic differentiation between sites, with $\log(d)$ explaining less than 1 per cent of the variation in $\log(\hat{M})$ in the regressions ($P = 0.66$ for all sites, and $P = 0.92$ for P sites only).

Mitochondrial genetic structure

Six restriction enzymes either failed to cut the 4-kb PCR product (*EcoRV*, *EcoRI*, *KpnI*, *RsaI* and *XbaI*) or cut the product identically among all individuals (*HindIII*). The remaining seven enzymes produced variable banding patterns among individuals that constituted a total of four composite mtDNA haplotypes (designated A, B, C and D).

Haplotype frequencies at the Georgia and Mississippi study sites are shown in Table 3. In Georgia, haplotype A predominates in the **M** form, where it is present at similar frequencies at all three sites. This haplotype is highly variable in frequency in the **P** form, ranging from being almost completely absent at the Cemetery site to being virtually fixed at the Tree Farm site. Haplotype C is the most common

haplotype overall in the **P** form in Georgia (though variable from site to site) and in Mississippi, but it is completely absent in the **M** form. Pooling all sites of each form in Georgia, the 95 per cent CIs for the frequencies of three of the four haplotypes do not overlap between the forms (Table 3).

Results of the hierarchical analyses of mtDNA structure are presented in Table 4. In contrast to the nuclear markers, a large proportion of the mtDNA diversity exists at all three levels analysed: within sites, among sites, and between the forms. Considering all samples together, the greatest amount of haplotype diversity (about 40 per cent) resides within sites, with a somewhat lesser amount found among the sites within each form. Significantly, one-quarter or more of the total diversity is found between the social forms. Values for all of the

Table 3 Frequencies of four mtDNA haplotypes in the monogyne and polygyne forms of *Solenopsis invicta* sampled from nine sites in northern Georgia and a single (polygyne) site in Mississippi

Site	Haplotypes			
	A	B	C	D
GEORGIA				
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 Georgia M 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)	
Snow's Mill	0.769	0	0.231	0
<i>N</i> = 65	(0.677–0.862)		(0.138–0.323)	
Tree Farm	0.935	0.016	0.049	0
<i>N</i> = 61	(0.869–0.984)	(0–0.049)	(0–0.115)	
All Georgia P sites	0.480	0.008	0.512	0
<i>N</i> = 356	(0.430–0.531)	(0–0.020)	(0.455–0.559)	
MISSISSIPPI (P form)				
<i>N</i> = 33	0.455	0	0.545	0
	(0.303–0.636)		(0.364–0.697)	

N is the number of nests (= individuals) sampled. The 95 per cent confidence intervals about the estimates are shown in parentheses.

Φ -statistics are significantly greater than zero, indicating significant mtDNA differentiation both among sites and between the forms in Georgia.

Considering the forms separately, virtually none of the mtDNA diversity in the **M** form occurs among sites and values of Φ_{SF} are indistinguishable from zero. In contrast, over half of the diversity in the **P** form occurs among sites and values of Φ_{SF} are significantly greater than zero (Table 4). The strong mtDNA differentiation between the forms and among **P** sites contrasts markedly with the relative lack of structure revealed by the nuclear markers.

One striking feature of the mtDNA data is the existence of a cline in the frequency of haplotype C from the centre of the core **P** population to the more easterly (downwind) **P** sites (Fig. 2). This haplotype is present at high frequencies near the location where the **P** form first appeared and declines steadily in frequency at **P** sites increasingly distant in the downwind direction from this original location. At the **P** site farthest downwind from the core population (Tree Farm), fewer than 5 per cent of the ants possess this haplotype, a frequency statistically indistinguishable from zero, the frequency in the **M** form. Survey records indicate that polygyny

has spread eastward from the original site of establishment according to the time sequence depicted in Fig. 2. Thus, the frequency of haplotype C is related to the recency of appearance of polygyny at our study sites, with sites that developed polygyny most recently least resembling the original polygyne founder population.

Relationships among sites based on mtDNA haplotype composition

The tree describing hierarchical relationships among the Georgia sites based on mtDNA haplotype composition is shown in Fig. 3. The two major groups evident in this tree are distinguished by whether or not the included sites have a high frequency of haplotype C. One of the groups includes sites located around the original locus of polygyny near Monroe; all of these sites have haplotype C present at a frequency greater than 0.8. The other group includes all of the **M** sites, as well as the three **P** sites where polygyny appeared subsequent to its initial establishment; all of these sites have haplotype C present at a frequency of 0.3 or less. Within the latter group, the **P** site in which polygyny

Table 4 Hierarchical analysis of mtDNA haplotype diversity in the monogyne and polygyne forms of *Solenopsis invicta* sampled from nine sites in northern Georgia

	Equidistant metric			Euclidean metric		
	Per cent of total diversity	Φ -statistics	<i>P</i>	Per cent of total diversity	Φ -statistics	<i>P</i>
All samples						
Between social forms	24.5	$\Phi_{FT} = 0.245$	0.045	28.7	$\Phi_{FT} = 0.287$	0.022
Among sites						
Within forms	33.5	$\Phi_{SF} = 0.443$	<0.001	33.2	$\Phi_{SF} = 0.465$	<0.001
All sites	(58.0)	$\Phi_{ST} = 0.579$	<0.001	(61.9)	$\Phi_{ST} = 0.619$	<0.001
Within sites	42.0			38.1		
Monogyne (M) form						
Among sites	0	$\Phi_{SF} = 0$	0.444	0.5	$\Phi_{SF} = 0.005$	0.294
Within sites	100			99.5		
Polygyne (P) form						
Among sites	50.3	$\Phi_{SF} = 0.503$	<0.001	50.2	$\Phi_{SF} = 0.502$	<0.001
Within sites	49.7			49.8		

The equidistant metric assumes that all four observed haplotypes are equally divergent from one another, whereas the Euclidean metric is a distance measure that equals the square of the number of restriction site differences between haplotypes. The total haplotype diversity is partitioned among three levels in the upper part of the table: between forms, among sites, and within sites. The proportion of diversity among all sites (in parentheses) is the sum of the diversity between forms and the diversity 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 that the estimates of the Φ -statistics do not differ from zero (no differentiation) are shown under the heading '*P*'.

Fig. 3 Tree describing the hierarchical genetic relationships of *Solenopsis invicta* sites in northern Georgia based on mtDNA haplotype composition. The probability of the genetic differentiation at each node occurring by chance is shown. The social form at each site (M = monogyne, P = polygyne) is indicated next to the site name.

appeared most recently (Tree Farm) is more closely allied to the M sites than to other P sites. Furthermore, the Snow's Mill and Mt. Vernon P sites, which acquired polygyny before Tree Farm but were not part of the original P founder population, also show greater affinity to the M form than to the sites in the core P population. In general, the mtDNA tree features groupings of sites that are affiliated on the basis of geographical proximity and/or a common social organization.

Discussion

Queens of the two social forms of *S. invicta* apparently differ strongly in their vagility. As is frequently the case in polygyne ants (Bourke & Franks, 1995; Ross & Keller, 1995), queens of the P form seem often not to disperse very far from their natal nests. Young queens may mate and be recruited as new egg layers in their natal nest, a strategy that occurs at least occasionally in introduced populations (Porter, 1991) and is common in native populations (Ross *et al.*, 1996). Even if they do not routinely mate within their nest in Georgia, P queens may nonetheless have restricted mating flights and be adopted into P nests close to the nest in which they

eclosed (see Bourke & Franks (1995) for examples from other polygyne ants). Limited female dispersal in the P form is suggested further by the presence of budding as an important mode of colony multiplication (Vargo & Porter, 1989). In contrast, queens of the M form disperse widely during their mating flights (up to 10 km — Markin *et al.*, 1971) and these queens only take up egg laying following such a flight, typically in conjunction with independent initiation of a new colony. Colony budding is not known in the M form. Assuming minimal female-mediated gene flow between the forms, a clear prediction emerging from the distinctive queen habits is that strong among-site differentiation in mtDNA variation should occur in the P form but not the M form, as we found.

Males of both forms presumably disperse widely prior to mating, and most P queens appear to mate with males originating from M nests rather than P nests (Ross & Shoemaker, 1993). Extensive male-mediated gene flow both within and between the forms is expected to reinforce nuclear genetic homogeneity across M sites resulting from high queen vagility, and largely to erase any signature of microgeographical structure in the P form caused by limited queen vagility. The results of our hierarchical analyses of nuclear genetic structure indeed show a lack of significant differentiation among sites, although some slight allelic differentiation among sites was detected in the P form using exact tests. Thus, low vagility of P queens, perhaps coupled with limitations on the dispersal of P males (Sundström, 1995), appears to have left a weak imprint in the patterning of nuclear variation in the polygyne form. Nonetheless, nuclear gene flow is sufficiently extensive at all levels to prevent detection of any signal of isolation by distance among sites.

Significant local genetic structure has been detected in a diversity of polygyne ants using nuclear or mtDNA markers (Ross & Keller, 1995), although the two sets of markers have not previously been employed in concert to study microgeographical structure in social insects. Studies using nuclear markers have documented the occurrence of local structure in polygyne populations and its absence in monogyne populations of the same or closely related species in the ant genera *Formica* (Pamilo & Rosengren, 1984; Sundström, 1993) and *Myrmica* (Seppä & Pamilo, 1995; Bansbach & Herbers, 1996), as well as in native *S. invicta* (unpublished data). Such studies constitute the most compelling evidence that differences in social organization and its correlates lead to important differences in gene flow regimes and consequent variation in genetic

structure. Even greater structure is to be expected for mtDNA markers in such polygyne ants, given that dispersal often may be accomplished primarily by males rather than queens (Sundström, 1995).

Physiological and behavioural differences in young queens of the two forms of *S. invicta*, coupled with preferences of **P** workers during the process of queen adoption, make it unlikely that queens often become egg layers in nests of the alternate type (thereby effecting interform gene flow). Young **M** queens acquire extensive nutrient reserves essential for long-distance mating flights and independent colony foundation (Toom *et al.*, 1976). Young **P** queens generally acquire far fewer reserves and forego attempts at independent nest foundation, instead seeking adoption into their own or another **P** nest (Keller & Ross, 1993a; Ross & Keller, 1995). Lack of reserves is advantageous to young **P** queens seeking adoption because **P** workers have evolved a strong preference for queens with such phenotypes (Keller & Ross, 1993b), but it is strongly disadvantageous to queens attempting to start a single-queen nest independently (Porter *et al.*, 1988). Thus, young **P** queens are physiologically ill-suited to initiate **M** nests, and young **M** queens possess inappropriate phenotypes for adoption into **P** nests. The specialized queen phenotypes produced by each form thus seem to generate significant obstacles to queen-mediated gene flow between them, leading to the strong interform mtDNA differentiation we report.

Few obstacles to interform gene flow occurring via males mating with queens of the alternate form are apparent from the natural history of these ants, and indeed, studies employing nuclear markers under differential selection in the two forms have demonstrated that **P** queens commonly mate with **M** males (although the converse type of mating, **M** queens with **P** males, seems to be rare; Shoemaker & Ross, 1996). Extensive interform gene flow mediated by dispersing males is expected to homogenize the two forms at neutral nuclear genes, and as predicted, no evidence of differentiation between the forms was discerned at six allozyme loci. That these six loci are sufficiently sensitive markers for detecting structure caused by restricted gene flow is indicated by the significant differentiation found between the Georgia and Texas ants.

Fire ant populations in northern Georgia thus are structured in fundamentally different ways at their mitochondrial and nuclear genomes. Restricted queen dispersal in the **P** form and the inability of queens of either form to become reproductives in nests of the alternate type generate extensive

mtDNA differentiation both among **P** sites and between the forms. Extensive male dispersal coupled with the success of **M** males in inseminating **P** queens lead to a lack of substantial nuclear gene differentiation at either level.

Scenario for the initiation and spread of polygyny

The clinal distribution of mtDNA variation in the **P** form in our study area, combined with recent data on the genetic basis of polygyny in *S. invicta*, suggest a mechanism by which polygyny arises and spreads in introduced fire ants. A single Mendelian factor, designated here as *pf*⁺ (for *polygyny factor*), appears to be required for the expression of polygyny in *S. invicta*. This has been shown by virtue of the fact that all egg-laying **P** queens possess the single heterozygous genotype *Bb* at the marker gene *Gp-9*, whereas all **M** queens possess the homozygous genotype *BB* at this locus (Ross, 1997). Thus, allele *Gp-9*^b is in complete gametic disequilibrium with *pf*⁺ and *Gp-9*^B is in disequilibrium with the allelic factor *pf*⁻. Polygyny has spread rapidly eastward from its site of origin near Monroe, and the points where it has arisen more recently show a decreasing resemblance to the founder population in their frequency of haplotype C (Fig. 2), with the frequency at the site where polygyny appeared most recently (Tree Farm) statistically indistinguishable from the frequency in the **M** form (zero). This suggests that polygyny has spread by means of monogyne queen lineages converting to polygyny, most likely as a result of acquiring *pf*⁺.

Monogyne queen lineages presumably acquire *pf*⁺ when **M** queens mate with **P** males that bear this factor (step 1 in Fig. 4). Such matings appear to be rare (Shoemaker & Ross, 1996), but should be most common downwind (east) of the core **P** population and at zones of contact between large 'pure' stands of each social form. **M** queens mated to **P** males bearing *pf*⁺ establish monogyne nests in which all female progeny have the genotype *pf*⁺/*pf*⁻. Thus, the new female sexuals produced by such a colony can form multiple-queen colonies that will accept other *pf*⁺/*pf*⁻ queens (step 2). Indeed, the fact that such heterozygous queens lack the extensive reserves required for independent founding (L. Keller & K. G. Ross, unpublished) and that they have never been found heading mature **M** colonies (Shoemaker & Ross, 1996) suggests that they *must* associate to found polygyne nests or join previously established polygyne nests.

Once polygyny originates in a monogyne maternal lineage, the new polygyne nests will continually

Fig. 4 Scenario for the spread of the polygyne social organization of *Solenopsis invicta* in the study area. Monogyne queens never possess mtDNA haplotype C nor the nuclear factor inducing polygyny, pf^+ . These queens occasionally mate with polygyne males that possess pf^+ (step 1), and the female sexual offspring from such matings, which possess genotype pf^+/pf^- on a monogyne mtDNA background (non-C haplotype), start polygyne colonies or join previously established polygyne colonies (step 2). Such new polygyne populations, which contain mostly monogyne mtDNA, increasingly adopt immigrant polygyne queens from upwind polygyne sites with higher frequencies of haplotype C. Thus, new polygyne populations eventually shift from high frequencies of non-C haplotypes to high frequencies of haplotype C.

accept queens with genotype pf^+/pf^- . Early on, these will be mostly queens with monogyne mtDNA derived from the original conversion of monogyne maternal lineages with pf^+ , but as the incipient polygyne population develops and P sites with high frequencies of polygyne mtDNA encroach from the west, an increasing frequency of adopted queens will be P queens with polygyne mtDNA immigrating from an upwind source (step 3). Thus, newly arisen polygyne populations are expected to contain mostly monogyne mtDNA when young, but to acquire polygyne mtDNA at increasing rates as they age. This explains the association between date of appearance of polygyny and frequency of haplotype C in the sites downwind of the core P population. This scenario implies that new P sites are more closely related to the local M form than to nearby P sites at their mtDNA when they first arise, but that they become increasingly similar to the source population for immigrant P queens over time. The tree of relationships among our study sites based on mtDNA haplotype composition (Fig. 3) accords with this expectation.

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