Evolution, 41(5), 1987, pp. 979–990

COMPARATIVE BIOCHEMICAL GENETICS OF THREE FIRE ANT SPECIES IN NORTH AMERICA, WITH SPECIAL REFERENCE TO THE TWO SOCIAL FORMS OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE)

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Abstract.—An electrophoretic study of the genetics of three fire ant species in North America was undertaken with the primary objective of further clarifying the genetic relationship between two social forms of *Solenopsis invicta*. Such social forms are common in many groups of ants and may, in some cases, represent significant intermediate stages in the speciation process. The monogyne and polygyne forms of *S. invicta*, while differing in a number of important biological traits, are genetically indistinguishable, in contrast to the substantial genetic differentiation observed between *S. invicta* and a second, closely related, introduced species, *S. richteri*. The native fire ant, *S. geminata*, is genetically the most distinct of the three species studied, in accord with its taxonomic placement in a different species complex. Hypotheses concerning the derivation of the polygyne form of *S. invicta* from the monogyne form which invoke their long-term reproductive isolation in South America and separate introductions to North America appear unfounded.

Although *S. invicta* and *S. richteri* are known to hybridize in North America, our study provided no evidence of gene introgression between *S. invicta* and the native species, *S. geminata*, in areas where our samples were collected. Analyses of population structure in *S. invicta* failed to reveal significant differentiation of populations or local inbreeding. Levels of genetic diversity in the three species studied, although not significantly different, were in the order predicted from knowledge of the population biology and recent history of the taxa, with *S. richteri* exhibiting the least diversity, *S. geminata* the greatest, and *S. invicta* having an intermediate level.

Received December 30, 1986. Accepted May 19, 1987

Ants comprise a diverse and successful lineage of highly social insects that display a remarkable array of life histories and habits. The presence, in several disparate taxa, of clusters of closely related social forms differing profoundly in colony social organization and reproductive strategies, while exhibiting only slight morphological differentiation, suggests that behavioral evolution is decoupled from morphological evolution in these groups and that such behavioral evolution may be an important component in the process of speciation (Wilson, 1971; Crozier, 1977*a*, 1981; Brian, 1983). The existence of these behaviorally distinct groups, while presenting numerous taxonomic challenges, also affords the opportunity for investigating in a comparative manner the evolution of reproductive strategies in ants, as well as the relationship between social evolution and speciation.

The *Solenopsis saevissima* complex of fire ants of the New World tropics and subtropics includes a profusion of endemic and broadly distributed forms which are rather indistinct morphologically and whose taxonomic status thus remains unsettled (e.g., Creighton, 1930; Wilson, 1952; Brand et al., 1972; Buren, 1972; J. C. Trager, unpubl.). At least two morphologically recognizable forms were introduced to the southeastern U.S.A. earlier in this century and are now regarded as significant pests in many areas. The two forms, originally considered subspecies by Wilson (1953), were later accorded species status as *Solenopsis invicta* and *richteri* by Buren (1972). Recent biochemical and genetic data (Vander Meer et al., 1985; Ross et al., 1987) indicate that the two species are distinct over the greater part of their North American ranges but that substantial hybrid populations do occur in some areas.

Solenopsis invicta, the more widespread and successful of the two introduced fire ant species, is in turn comprised of two distinctive social forms. The monogyne form features colonies containing only a single inseminated egg-laying queen, while the

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polygyne form is characterized by colonies containing several to hundreds of functional queens (Glancey et al., 1973; Fletcher et al., 1980; Fletcher, 1983; Lofgren and Williams, 1984; Ross and Fletcher, 1985*a*). Colonies of the polygyne form most often occur in discrete populations, which are widely distributed throughout the North American range. Polygyne populations of *S. invicta* are invariably embedded within the surrounding monogyne populations, although the origin of polygyne populations may take place in relative geographic isolation from the monogyne form (Fletcher, 1983). The polygyne form differs from the more common monogyne form not only in its distinctive social organization, but also in the diminutive size of workers and the likely adoption of budding as a mode of colony reproduction (Fletcher, 1983; Greenberg et al., 1985). Given the significant behavioral and subtle morphological differences between the forms, questions have arisen over their genetic and evolutionary relationship, including whether they are reproductively isolated on this continent, whether they have been reproductively isolated over evolutionary time in South America, and whether they may be the products of at least two distinct introductions, as are monogyne *S. invicta* and *S. richteri.* Answers to questions such as these may further inform discussions of the apparent link between shifts in social organization and speciation in ants.

In this paper we take a step toward resolving these questions by means of a comparative biochemical genetic survey of the two social forms of *S. invicta.* Meaningful interpretation of the results of such a study can only be accomplished by establishing a system of reference for assessing observed genetic differentiation between the groups of interest. This is done by two means here: 1) the range of genetic variability observed within each social form of *S. invicta* over a broad geographical area is compared to the extent of genetic differentiation between the forms; and 2) this latter difference is in turn compared to the extent of genetic differentiation between *S. invicta* and *S. richteri.* This second species is an ideal outgroup for evaluating genetic differentiation between the two social forms of *S. invicta* because of the obvious affinities between *S. invicta* and *S. richteri* (e.g., they produce viable F_1 hybrids [Ross et al., 1987]).

The existence of substantial hybrid populations of the two introduced species, while serving to indicate high genetic compatibility between them, also points to a related area of inquiry relevant to the main topic: the possible occurrence of introgression between the introduced species and native fire ants. This is of particular importance for *S. invicta* and *S. geminata,* which are broadly sympatric over a large part of the southern U.S.A. (Bass and Hays, 1976; Wojcik et al., 1976). Thus *S. geminata* was included in this study to look for evidence of possible introgression, as well as to provide another point of reference for genetic differentiation in this group of ants, in this case between species in different species complexes.

Materials and Methods

Collection of Samples

The collection protocol for monogyne (M) and polygyne (P) *S. invicta* was designed to reveal the extent of genetic diversity present throughout the North American distributions of the two forms, while at the same time taking into account any large-scale geographic structure that might influence interpretations of interform differentiation. This was accomplished by sampling five discrete, widely separated P populations and pairing each with a sample from the nearest M population that could be located (Fig. 1). These paired samples were collected in Tangipahoa Parish, LA; Jackson Co., MS; Tuscaloosa Co., AL; Morgan Co. (M) and Walton Co. (P), GA; and Alachua Co. (M) and Marion Co. (P), FL. Polygyne populations were unambiguously distinguished from monogyne populations on the basis of worker size and mound density (Fletcher, 1983; Greenberg et al., 1985), observation of dealate queens, colony genotype distributions (Ross and Fletcher, 1985*a*), and presence of diploid males (Ross and Fletcher, 1985*b*; Ross, unpubl.). In addition to the five pairs of samples, four other samples from M populations were collected, one each from Webster Parish, LA; Turner Co., GA; Bamberg Co., SC; and Brunswick Co., NC (Fig. 1). *Solenopsis richteri* presently occurs

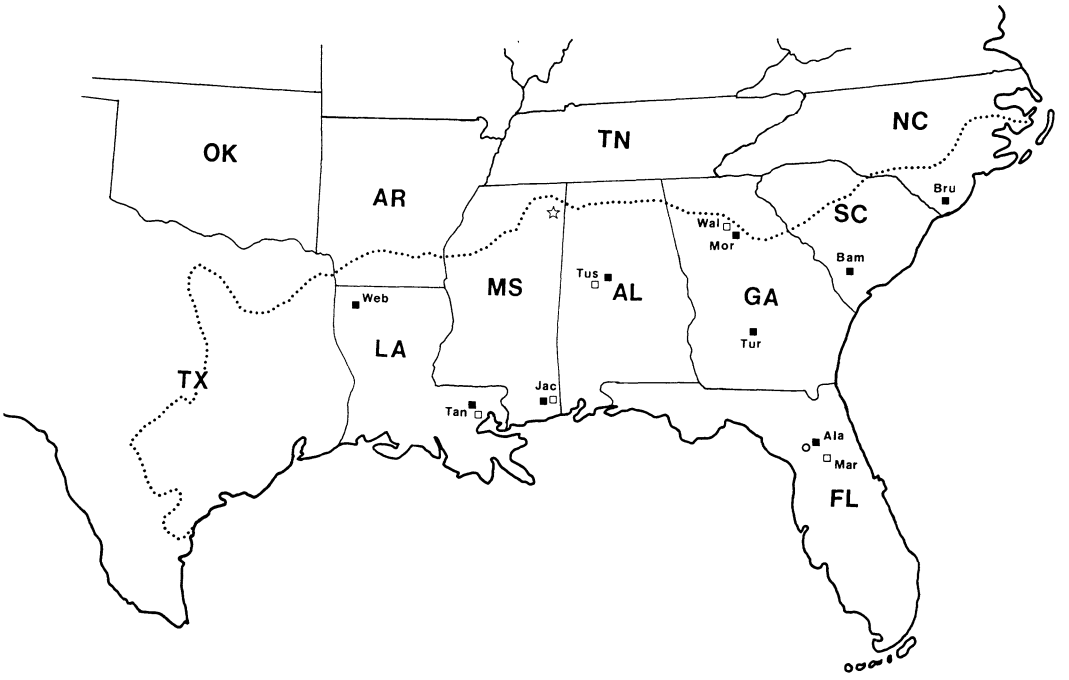

FIG. 1. Approximate limit of distribution of introduced fire ants in North America (dotted line) and locations of samples of monogyne (■) and polygyne (□) *S. invicta, S. richteri* (☆), and *S. geminata* (○) for electrophoretic analysis. Sampled *S. invicta* populations are identified by the first three letters of the county where samples were collected.

in a rather limited area of northern Mississippi and Alabama (Buren et al., 1974; Vander Meer et al., 1985), so that only a single sample was collected from northeastern Mississippi (Prentiss, Itawamba, and Tishomingo counties). A single sample of the "red form" of *S. geminata* (sensu Wilson, 1971; Wojcik et al., 1976) was collected from Alachua Co., FL. Nests sampled for each *S. invicta* population were generally located within an area no greater than 3–5 hectares, while nests included in the *S. richteri* and *S. geminata* samples were distributed over much larger areas (ca. several hundred hectares).

For each sample of *S. invicta* and for *S. geminata,* several winged queens were collected from each of 30 nests (31 nests for the Alachua, FL, M population), whereas queens were collected from 58 nests in the *S. richteri* population. Queens were held in liquid nitrogen in the field until they were returned to the laboratory; then, they were held in a freezer at −60°C until electrophoresis. Only a single queen genotype per locus was scored for each nest because of the high correlation between genotypes of female nestmates in M colonies (Ross and Fletcher, 1985*a*; Ross et al., 1987, unpubl.). Thus 60 genomes were studied for each sample of *S. invicta* (62 for Alachua Co., FL, sample; total of 842) and for *S. geminata,* while 116 genomes were studied for *S. richteri.*

Gel Electrophoresis

Horizontal-gel electrophoresis was conducted on 14% starch gels using standard procedures (Harris and Hopkinson, 1976; May et al., 1979; Ross et al., 1985). Four buffer systems were utilized (C, M, 4, and R; see Ross et al. [1985] for references); staining followed Brewer (1970), Shaw and Prasad (1970), and Ross and Fletcher (1985*a*). The gasters and alitrunks of queens were separated to extend the material and improve resolution of the staining bands. Nineteen enzyme systems and general protein (PRO) were studied, yielding the products of 26 presumptive loci (as indicated by discrete zones of activity). The enzymes

studied (with body part and buffer system utilized) were: aldolase (ALD, EC 4.1.2.13, alitrunk, "C"); alpha-glycerophosphate dehydrogenase (AGP, 1.1.1.8, alitrunk and gaster [2 loci], "C"); lactate dehydrogenase (LDH, 1.1.1.27, alitrunk [2 loci], "C"); phosphogluconate dehydrogenase (PGD, 1.1.1.44, alitrunk, "C"); esterase with either naphthol AS acetate or alpha- and beta-naphthyl acetates as substrates (EST, 3.1.1.1, alitrunk [3 loci], "C"); octanol dehydrogenase (ODH, 1.1.1.73, alitrunk, "C"); superoxide dismutase (SOD, 1.15.1.1, alitrunk, "C"); phosphoglucomutase (PGM, 2.7.5.1, gaster,"C"); glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.2.1.12, gaster, "C"); malate dehydrogenase (MDH, 1.1.1.37, gaster, "C" and "4" [2 loci]); glutathione reductase (GR, 1.6.4.2, gaster, "M"); diaphorase (DIA, 1.6.4.3, gaster, "4"); glucosephosphate isomerase (GPI, 5.3.1.9, gaster, "4"); peptidase with phenylalanyl-proline as substrate (PEP-PAP, 3.4.13.9, alitrunk and gaster [2 loci], "R"); peptidase with glycyl-leucine as substrate (PEP-GL, 3.4.13.2, alitrunk, "R"); leucine aminopeptidase (LAP, 3.4.11.1, alitrunk, "R"); and mannosephosphate isomerase (MPI, 5.3.1.8, gaster,"R"). The general protein stain yielded the products of three loci using the alitrunk and gaster with the "M" buffer system. Mendelian inheritance of the products of polymorphic loci (i.e., those loci for which the most common allele occurs at a frequency of less than 0.99) in *S. invicta* and *S. richteri* has been demonstrated by Ross and Fletcher (1985*a*) and Ross et al. (1987). Mendelian inheritance for polymorphic loci of *S. geminata* and colony genetic structures for *S. richteri, S. geminata,* and hybrid *S. invicta/richteri* will be reported elsewhere.

Data Analyses

Gene diversity (expected heterozygosity, H_{exp}) estimates for the taxa were calculated from allele frequencies using the formulas of Nei (1978). Because homologous loci were studied for all taxa, heterozygosity values were compared using paired-sample t tests on the arcsine square-root transformed data (Archie, 1985). Observed heterozygosity (H_{obs}) is the mean proportion of heterozygotes observed at the 26 loci studied.

Single-locus population structure of *S. invicta* was investigated using Wright's (1951) F statistics, as estimated by the procedures of Weir and Cockerham (1984). These estimates are relatively insensitive to sampling biases introduced via limited numbers of samples and individual sample sizes. Variances of the estimates were generated using a jackknife procedure over populations (Weir and Cockerham, 1984), because of the low number of polymorphic loci. Confidence limits (95%) were generated from the jackknife variances based on the t distribution. Observed genotype frequencies for polymorphic loci in all populations of *S. invicta,* as well as in *S. richteri* and *S. geminata,* were tested for conformity to frequencies expected under Hardy-Weinberg equilibrium using the goodness-of-fit χ^2 statistic.

Genetic distances (Nei, 1972) between pairs of *S. invicta* populations and among the three *Solenopsis* species were calculated using the jackknife approach of Mueller and Ayala (1982), as implemented in the program of Sattler and Hilburn (1985). These estimates are corrected for biases from limited sample sizes (Nei, 1978) and limited numbers of loci studied (Mueller and Ayala, 1982), as well as for unequal rates of evolution at the loci (Hillis, 1984). Variances were generated from the jackknife estimates over the loci. The program also provides for a test of significant genetic differentiation between two groups of populations (in this case the M and P forms of *S. invicta*) by comparing genetic distances between and within the two groups (see Mueller and Ayala, 1982). The test statistic is U, which differs significantly from zero only if the two groups of populations are genetically distinct.

RESULTS

Genetic Diversity

Solenopsis invicta is characterized by a predominance of monomorphic loci, as are its two congeners (Table 1). Only two loci exhibit substantial variability in *S. invicta*; both *Agp-1* and *Est-4* are diallelic, with the most common alleles present at frequencies of 0.669 and 0.586, respectively (Table 2). These alleles occur at similar frequencies in

TABLE 1. Proportion of loci polymorphic (P), gene diversity (expected heterozygosity, H_{exp}), and average observed heterozygosity per locus (H_{obs}) for three fire ant species. A locus is considered polymorphic when the most common allele is present at a frequency of less than 0.99. Number of genomes studied is indicated in parentheses.

Species	P	H_{exp}	H_{obs}
Solenopsis richteri (N = 116)	0.077	0.020	0.021
Solenopsis invicta			
Monogyne form (N = 542)	0.077	0.037	0.032
Polygyne form (N = 300)	0.077	0.037	0.037
All populations (N = 842)	0.077	0.037	0.034
Solenopsis geminata (N = 60)	0.192	0.048	0.044

the M and P forms (see below; Table 2). Rare alleles (all frequencies < 0.006) were found at seven loci (*Pro-1, Pro-3, Mdh-2, Dia, Gapdh, Lap,* and *Mpi*) and were generally distributed evenly between the two forms and sporadically throughout the geographic range. An exception is *Lap,* where three of the five observed heterozygotes bearing the rare allele were from the Morgan Co., GA, monogyne population.

Two loci (*Est-2* and *Pro-3*) are defined as polymorphic in *S. richteri* (Table 3), although only the diallelic locus *Est-2* exhibits useful variability. A rare alternate allele at the locus *Agp-1* is present at a frequency of 0.009. Five loci of *S. geminata* are defined as polymorphic (*Ldh-1, Pgd-2, Odh, Gpi,* and *Mpi*; Table 3), although only *Ldh-1, Odh,* and *Gpi* exhibit individual locus heterozygosities (h_i) greater than 0.200. Polymorphisms were found at three additional loci (*Agp-1, Est-1,* and *Pro-3*) in a limited sample of three colonies of "dark form" *S. geminata* collected alongside the 30 "red form" colonies (Ross, unpubl.), suggesting that the two color-forms of this "species" may be genetically distinct. Therefore, only the red form is considered in this paper.

Among the three species studied, *S. geminata* exhibits the greatest heterozygosity and *S. richteri* exhibits the least, while *S. invicta* exhibits an intermediate level (Table 1). Although none of the differences in expected

TABLE 2. Allele frequencies at two diallelic loci (*Agp-1, Est-4*) for two social forms of *S. invicta.* Populations are identified as monogyne (M) or polygyne (P) as well as by the first three letters of the county in which they were sampled (see Fig. 1). Sixty genomes were sampled from each population (62 from Ala M). Subscripts denote allelic designations at each locus.

	Agp-1		*Est-4*	
	p_F	q_S	p_A	q_B
Ala M	0.694	0.306	0.387	0.613
Tur M	0.450	0.550	0.750	0.250
Mor M	0.650	0.350	0.550	0.450
Tus M	0.750	0.250	0.650	0.350
Jac M	0.717	0.283	0.617	0.383
Tan M	0.750	0.250	0.567	0.433
Web M	0.700	0.300	0.817	0.183
Bam M	0.800	0.200	0.533	0.467
Bru M	0.650	0.350	0.283	0.717
All M populations	0.685	0.315	0.573	0.427
Mar P	0.617	0.383	0.450	0.550
Wal P	0.667	0.333	0.733	0.267
Tus P	0.600	0.400	0.533	0.467
Jac P	0.617	0.383	0.717	0.283
Tan P	0.700	0.300	0.617	0.383
All P populations	0.640	0.360	0.610	0.390
All populations	0.669	0.331	0.586	0.414

heterozygosity is statistically significant (paired t tests, all $P > 0.05$), these differences may, nonetheless, be biologically relevant, given the limited sensitivity of such tests at low heterozygosity levels (see Archie, 1985). The close matches between values for expected and observed heterozygosity within all of the taxa (Table 1) suggest that deviations from panmixis are relatively unimportant in the populations (see also below).

Population Structure

The sampling design for this study lends itself to a first analysis of large-scale population structure in North American *S. invicta. F* statistics for the polymorphic loci *Agp-1* and *Est-4* for the monogyne and polygyne populations, as well as for all populations considered together, are presented in Table 4. F_{ST}, which is the correlation between genes of different individuals within populations, indicates the extent of genetic differentiation among the populations (Nei, 1977; Weir and Cockerham, 1984). Values are relatively low for both loci for

TABLE 3. Allele frequencies at two polymorphic loci (*Est-2* and *Pro-3*) for a single population of *S. richteri* from northeastern Mississippi (116 genomes sampled) and at five polymorphic loci (*Ldh-1, Pgd-2, Odh, Gpi,* and *Mpi*) for a single population of *S. geminata* from Alachua Co., Florida (60 genomes sampled). Subscripts denote allelic designations at each locus.

S. richteri			
Est-2		*Pro-3*	
p_F	q_S	p_1	q_2
0.612	0.388	0.983	0.017

S. geminata											
Ldh-1				*Pgd-2*		*Odh*		*Gpi*		*Mpi*	
p_1	q_2	r_3	s_0	p_2	q_3	p_8	q_9	p_1	q_2	p_2	q_1
0.866	0.017	0.067	0.050	0.983	0.017	0.567	0.433	0.783	0.217	0.933	0.067

both social forms, but they are consistently and significantly greater than zero for the M form and for all populations considered together. These results may reflect a moderate degree of large-scale geographic structure in *S. invicta* but should be viewed with caution in light of the limited sample sizes available for each population. In any case, it is clear that more than 90–95% of the observed genetic variability resides within small populations, such as those which we sampled. Allelic differentiation among populations at the polymorphic loci *Agp-1* and *Est-4* is shown in Table 2.

The parameters F_{IS} and F_{IT} are the correlations of genes within individuals within populations and over the entire range, respectively (Weir and Cockerham, 1984). They may also be viewed as measures of deviations of genotype frequencies from those expected under Hardy-Weinberg equilibrium within the populations (F_{IS}) and over the range (F_{IT}) (Nei, 1977). With the exception of the *Est-4* locus in the Tangipahoa Parish, LA, monogyne population, all populations were in Hardy-Weinberg equilibrium for both polymorphic loci (96.4% of χ^2 tests with $P > 0.05$). Cases of heterozygote deficiency outnumbered cases of heterozygote excess for both loci in the M populations, as is reflected in the significant positive F_{IS} values for this form. In P populations, observed frequencies of heterozygotes were generally quite close to those predicted. When genotypes from all M populations were pooled, significant deficiencies of heterozygotes were observed at both loci (χ^2 tests, both $P < 0.05$), as was also true for the *Est-4* locus when genotypes from all populations of both forms were pooled. This is reflected in the rather substantial F_{IT} values for the M form and pooled populations, which result from the combined effects of slight heterozygote deficiencies within M populations as well as a mild Wahlund effect associated with some population differentiation.

Genotype frequencies at the highly poly-

TABLE 4. *F* statistics for monogyne and polygyne *S. invicta* populations for two diallelic loci. Standard errors from jackknife estimates of variances are included in parentheses. Asterisks indicate values significantly different from zero.

	Agp-1			*Est-4*		
	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}
Monogyne populations ($N = 9$)	0.029* (0.009)	0.098* (0.006)	0.124* (0.007)	0.095* (0.011)	0.063* (0.008)	0.152* (0.008)
Polygyne populations ($N = 5$)	−0.006 (0.001)	−0.060 (0.025)	−0.069 (0.025)	0.044* (0.007)	0.000 (0.012)	0.045* (0.004)
All populations ($N = 14$)	0.016* (0.007)	0.038* (0.004)	0.053* (0.004)	0.073* (0.003)	0.040* (0.002)	0.110* (0.003)

morphic loci of *S. richteri* (*Est-2*) and *S. geminata* (*Gpi* and *Odh*) were in relatively good agreement with expected Hardy-Weinberg frequencies (χ^2 tests, all $P > 0.05$). Slight excesses of heterozygotes were observed at *Est-2* in *S. richteri* and *Gpi* in *S. geminata* ($F_{IS} = -0.044$ and -0.062, respectively), whereas a deficiency of heterozygotes was observed at *Odh* in *S. geminata* ($F_{IS} = 0.202$).

Genetic Differentiation

With few exceptions, the M and P forms of *S. invicta* share identical alleles at the 26 loci studied (see also Ross et al. [1985]). The exceptions include extremely rare alleles at five loci that occurred only in single heterozygous individuals, and a rare alternate allele at the *Lap* locus that was present in five heterozygotes from M populations. At the polymorphic loci *Agp-1* and *Est-4*, allele frequencies are similar between the forms (Table 2; also Ross and Fletcher [1985*a*]), with the range of values for each locus from P populations completely bracketed by values from individual M populations.

Because a majority of the *S. invicta* loci studied are monomorphic and populations exhibit relatively similar allele frequencies at *Agp-1* and *Est-4*, genetic distances between pairs of populations are extremely low (Table 5), with a mean value for all populations of 0.003. The mean value for distances between population pairs with contrasting social organization (0.002) is not particularly large relative to mean values within social forms (0.004 and 0.001), and, not surprisingly, no significant genetic differentiation between the two types of populations was found using the method of Mueller and Ayala (1982) (95% confidence limits around U: -0.0030–0.0009). Of particular interest is the exceptionally low mean genetic distance between the five geographically paired M and P populations (0.0004, range 0–0.001). To test the possibility that this value is significantly less than the mean genetic distance between populations within social forms, a randomization test was conducted in which a distribution was constructed from the means of 1,000 sets of five distance values. Each set was drawn randomly and independently from the matrix of within-form distance values, and each draw in a set was taken without replacement. The mean genetic distance between the paired M and P populations falls well within the 5% tail at the low end of the constructed distribution ($\bar{x} = 0.0033$, SD = 0.0017), indicating that geographical proximity of the populations is a more important determinant of genetic similarity than is possession of a common social organization.

TABLE 5. Mean genetic distances ($\bar{D} \pm$ SE) (Nei, 1972) within and between the monogyne (M) and polygyne (P) social forms of *S. invicta* and between the three fire ant species studied. Numbers of pairwise values averaged for *S. invicta* are indicated in parentheses, as are ranges of distance values.

Species and forms	$\bar{D}$
S. invicta	
M populations ($N = 36$)	0.004 ± 0.001 (0–0.016)
P populations ($N = 10$)	0.001 ± 0.001 (0–0.005)
M populations/P populations ($N = 45$)	0.002 ± 0.001 (0–0.012)
All populations ($N = 91$)	0.003 ± 0.001 (0–0.016)
S. invicta/S. richteri	0.176 ± 0.084
S. invicta/S. geminata	0.471 ± 0.155
S. richteri/S. geminata	0.597 ± 0.182

In contrast to the lack of differentiation between the two social forms of *S. invicta*, this species and *S. richteri* are well differentiated genetically (Table 5). The two species share no alleles at four (15.4%) of the loci (*Gpi, Ald, Odh,* and *Est-2*), and the most common alleles at *Agp-1* and at *Est-4* in *S. invicta* are essentially fixed in *S. richteri* (see also Ross et al. [1987]).

As expected by virtue of its taxonomic placement in a separate species complex, the genetic similarity of *S. geminata* to its two congeners is considerably less than their similarity to one another (Table 5). *S. geminata* is completely distinct from *S. invicta* (i.e., they have no alleles in common) at nine (34.6%) of the loci, and it is completely distinct from *S. richteri* at 11 (42.3%) of the loci. The fact that no alleles are shared between *S. invicta* and *S. geminata* at one-third of the loci studied indicates that no significant introgression occurs between these species in the areas of sympatry where

our samples were taken (Alachua Co. and Marion Co., FL; Bamberg Co., SC [see Wojcik et al., 1976]). The ranges of *S. richteri* and *S. geminata* do not overlap as far as is known, thus precluding the possibility of hybridization between these species.

Discussion

The results of this study indicate that the monogyne and polygyne forms of *Solenopsis invicta* are genetically quite indistinguishable. This conclusion rests, in large part, on the comparatively much greater genetic differentiation observed between *S. invicta* and *S. richteri,* the outgroup species chosen for comparison. A close genetic relationship between *S. richteri* and *S. invicta* is evidenced by the fact that they were, until recently, considered to be conspecific (Wilson, 1953), as well as by the fact that they produce viable F_1 hybrids in areas where introgression is occurring (Ross et al., 1987). Thus, the genetic similarity between these two species can serve as an approximate standard for the expected level of genetic differentiation between cryptic species in the *Solenopsis saevissima* species complex. The genetic distance between *S. richteri* and *S. invicta* (0.176) is conspicuously greater than the average genetic distance between the M and P forms of *S. invicta* (0.002). The former value is rather representative for cryptic species in ants (Crozier, 1977*b*; Pamilo et al., 1979; Ward, 1980; Ross, 1987), whereas the latter value appears low even for conspecific ant populations (Crozier, 1977*b*; Pamilo et al., 1979; Ward, 1980; Halliday, 1981; Ross, 1987), presumably due to the lack of genetic variability in introduced *S. invicta* (see also Pamilo [1983]).

Several other independent lines of evidence support a close genetic and evolutionary relationship between the M and P forms of *S. invicta.* These include the fact that P populations invariably occur in association with the M form (e.g., Fletcher, 1983; Lofgren and Williams, 1984; Greenberg et al., 1985), that queens of each form are readily adopted into colonies of the alternate form in the laboratory (Fletcher et al., 1980; Fletcher and Blum, 1983; Ross and Fletcher, 1986), that sexuals of the two forms exhibit virtually identical morphologies (J. C. Trager, pers. comm.), and that similar frequencies of diploid male producing queens occur in the two forms (Ross and Fletcher, 1985*b,* 1986).

Results of the present study permit some critical evaluation of hypotheses seeking to explain the evolution of the polygyne form, which is presumed to be secondarily derived from the monogyne form in this and other ants (Crozier, 1977*a*; Hölldobler and Wilson, 1977; Brian, 1983; Higashi, 1983; Fletcher and Ross, 1985; but see also Elmes [1980]). Our data suggest that the two forms do not represent distinct lineages that have been reproductively isolated over evolutionary time in their native habitats and that were introduced on separate occasions to this continent (as appears to be the case for *S. richteri* and *S. invicta*). This conclusion is consistent with a de novo origin of the P form subsequent to the introduction of *S. invicta* to North America, although such conjecture can most readily be tested by a thorough search for the P form in South America.

While the results of this study are useful in assessing the genetic relationship between the social forms of *S. invicta,* they are of only limited value in aiding our understanding of the evolutionary origin of the P form, that is, the mechanisms whereby such a fundamental change in social organization can be achieved. Such changes in social organization are a common feature in social-insect evolution, particularly in ants, with the best known examples being shifts from monogyne to polygyne forms, from a queen-reproductive to worker-reproductive (gamergate) system, or from a free-living to socially parasitic existence. The groups involved (i.e., M form/P form, queenright form/gamergate form, host/social parasite) exhibit a range of differentiation from conspecific populations to distinct species (Wilson, 1971; Elmes, 1978*a*; Pearson and Child, 1980; Brian, 1983; Ward, 1983; Pamilo and Rosengren, 1984), suggesting that shifts in social organization may, in some manner, be associated with speciation and that they constitute an ongoing process important in the diversification and success of ants. The observation that these shifts may, in some instances, be accompanied by divergence in mating systems, strategies of colony reproduction, and niche occupation (e.g., Talbot,

1948; Elmes, 1978*b*; Higashi, 1979, 1983; Pamilo and Rosengren, 1984; Bolton, 1986) further supports the idea that they may often be fundamental to the development of reproductive isolation between populations.

Based on the obvious affinities between the M and P forms of *S. invicta,* the discreteness and stability of P populations, the likely origin of P populations in isolation from M populations, and the probable divergence of reproductive strategies in P populations from those characterizing the M form, Ross and Fletcher (1985*a*) speculated that the two forms are reproductively isolated and, thus, in the incipient stages of speciation. In the absence of complete data bearing on the occurrence of the P form in South America, it is at present impossible to decide whether its origin may be linked to some genetic disruption following colonization of North America (such as loss of genetic variability [Tschinkel and Nierenberg, 1983; Ross and Fletcher, 1985*b*]) or perhaps to some ecological feature of the novel environment (such as an abundance of disturbed habitats). Results of the present study indicate either that the forms are not reproductively isolated or that reproductive isolation has been initiated only recently, perhaps following the introduction. Genetic studies of the two forms in Texas (R. S. Dunton, J. S. Johnston, E. M. O'Brien, and S. B. Vinson, unpubl.) suggest that gene flow between them may be greatly restricted in this area. This important issue of reproductive isolation can only be resolved in full by detailed studies of the mating systems of the two forms, by development of additional genetic markers, or by obtaining very large samples of geographically paired populations and using the occurrence of rare ("private") alleles for tracking gene flow (Slatkin, 1985).

No evidence for introgression between *S. invicta* and the native fire ant *S. geminata* was found in this study. Only two native species in the subgenus *Solenopsis, S. geminata* and *S. xyloni,* are broadly sympatric with *S. invicta* in North America (Creighton, 1930) and, thus, are likely candidates for hybridization. Both of these species are genetically quite distinct from *S. invicta* (see above; see also Hung and Vinson [1977] and Hung [1985]), so that any significant introgression should be readily detected. That the techniques we use are sufficiently sensitive to detect introgression involving these native species, if it were occurring, is demonstrated by our earlier confirmation of hybridization between the genetically more similar species, *S. invicta* and *S. richteri* (Ross et al., 1987). The absence of detectable introgression between the introduced and native fire ant faunas is intriguing in light of the substantial hybridization occurring between the two introduced species and may be related to the considerable genetic divergence between the two groups (low genetic compatibility) or, alternatively, to the existence of premating barriers, such as differences in flight times (the introduced species fly in early afternoon whereas the native species fly in late afternoon [Fletcher and Ross, unpubl.]). Hybridization between introduced *S. invicta* and *S. richteri* appears to have been initiated by a breakdown of premating barriers upon colonization of a novel habitat in the absence of significant postmating barriers (Ross et al., 1987).

Large-scale geographic structure of *S. invicta* populations proved not to be an important variable in interpreting the genetic relationship between the two social forms of this species. The modest level of population differentiation observed may be explained by reference to the somewhat limited vagility of fire ants, the natural dispersal of which is confined to passive distribution of winged sexuals by wind currents. The study of Markin et al. (1971) suggests that newly mated queens normally disperse up to ca. 2 km, but that occasional longer-distance dispersal of up to 10–15 km may occur. Also, transport of the ants by human agency seems to have been commonplace earlier (Lofgren et al., 1975) and must be presumed to effect some degree of long-distance gene flow even at the present time.

Genotype proportions in the study populations were generally in good agreement with Hardy-Weinberg expectations, suggesting that inbreeding is not important in these populations and that there is no significant structure at a lower level (other than the nest in M populations [Ross and Fletcher, 1985*a*; Ross et al., 1987]). The lack of evidence for inbreeding is particularly noteworthy in the case of the P populations of

S. invicta, because of earlier speculation that these might be highly inbred groups, the origin of which was mediated by kin selection (Brian, 1983; Fletcher, 1983). An earlier detailed study of the Walton Co. P population in northern Georgia similarly failed to find evidence for significant inbreeding (Ross and Fletcher, 1985*a*).

A final point bearing discussion is the order of the heterozygosity values for the three fire ant species. These values, while typical for ants ($\bar{H}_{exp}$ = 0.034; Graur, 1985), are quite low relative to other insects and invertebrates, in accord with the accumulating evidence that social Hymenoptera are generally deficient in genetic variability as determined electrophoretically (Metcalf et al., 1975; Pamilo and Crozier, 1981; Graur, 1985). Of greater interest here is the observation that the hierarchy of heterozygosity values in the three species matches predictions on the basis of the population biology and recent history of the taxa. *S. richteri,* with the lowest diversity, was recently introduced to this continent and is presently confined to a limited range where effective population size may be relatively low (Buren et al., 1974; Fletcher and Ross, unpubl.). *S. invicta,* with intermediate heterozygosity, is an immensely successful colonizer that would seem to possess enormous effective population sizes, but which has also suffered a recent bottleneck. *S. geminata,* with the highest heterozygosity, appears to have large effective population sizes, and, as a native species, it presumably has not experienced recent major population bottlenecks. Future studies of *S. geminata* over its entire range in the U.S.A. may reveal even greater genetic diversity than we were able to detect in our very limited sample.

Acknowledgments

We thank R. Crozier, J. Trager, and J. Carpenter for comments on the manuscript, M. A. Moran for help with the analyses, and J. Robertson, T. Brooks, S. Hall, and J. Wicker for assistance in the laboratory. This research was funded, in part, by grants from the American Farm Bureau Federation.

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