

SYSTEMATICS AND POPULATION GENETICS OF FIRE ANTS (*SOLENOPSIS SAEVISSIMA* COMPLEX) FROM ARGENTINA

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Abstract.—Specimens of seven fire ant species collected from their native ranges in Argentina were studied by protein electrophoresis and morphological analysis. Concordance between the genetic and morphological character sets is strong (96% agreement on identifications), suggesting that recognition of reproductively isolated populations and partitioning of intra- and interspecific variation can in most cases be achieved using appropriate characters of either type in this taxonomically difficult group. Genetic differentiation between native (Argentina) and introduced (USA) conspecific populations of two species, *Solenopsis invicta* and *S. richteri*, is rather typical of the differentiation existing between conspecific populations found within either country. Furthermore, there appears to have been little reduction of variability (heterozygosity) at enzyme loci following colonization by either species of the United States, although some rare alleles have been lost in the introduced populations. Hybridization is rare between *S. invicta* and *S. richteri* where their native ranges overlap in central Argentina, in contrast to the extensive hybridization of these species in the United States, suggesting that prezygotic barriers to gene flow have been compromised in introduced populations. Phylogenetic analysis of the seven species indicates that *S. invicta* and *S. richteri* are relatively distantly related within the *S. saevissima* complex. Given that hybrids between these species in the United States suffer little apparent loss of fitness, genomic incompatibilities generally may be insufficient to create effective postzygotic barriers to interspecific gene flow in this group of ants.

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The ants (family Formicidae) represent a remarkably successful lineage of ecologically diverse, highly eusocial species, the systematics of which has suffered relative neglect given the ecological and economic importance of the group. This neglect stems in large part from considerable taxonomic difficulties at the species level. For instance, intraspecific geographic variability often transcends interspecific variability in ants (e.g., Ward, 1984, 1989), leading to difficulties in deciding the taxonomic status of closely related populations when traditional characters are studied in a limited number of specimens. Furthermore, morphologically cryptic species, that is, reproductively isolated populations in which morphological divergence is not evident, are sufficiently common that most routine electrophoretic surveys have revealed previously undescribed species (Crozier, 1977a, 1981; Ward, 1980; Halliday, 1981; Crozier et al., 1986; Heinze, 1989; see also Ross et al., 1987a). These findings have been taken to suggest that many groups of ants are in phases of active radiation and that the process of speciation may often be decoupled from significant morphological evolution in these insects (see Crozier, 1977b; also Larson,

1989). Resolution of the taxonomic difficulties created by such patterns of cladogenesis may in many instances require the use of new character sets (e.g., molecular markers) to define genetically isolated groups and to assess the distribution of genetic and morphological variability within and between species. Because ant species comprising taxonomically difficult groups are likely to be evolutionarily young, comparative population genetic and systematic studies of such groups may be particularly informative with respect to the processes leading to reproductive isolation.

One group of ants in which the species-level taxonomy has posed continuing problems is the *Solenopsis saevissima* species complex (subfamily Myrmicinae), members of which are commonly known as fire ants. This complex, which includes relatively large-bodied forms with well developed worker polymorphism, is distributed naturally over a large part of the South American continent east of the Andes. The taxonomy of the group has a history of confusion and disagreement, with the number of formally recognized taxa (species or subspecies) ranging from two to 17 (Buren, 1972). A recent resurgence of interest in the

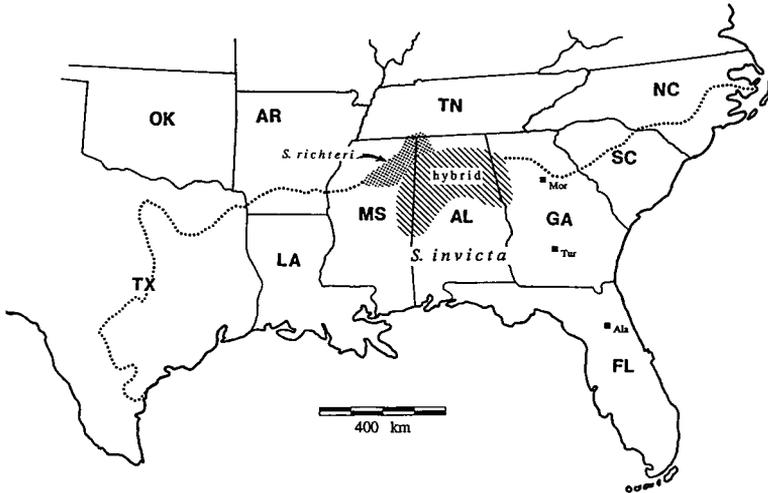


FIG. 1. Distribution of introduced fire ants (*Solenopsis saevissima* complex) in the United States (modified from Diffie et al., 1988). Dotted line indicates approximate northern and western limits of expansion of introduced populations. Locations of three study populations of Ross et al. (1987a) resampled for this study are also indicated (Mor, Tur, Ala).

systematics of these ants has come about for several reasons. First, fire ants introduced to the United States have emerged as significant pests (Lofgren et al., 1975). Second, the introduced species provide one of the few well-documented examples of an animal hybrid zone forming in historical times (Vander Meer et al., 1985; Ross et al., 1987b). Third, fire ants have become important model systems for studying the evolution of social organization, the origin of reproductive isolation, and the relationship between these two facets of evolution in advanced eusocial Hymenoptera (e.g., Wilson and Brown, 1958; Crozier, 1979; Ross et al., 1987a, 1988; Ward, 1989). Continued progress of research on all fronts will depend on an improved understanding of the taxonomic status and phylogenetic affinities of introduced and native fire ant populations, as can be obtained through an integrated systematics/population genetics approach. Furthermore, comparative population genetic data can shed light on the extent to which population bottlenecks have influenced genetic structure in introduced populations, an important consideration given that founder effects have been invoked to explain the origin of such intriguing features of introduced fire ants as polygyny (multiple queens per colony), frequent male diploidy

(in an order characterized by male haploidy), and loss of premating isolating mechanisms associated with interspecific hybridization (Brian, 1983 p. 259; Tschinkel and Nierenberg, 1983; Ross and Fletcher, 1985a; Ross et al., 1987b).

Fire ants of the *S. saevissima* complex were introduced to southern Alabama, in the early part of this century and have since become widespread throughout the southeastern and south-central parts of the United States (Fig. 1). Introduced populations, originally assigned to the single variable species *Solenopsis saevissima* (Creighton, 1930; Ettershank, 1966), have been considered since the revision of Buren (1972) to comprise two species, *S. invicta* and *S. richteri*. The work of Buren (Buren, 1972; Buren et al., 1974), based on morphological analyses of specimens from the United States and central South America, suggested that *S. invicta* and *S. richteri* are phenotypically distinctive and internally quite uniform. Buren (1972) further concluded that hybridization between the two species is rare or absent, despite the abundant opportunity for gene exchange between introduced populations in the United States.

A recent challenge to Buren's view has come from the data of Vander Meer et al. (1985). These authors, using gas chromat-

graphic analyses of diagnostic micromolecular characters (hydrocarbons and venom alkaloids), provided evidence that hybridization commonly occurs between *S. invicta* and *S. richteri* in east-central Mississippi. This has since been confirmed using electrophoretic markers (Ross et al., 1987b), and the zone of hybridization between these introduced forms is now known to encompass a large area extending from eastern Mississippi to northwestern Georgia (Diffie et al., 1988; see Fig. 1). Genetic analyses of the zone at its eastern and western extremes have revealed that genotype distributions at informative loci do not depart substantially from distributions expected under Hardy-Weinberg equilibrium and that a diverse array of recombinant multilocus genotypes is present (Ross et al., 1987b; Ross and Robertson, 1990). These findings are consistent with panmixia in introgressed populations and suggest that there is no large-scale breakdown in hybrid viability or fertility.

These results from hybrid populations of introduced *S. invicta* and *S. richteri* again raise questions concerning our concepts of species in fire ants and highlight our ignorance of evolutionary and genetic relationships among morphologically recognizable forms comprising the *S. saevissima* complex. However, because interactions between introduced fire ant populations may have been influenced by their colonization of novel habitats or by the genetic effects of recent population bottlenecks, systematic uncertainties surrounding this group of ants cannot be resolved by exclusive study of populations in the United States. Thus, the comparative genetic study of native populations reported here was undertaken to accomplish the following objectives: 1) to determine the extent of congruence between species assignments (concepts) based on morphology and those based on genetic markers for fire ants in their native South American ranges; 2) to identify the extent of geographically based genetic variability within these fire ant species; 3) to link genetically the native populations of *S. invicta* and *S. richteri* with fire ant populations established in the United States; 4) to compare levels of genetic variability in native populations of *S. invicta* and *S. richteri* with levels in conspecific introduced populations

in the United States; 5) to obtain evidence bearing on the possibility of gene flow between fire ant species in their native ranges, particularly with reference to *S. invicta* and *S. richteri*; and 6) to infer phylogenetic relationships among the species. A related study in which morphological characters provide the basis of a formal revision of the *S. saevissima* complex and allied taxa is presented elsewhere (Trager, 1990).

MATERIALS AND METHODS

Sample Collections

Samples were collected from 201 nests of *Solenopsis* distributed over 48 sites in northern Argentina (Buenos Aires, Santa Fe, Corrientes, Chaco, Formosa, Santiago del Estero, and Cordoba Provinces; see Fig. 2). Wherever possible a series of nests (up to eight) of the same species was sampled at a single site (preliminary morphological identification was made in the field). Several winged female sexuals and workers were taken from each nest, the former for electrophoresis and the latter for subsequent detailed morphological analysis. Collected specimens were placed immediately in a dry cryogenic refrigerator for transport to the Athens laboratory, where they were transferred to an ultra-low-temperature freezer (-60°C) for storage.

Collection sites were situated so that geographic variability in *S. invicta* (and to a lesser extent in *S. richteri* and *S. quinquecupis*) could be studied (Fig. 2). Samples of other species were collected opportunistically. Because introduced *S. invicta* and *S. richteri* hybridize in North America, a special effort was made to locate any areas in South America where the ranges of these two species overlap. Such an area was found in southern Santa Fe Province, Argentina (near Rosario), so collecting activities were concentrated in this area. A total of 100 *S. invicta*, 57 *S. richteri*, 26 *S. quinquecupis*, 6 *S. interrupta*, and a single *S. macdonaghi* colony were sampled. A single colony of *S. electra* (a member of the *S. saevissima* complex but placed in a different subcomplex than the above species—Trager, 1990) was included to serve as a representative out-group taxon to the other species. Among the remaining samples, either species identifi-

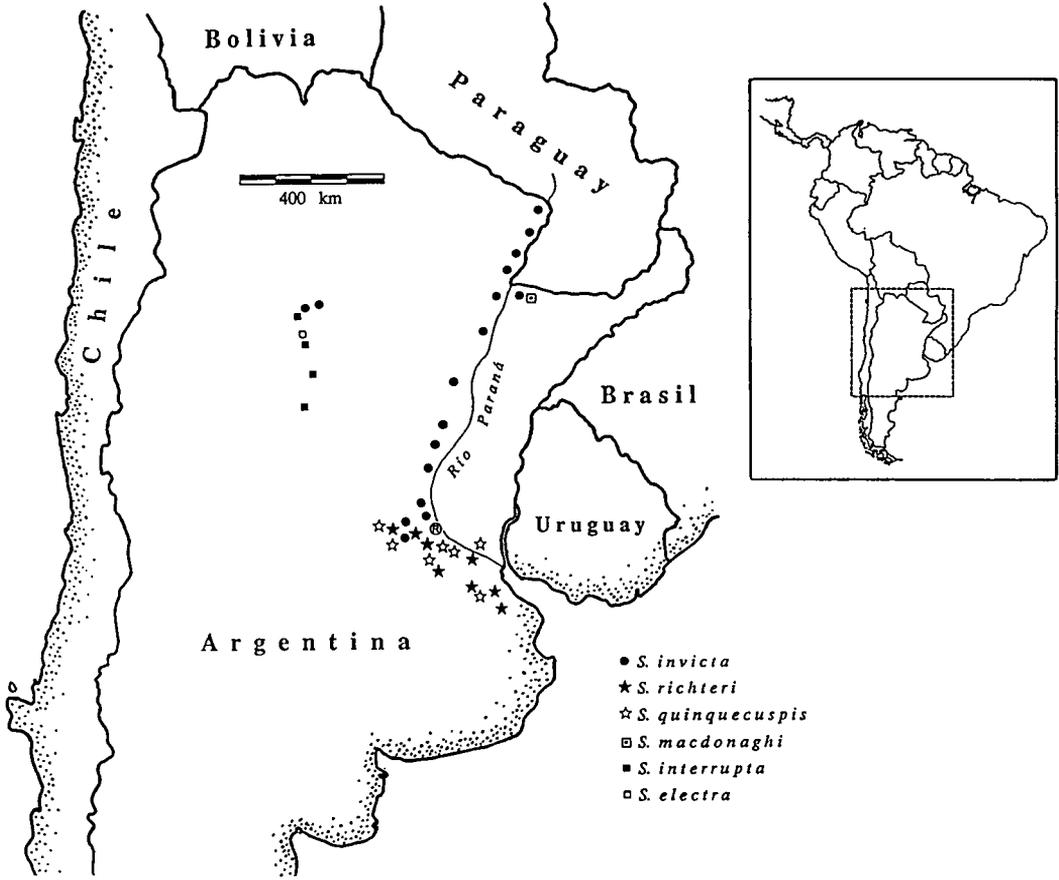


FIG. 2. Locations of collection sites of *Solenopsis* in Argentina. Not every collection site is figured in areas with a high density of sites. Location of the city of Rosario is indicated by '®.'

colonies) or these samples likely represent previously undescribed species (six colonies) (see below).

Morphological Analyses

Initial identification of specimens made in the field was later confirmed in the laboratory by examining morphological features of the major workers under a dissecting microscope at $25\times$ with bright incandescent illumination. Morphological determinations were made without knowledge of the results of the genetic analyses. Useful characters for identification include color patterns, head shape and proportions, and shape and surface sculpture of the postpetiole. Representative voucher specimens of the material examined are deposited in the Museum of Comparative Zoology at Harvard University. Detailed descriptions

and a key for identification of the species can be found in Trager (1990).

Genetic Analyses

Electrophoresis was conducted on 14% starch gels using procedures outlined in Ross et al. (1987a). The products of 26 presumptive loci were studied, these being identical to the markers studied in Ross et al. (1987a) with the following exceptions: 1) *Ald* was omitted because of poor resolution of bands; 2) a new polymorphic locus (*Pro-0*) visualized with a general protein stain was added; 3) previously unrecognized variability was discerned for the product of *Pgm* by altering running conditions. Because variability at the latter two markers was not characterized previously for *S. invicta* from the United States, three of the study populations of Ross et al. (1987a) from

Georgia and Florida were resampled (30 nests each from the Mor, Tur, and Ala populations; see Fig. 1) and allele frequencies estimated for *Pro-0* and *Pgm*. Mendelian inheritance of the products of eight of the study loci (*Agp-1*, *Est-2*, *Est-4*, *Gpi*, *Ldh-1*, *Mpi*, *Odh*, *Pgm*) has been demonstrated (Ross and Fletcher, 1985b; Ross et al., 1987b, 1988; K. Ross and D. Shoemaker, unpubl.).

For analyses of genetic data only a single genotype per colony was used because of the high correlation (non-independence) of genotypes within nests previously determined for several fire ant populations in the United States (Ross and Fletcher, 1985b; Ross et al., 1988). Comparisons of genotype frequencies at sufficiently polymorphic loci with those expected under Hardy-Weinberg equilibrium were conducted for *S. richteri* and *S. invicta* (total data sets or broken down regionally if sample size permitted) using χ^2 tests with Levene's (1949) correction for small sample sizes. For polymorphic loci with extreme allele frequencies, rare alleles were collapsed into a single class and Emigh's (1980) continuity-corrected χ^2 test was employed. The fixation indices F_{IS} and F_{IT} , which assess the magnitude of departures from Hardy-Weinberg genotypic proportions regionally and over the entire collection, were estimated using the methods of Weir and Cockerham (1984; see below) to complement the χ^2 tests.

To assess the extent of intraspecific geographically based genetic variability in native *S. invicta* and *S. richteri*, the eastern collections from Argentina were pooled into five 'populations' for *S. invicta*, each consisting of 18 colonies, and two 'populations' for *S. richteri*, consisting of 33 and 24 colonies (Fig. 3). The fixation index F_{ST} (standardized allele frequency variance), which measures the extent of genetic differentiation among populations, was estimated using the methods of Weir and Cockerham (1984) for all alleles at polymorphic loci present at frequencies greater than 0.01 (see Table 1). A single bias-corrected estimator and its variance were obtained by jackknifing over loci, with confidence limits (95%) generated assuming the *t* distribution. Values of F_{ST} for Argentine *S. invicta* populations were compared to values obtained

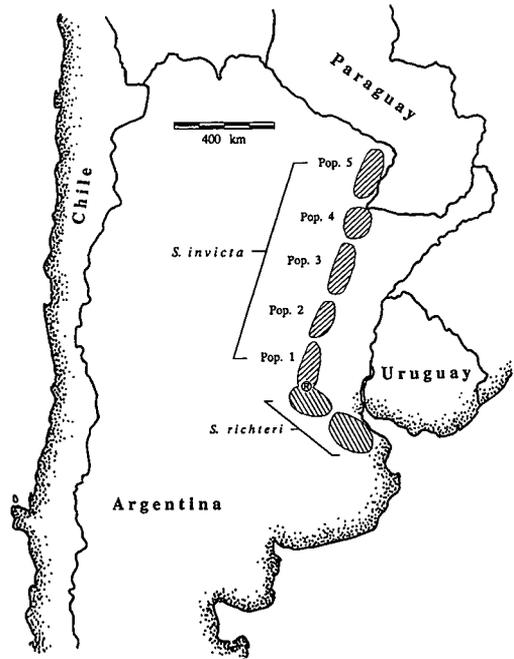


FIG. 3. Locations of pooled regional collections ('populations') for analysis of geographic variability in *S. invicta* and *S. richteri*. Location of the city of Rosario is indicated by '@.'

for three U.S. populations distributed on a similar spatial scale along a north-south transect in Georgia and Florida (see above and Fig. 1). Sample sizes from Argentina were not sufficiently large to examine geographic variability in species other than *S. invicta* and *S. richteri*.

Intraspecific genetic diversity (expected heterozygosity, H_{exp}) was estimated from observed allele frequencies using equation 8.4 in Nei (1987) for *S. invicta*, *S. richteri*, and *S. quinquecupis*. Diversity was compared between species in Argentina, as well as between U.S. and Argentine populations of *S. invicta* and *S. richteri*, using paired-sample *t* tests on the angular-transformed single-locus heterozygosity data (see Archie, 1985).

Genetic distances (Nei's D —Nei, 1987 pp. 222–227; see also Tomiuk and Graur, 1988) between the species and between conspecific populations of *S. invicta* and *S. richteri* were estimated using a jackknife procedure implemented in the program of Sattler and Hilburn (1985). This procedure corrects for limited sample size and number of loci studied as well as unequal rates of evolution.

TABLE 1. Allele frequencies at all loci exhibiting variability in *S. invicta* and *S. richteri* in Argentina and the United States (data for U.S. samples from Ross et al., 1987a). 'N' is the number of diploid genotypes studied (one genotype per nest). Dashes indicate the allele has not been found in this species.

	<i>S. invicta</i>			<i>S. richteri</i>	
	Argentina (all) N = 100	Argentina (northern)* N = 36	U.S. N = 421	Argentina N = 57	U.S. N = 58
<i>Agp-1</i>					
125	0.010	0.014	0	—	—
100	0.960	0.903	0.669	1.0	0.991
41	0.030	0.083	0.331	—	—
30	—	—	—	0	0.009
<i>Est-2</i>					
116	0.035	0	0	0.008	0
110	0.025	0	0	0.868	0.612
100	0.890	1.0	1.0	0.009	0
84	—	—	—	0.018	0
80	0.010	0	0	0.070	0.388
60	0.015	0	0	0.009	0
47	0.025	0	0	0.018	0
<i>Est-4</i>					
155	0.308	0.300	0.414	—	—
100	0.692	0.700	0.586	1.0	1.0
<i>Dia</i>					
113	0	0	0.001	—	—
100	0.995	1.0	0.999	1.0	1.0
69	0.005	0	0	—	—
<i>Gapdh</i>					
138	—	—	—	0.018	0
130	0	0	0.001	—	—
100	1.0	1.0	0.999	0.982	1.0
<i>Gpi</i>					
128	0.005	0	0	—	—
113	0.005	0	0	—	—
107	0.005	0	0	0.991	1.0
100	0.975	1.0	1.0	0.009	0
79	0.010	0	0	—	—
<i>Lap</i>					
113	0	0	0.006	—	—
100	1.0	1.0	0.994	1.0	1.0
<i>Ldh-1</i>					
121	0.005	0.014	0	—	—
100	0.985	0.986	1.0	1.0	1.0
63	0.010	0	0	—	—
<i>Ldh-2</i>					
150	—	—	—	0.009	0
100	1.0	1.0	1.0	0.991	1.0
<i>Mdh-2</i>					
100	0.995	1.0	0.999	1.0	1.0
87	0.005	0	0.001	—	—
<i>Mpi</i>					
100	1.0	1.0	0.999	1.0	1.0
95	0	0	0.001	—	—
<i>Odh</i>					
110	0.005	0	0	0	0
100	0.980	0.986	1.0	0.321	0.008
86	—	—	—	0.679	0.992

TABLE I. Continued.

	<i>S. invicta</i>			<i>S. richteri</i>	
	Argentina (all) N = 100	Argentina (northern)* N = 36	U.S. N = 421	Argentina N = 57	U.S. N = 58
<i>Odh</i>					
69	0.005	0.014	0	—	—
52	0.010	0	0	—	—
<i>Pep(gl)</i>					
121	—	—	—	0.009	0
100	1.0	1.0	1.0	0.982	1.0
79	—	—	—	0.009	0
<i>Pgd-2</i>					
433	0.005	0	0	—	—
100	0.995	1.0	1.0	1.0	1.0
<i>Pgm</i>					
105	0.111	0	0	0.009	0
100	0.702	0.861	0.922	0.991	1.0
95	0.141	0.111	0.078	—	—
85	0.046	0.028	0	—	—
<i>Pro-0</i>					
100	0.995	0.986	0.989	1.0	1.0
94	0.005	0.014	0.011	—	—
<i>Pro-1</i>					
107	0.005	0	0.001	—	—
100	0.995	1.0	0.999	1.0	1.0
<i>Pro-3</i>					
111	0	0	0.005	—	—
100	1.0	1.0	0.995	0.991	0.983
92	—	—	—	0.009	0.017

* Based on samples from two northernmost populations only.

Phylogenetic Analyses

Putative phylogenetic relationships among the species were studied using three approaches to tree-building: 1) a phylogenetic procedure for unrestricted fitting of branch lengths to the matrix of genetic distances; 2) a parsimony-based phylogenetic character analysis in which each locus was treated as a character, and allele frequency arrays were regarded as continuous character states; and 3) a parsimony-based phylogenetic character analysis in which each locus was treated as a character, and allelic complements at the locus were encoded as discrete character states. The undirected trees produced using all of these analyses were rooted by specifying *S. electra* as the outgroup.

The modified distance Wagner procedure of Farris (1981), which does not assume constant evolutionary rates, was chosen for analysis of the genetic-distance data. This procedure (implemented in the program

PHYSYS distributed by J. S. Farris and M. F. Mickevich) was employed to generate the 50 best-fitting trees, that is, those trees for which the derived matrix of distance values most closely resembled the observed distance matrix (as assessed by the percent standard deviation statistic).

For the first phylogenetic character analysis, the modified Wagner method of Swofford and Berlocher (1987) for analyzing allele frequency data was used. This procedure (implemented in the program FREQPARS distributed by D. L. Swofford) is a parsimony method for finding the tree with the least amount of allele frequency change (minimum total branch length) given the constraint that allele frequencies at each locus sum to one in hypothetical ancestors. Allele frequencies at 10 informative loci (weighted means over all conspecific populations in Argentina) constituted the input data set (Table 2).

For the second character analysis, the

TABLE 2. Input data set (allele frequencies) for phylogenetic character analysis of Argentine fire ants (*Solenopsis*) using the modified Wagner method of Swofford and Berlocher (1987).

	<i>S. richteri</i>	<i>S. quinquecupis</i>	<i>S. invicta</i>	<i>S. macdonaghi</i>	<i>S. interrupta</i>	'Species x'	<i>S. electra</i>
<i>Agp-1</i>							
150	0	0	0	0	0	1	0
125	0	0	0.010	0	0	0	0
100	1	1	0.960	1	1	0	1
41	0	0	0.030	0	0	0	0
<i>Agp-2</i>							
125	0	0	0	1	1	0	0
100	1	1	1	0	0	1	1
<i>Dia</i>							
106	0	0	0	0	0	0	1
100	1	1	0.995	1	1	1	0
69	0	0	0.005	0	0	0	0
<i>Est-2</i>							
121	0	0	0	0	1	0	0
116	0.008	0	0.035	0.500	0	0	0
110	0.868	1	0.025	0	0	0	0
100	0.009	0	0.890	0.500	0	1	0
84	0.018	0	0	0	0	0	0
80	0.070	0	0.010	0	0	0	0
60	0.009	0	0.015	0	0	0	0
47	0.018	0	0.025	0	0	0	0
16	0	0	0	0	0	0	1
<i>Est-4</i>							
155	0	0	0.308	0	1	0	0
100	1	1	0.692	1	0	1	0
56	0	0	0	0	0	0	1
<i>Gpi</i>							
128	0	0	0.005	0	0	0	0
113	0	0	0.005	0	0	0	0
107	0.991	0	0.005	0	0	0	0
100	0.009	0.981	0.975	1	1	1	1
79	0	0	0.010	0	0	0	0
10	0	0.019	0	0	0	0	0
<i>Gr</i>							
109	0	0	0	0	0	0.100	0
103	0	0	0	0	0	0.900	0
100	1	1	1	1	0	0	0
94	0	0	0	0	1	0	0
88	0	0	0	0	0	0	1
<i>Odh</i>							
110	0	0	0.005	0	0	0	0
100	0.321	1	0.980	0	1	1	0
86	0.679	0	0	0	0	0	0
81	0	0	0	1	0	0	0
69	0	0	0.005	0	0	0	0
52	0	0	0.010	0	0	0	0
null	0	0	0	0	0	0	1
<i>Pgd-2</i>							
433	0	0	0.005	0	0	0	0
133	0	0.019	0	0	0	0	0
100	1	0.981	0.995	1	1	1	1
<i>Pgm</i>							
105	0.009	0	0.111	0	0	0	1
100	0.991	1	0.702	1	0.750	1	0
95	0	0	0.141	0	0	0	0
87	0	0	0	0	0.250	0	0
85	0	0	0.046	0	0	0	0

TABLE 3. Input data set (character state values) for phylogenetic character analysis of Argentine fire ants (*Solenopsis*) in which allelic combinations were encoded as discrete states. Characters for which two equally parsimonious character state orderings exist were re-coded as two characters with the subscripts 'a' and 'b'. The weight given to each character appears in parentheses beside it.

	<i>S. richteri</i>	<i>S. quinquecupis</i>	<i>S. invicta</i>	<i>S. macdonaghi</i>	<i>S. interrupta</i>	'Species x'	<i>S. electra</i>
Agp-2 (100)	0	0	0	2	2	0	0
Est-2 _a (25)	0	1	7	8	3	7	5
Est-2 _b (25)	0	1	7	8	5	7	3
Est-4 _a (35)	1	1	0	1	3	1	5
Est-4 _b (35)	1	1	0	1	5	1	3
Gr (95)	0	0	0	0	1	3	2
Odh _a (30)	0	1	1	3	1	1	5
Odh _b (30)	0	1	1	5	1	1	3
Pgm (60)	4	4	2	4	5	4	0

combinations of alleles observed at a locus in each of the taxa were encoded so as to define discrete character states (Mickevich and Mitter, 1983; Buth, 1984). This discretization of the data was done without regard to the frequencies of constituent alleles, except that alleles present at frequencies less than 0.10 (weighted mean over all conspecific populations in Argentina) were excluded. Character states were coded additively (with the exception of *Gr*), the differences in integer values between two states reflecting the necessary number of allelic gain and loss steps separating them when all states

at a locus were ordered according to the minimum allele turnover model of Mickevich and Mitter (1983). This ordering scheme minimizes both gains and losses of alleles in a single-locus transformation series. In cases where two character state orderings were equally parsimonious, the locus was recoded as two characters receiving half the weight of the original character (resulting in nine characters in the input data set; see Table 3). Loci were weighted originally in inverse proportion to the observed extent of intraspecific polymorphism (Kluge and Farris, 1969). Most parsimonious trees

TABLE 4. Frequencies of diagnostic and informative alleles for identification of seven species of *Solenopsis* from Argentina.

	<i>S. richteri</i>	<i>S. quinquecupis</i>	<i>S. invicta</i>	<i>S. macdonaghi</i>	<i>S. interrupta</i>	'Species x'	<i>S. electra</i>
Agp-1 ¹⁵⁰	0	0	0	0	0	1.0	0
Dia ¹⁰⁶	0	0	0	0	0	0	1.0
Est-1 ⁸⁶	0	0	0	0	0	0	1.0
Est-2 ¹²¹	0	0	0	0	1.0	0	0
Est-2 ¹¹⁰	0.868	1.0	0.025	0	0	0	0
Est-2 ¹⁶	0	0	0	0	0	0	1.0
Est-4 ¹⁵⁵	0	0	0.308	0	1.0	0	0
Est-4 ¹⁰⁰	1.0	1.0	0.692	1.0	0	1.0	0
Est-4 ⁵⁶	0	0	0	0	0	0	1.0
Gpi ¹⁰⁷	0.991	0	0.005	0	0	0	0
Gpi ¹⁰⁰	0.009	0.981	0.975	1.0	1.0	1.0	1.0
Gr ¹⁰⁹	0	0	0	0	0	0.100	0
Gr ¹⁰³	0	0	0	0	0	0.900	0
Gr ⁹⁴	0	0	0	0	1.0	0	0
Gr ⁸⁸	0	0	0	0	0	0	1.0
Mpi ⁹³	0	0	0	0	0	0	1.0
Odh ⁸¹	0	0	0	1.0	0	0	0
Odh ^{null}	0	0	0	0	0	0	1.0
Pgm ¹⁰⁵	0	0	0.111	0	0	0	1.0
Pgm ⁹⁵	0	0	0.141	0	0	0	0
Pgm ⁸⁵	0	0	0.046	0	0	0	0

TABLE 5. Values of F_{ST} (\pm SE) for populations of *S. invicta* from Argentina (based on five or three populations; see text) and the United States, and for *S. richteri* from Argentina.

Argentine <i>S. invicta</i> (5)	Argentine <i>S. invicta</i> (3)	U.S. <i>S. invicta</i>	Argentine <i>S. richteri</i>
0.035 \pm 0.031	0.036 \pm 0.026	0.079 \pm 0.030	0.004 \pm 0.018

constructed using a branch and bound algorithm implemented in the program HENNIG86 (distributed by J. S. Farris). A single tree was generated from the resulting multiple trees of minimal length 1) by strict (Nelson) consensus criteria and 2) by successive approximations character weighting (e.g., Carpenter, 1988).

RESULTS

Species Identifications

A high degree of concordance exists between the assignment of samples to species on the basis of morphological characters and their identification from genetic markers, with 96% agreement between the methods over all of the samples. Each of the morphologically recognizable species, with the exception of *S. invicta*, is characterized by monomorphism for unique alleles at one or more loci, or by possession of unique combinations of alleles over two or more loci (Table 4). For instance, *S. richteri* is unique in being effectively monomorphic for the *Gpi*¹⁰⁷ allele, *S. macdonaghi* is defined by possession of the *Odh*⁸¹ allele, *S. interrupta* is fixed for the unique *Est-2*¹²¹ and *Gr*⁹⁴ alleles, and *S. electra* is distinguished by possession of the *Dia*¹⁰⁶, *Mpi*⁹³, and *Odh*^{null} alleles (among others). *Solenopsis quinquecuspis* is defined by joint possession (in effectively monomorphic condition) of the *Est-2*¹¹⁰ and *Gpi*¹⁰⁰ alleles. *Solenopsis invicta* is characterized by the absence of these alleles or allelic combinations diagnostic of the other species, and by possession of characteristic polymorphisms at *Est-4* and *Pgm* (Table 4).

Discordance between morphological identifications and those based on the genetic data involve one colony each of *S. invicta* and *S. interrupta* that can be identified readily from their genotypes but were judged as ambiguous in terms of morphology, and six colonies that possess novel alleles at one or two loci but were not rec-

ognized as being distinct on the basis of their morphology. The latter most likely represent colonies of heretofore unrecognized species. One of these unique colonies included only females homozygous for the *Pep(pap)-2*⁸⁹ allele, in contrast to all other ants from this study which were homozygotes for the *Pep(pap)-2*¹⁰⁰ allele. More compelling as an example of a new cryptic species are the five colonies monomorphic for the *Agp-1*¹⁵⁰ allele and possessing the *Gr*¹⁰⁹ and *Gr*¹⁰³ alleles, all of which are unique to these colonies (see Table 4; 'species x'). These five colonies, collected in northern Buenos Aires and southern Santa Fe Provinces at sites where *S. richteri* and *S. quinquecuspis* were also collected, were identified initially as hybrids between these two species on the basis of their morphology.

Intraspecific Genetic Variability and Relationship of Native to Introduced Populations

Comparisons of genotype distributions observed with those expected under Hardy-Weinberg equilibrium were conducted for two loci in *S. richteri* and for 11 locus/population combinations in *S. invicta*. For *S. richteri*, observed genotypes at both loci occur at frequencies virtually identical to those expected (both $P > 0.25$), and values of F_{IT} and F_{IS} do not differ significantly from zero. For *S. invicta*, homozygotes generally occur in excess of the frequency expected at both the species and regional levels, with heterozygote deficiencies significant in two of 11 (18%) of the cases (both $P < 0.025$), both of these at the species level. On the other hand, the 95% confidence limits for F_{IT} and F_{IS} for *S. invicta* encompass zero, suggesting that overall departures from Hardy-Weinberg ratios are unlikely to be of importance at either level.

Geographically based intraspecific genetic variability in Argentine *S. invicta* was studied by examining allele frequency dis-

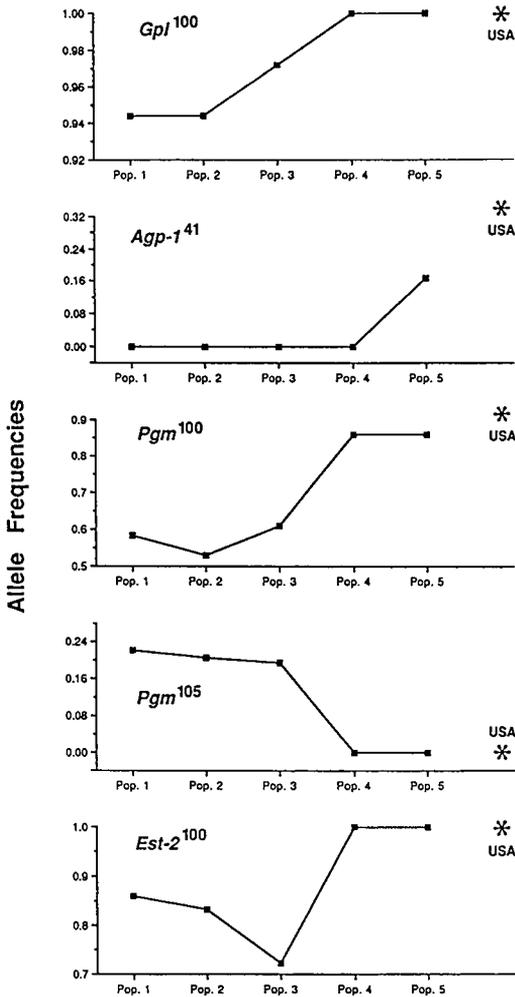


FIG. 4. Frequencies of five informative alleles for five *S. invicta* populations located along a north-south transect extending from southern Santa Fe Province, Argentina, to the Paraguayan border (see Fig. 3). Mean frequencies of these alleles in U.S. populations are also indicated (asterisks).

tributions and estimating F_{ST} for the five groups of colonies ('populations') distributed on a north-south transect along the Río Paraná and Río Paraguay from southern Santa Fe Province to the Paraguayan border (Fig. 3). Values of F_{ST} are quite low and statistically indistinguishable from zero (Table 5). No difference in F_{ST} is evident between the Argentine and U.S. populations of *S. invicta*, regardless of whether all five Argentine populations are included in the analysis or only populations 1, 3, and 5 are included (Table 5). The latter analysis

TABLE 6. Pairwise genetic distance values (Nei's D) for *S. invicta* populations in Argentina and the United States.

	Argentina				U.S. Pops.
	Pop. 4	Pop. 3	Pop. 2	Pop. 1	
Pop. 5	0	0.0012	0.0028	0.0023	0.0016
Pop. 4		0.0007	0.0031	0.0014	0.0038
Pop. 3			0	0	0.0065
Pop. 2				0.0009	0.0087
Pop. 1					0.0077

was done to facilitate comparison of geographic structure in native and introduced *S. invicta*, as the U.S. samples were collected from relatively discrete localities separated by 240–290 km (see Ross et al., 1987a). The value of F_{ST} for *S. richteri* from Argentina, based on only two populations, is very close to zero.

The modest geographically based variability characterizing Argentine *S. invicta* appears, in the case of several alleles, to be clinally distributed (Fig. 4), with the more northerly samples most closely resembling introduced conspecific populations in the United States. This resemblance is due to relatively greater similarities between northern Argentina and U.S. populations in allele frequencies at several loci (see Fig. 4, Tables 1, 6), but is especially significant for the *Agp*-141 allele. This is a common allele throughout the range of *S. invicta* in the United States ($\bar{p} = 0.331$), but in our Argentine samples it is present only in ants collected from Formosa Province (Population 5), where it occurs at a frequency of 0.167. Among our samples, specimens of *S. invicta* from northern Argentina also are most similar to introduced *S. invicta* in the United States in terms of color patterns and head shape (see also Trager, 1990).

Despite some geographic heterogeneity, *S. invicta* and *S. richteri* from Argentina exhibit high overall genetic similarity to conspecific populations introduced to the United States, in comparison with their similarity to their closest heterospecific relatives. For both species genetic distances between native and introduced populations fall close to or within the range of distances estimated between *S. invicta* populations within Argentina or within the United States (Fig. 5). In *S. invicta*, all alleles present at a

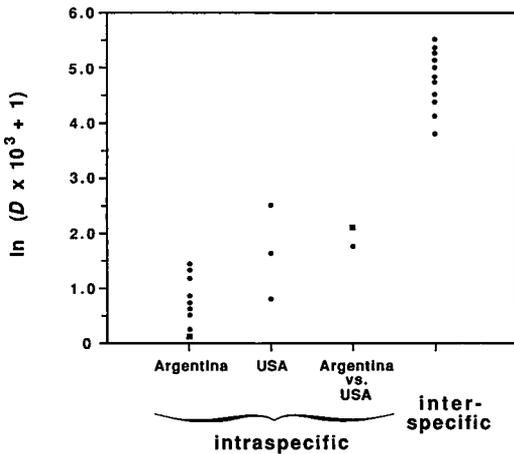


FIG. 5. Intra- and interspecific genetic distances (In $[D \times 10^3 + 1]$) for the *S. saevissima* complex. In the first column are intraspecific distances for *S. invicta* (dots) and *S. richteri* (square) in Argentina. In the second column are intraspecific distances for three *S. invicta* populations in the United States. In the third column are distances between native Argentine and introduced U.S. populations of *S. invicta* (dot) and *S. richteri* (square). Interspecific distances for pairs of *S. saevissima* complex species in Argentina (excluding *S. electra*) are shown in the fourth column.

within Argentina or within the United States (Fig. 5). In *S. invicta*, all alleles present at a frequency greater than 0.05 in the two northernmost Argentina populations are present also in U.S. populations (see Table 1). Furthermore, allele frequencies in these northern populations at the two robustly polymorphic loci *Agp-1* and *Est-4* are similar to frequencies found in several U.S. populations (Ross et al., 1987a). In *S. richteri*, the two most common alleles (out of seven total) at the locus *Est-2* in Argentine samples are the only alleles present in the U.S. population (Table 1). At the locus *Odh*, the only other robustly polymorphic locus in this species (i.e., frequency of the most common allele < 0.95), two alleles are present in Argentine populations but the most common one ($p = 0.679$) is virtually fixed in the U.S. population ($p = 0.992$).

and *S. quinquecupis* in Table 7, along with an estimate of this parameter for a Florida population of *S. geminata* (see Ross et al., 1987a), a fire ant species thought to be native to the United States. Heterozygosity is little diminished in introduced *S. invicta* and *S. richteri* relative to native conspecific populations, with neither of the differences be-

TABLE 7. Heterozygosity (H_{exp} , gene diversity) in native and introduced populations of *Solenopsis* (data for introduced populations and *S. geminata* from Ross et al., 1987a). 'N' is the number of diploid genotypes studied (one genotype per nest).

	Native populations	Introduced populations
<i>S. richteri</i>	0.032 (N = 57)	0.021 (N = 58)
<i>S. invicta</i> (all)	0.052 (N = 100)	0.044 (N = 421)
<i>S. invicta</i> (northern)*	0.036 (N = 36)	
<i>S. quinquecupis</i>	0.003 (N = 26)	
<i>S. geminata</i>	0.048 (N = 30)	

* Based on samples from two northernmost populations only.

tween introduced and native populations statistically significant. Values for *S. invicta* are even more similar when only the northernmost Argentine samples are compared with the U.S. populations. Among the Argentine species, *S. quinquecupis* exhibits the lowest and *S. invicta* the highest heterozygosity (the difference is significant at $P = 0.014$). *Solenopsis quinquecupis* is unique among all fire ants studied to date for which reasonable sample sizes have been obtained in that it is effectively monomorphic (0.95 criterion) at every locus surveyed.

The rather insignificant effect of colonization on overall loss of allozyme variability in *S. invicta* and *S. richteri* is further reflected in the similar distributions for numbers of effective alleles (n_e) at the 26 marker loci in native and introduced populations (Fig. 6). The great preponderance of loci in all of these populations has fewer than 1.05 effective alleles.

While genetic variability as assessed by measures of allelic evenness (H_{exp} , n_e) appears little affected by colonization in the two species, consideration of the total numbers of alleles and distributions of their population frequencies (Fig. 7) suggests that rare alleles present in the native ranges were lost during colonization, leading to reduced allelic richness in the introduced populations. For *S. invicta* in Argentina, over 40% of the observed alleles are present at a frequency of 0.05 or less, but only half of this percentage of rare alleles is found in the United States. The distribution of allele frequencies

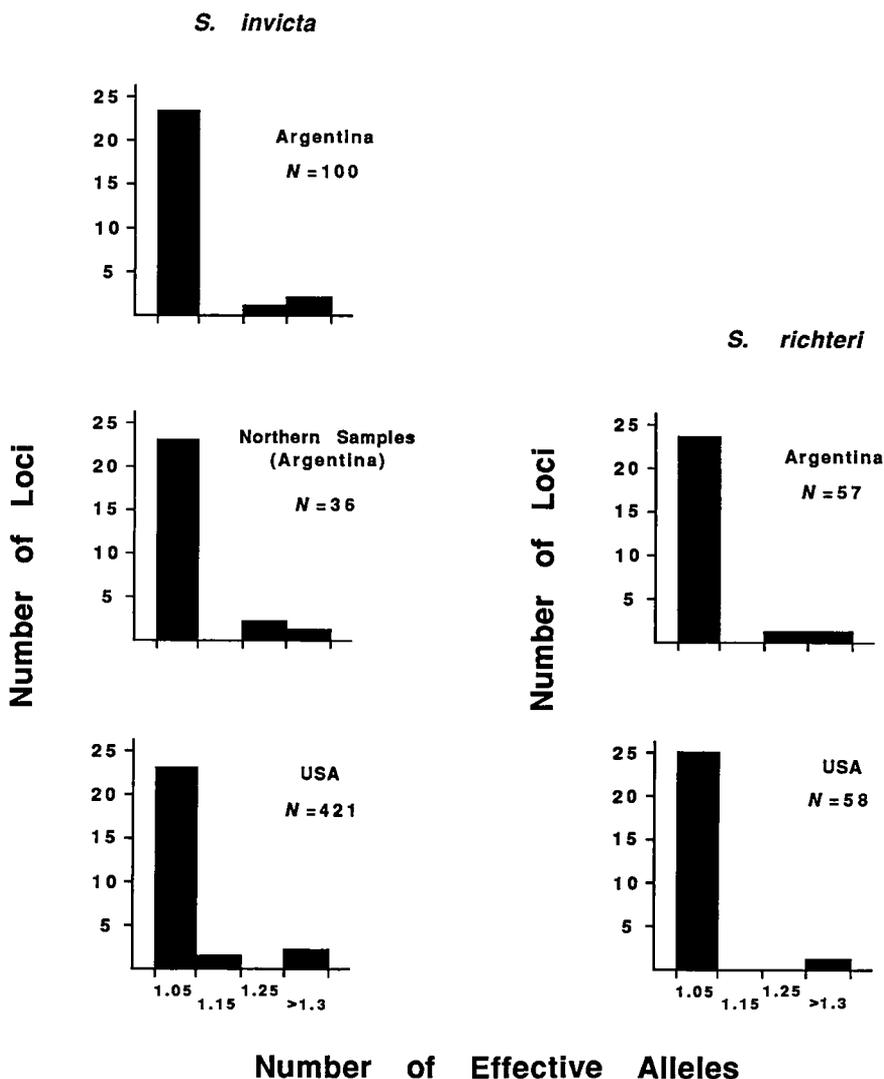


FIG. 6. Number of effective alleles at 26 marker loci for *S. invicta* and *S. richteri* in Argentina and the United States (data for U.S. samples from Ross et al., 1987a). 'N' is the number of diploid genotypes studied (one genotype per nest). Labels on the abscissa indicate interval midpoints, except for the final column, which includes all values greater than 1.3.

in the two northernmost Argentine samples is similar to that for the U.S. populations (Fig. 7), suggesting that the relative absence of rare alleles in introduced *S. invicta* may be due more to an effect of the geographically restricted sampling of colonists than to a population bottleneck per se (see also Table 1). On the other hand, the small sample size for the northernmost collections can be expected to result in underrepresentation of rare alleles through sampling error. For

introduced *S. richteri*, the proportional representation of rare alleles is only one-third that in native populations, based on nearly identical sample sizes from each area.

*Evidence for Reproductive Isolation
between S. invicta and S. richteri*

The low level of intraspecific genetic differentiation found among geographic populations of *S. invicta* and *S. richteri* in Ar-

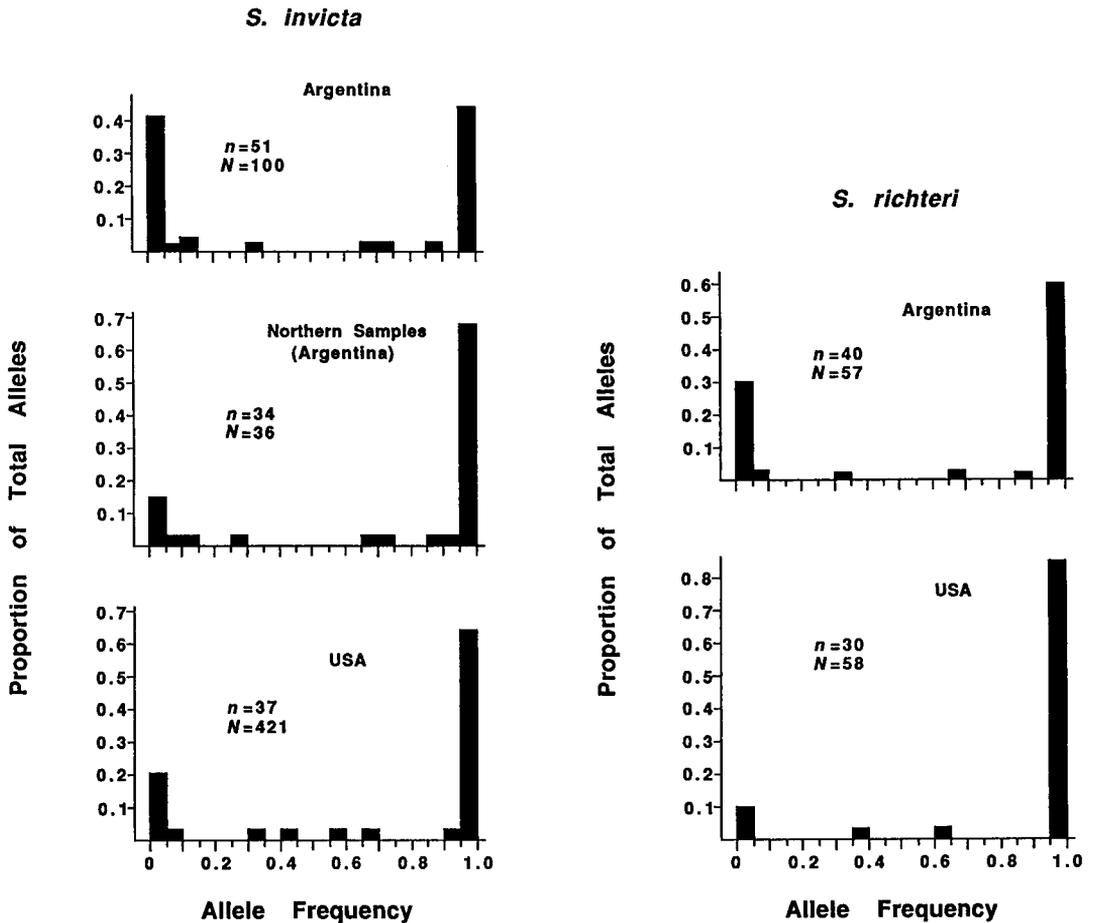


FIG. 7. Distributions of all alleles of *S. invicta* and *S. richteri* in Argentina and the United States by allele frequency class (data for U.S. samples from Ross et al., 1987a). For each panel, 'n' is the total number of alleles observed at the 26 marker loci and 'N' is the number of diploid genotypes studied (one genotype per nest).

gentina contrasts sharply with the genetic differentiation existing between these species and, indeed, with that occurring among all of the species studied. This is reflected in the substantial gap between intra- and interspecific genetic distance values (Fig. 5), as well as by the fact that the species generally are characterized by diagnostic alleles or allelic combinations (see above).

An effort was made to identify areas where the ranges of *S. invicta* and *S. richteri* come into contact and, thus, where hybridization between these two taxa is possible. Such an area exists in southern Santa Fe Province in the city of Rosario and its environs, where colonies of both species are common (see Fig. 2). Thirty-nine colonies collected with-

in 30 km of Rosario (almost all within 20 km) constitute the focal sample for determining the extent of reproductive isolation between *S. invicta* and *S. richteri* in this zone of parapatry. Twenty-three of these colonies were identified as *S. richteri* from the morphology, 13 as *S. invicta*, and three as uncertain, the latter including specimens suspected of being hybrids between *S. richteri* and either *S. invicta* or *S. quinquecupis*. That these may indeed represent products of hybridization is further suggested by heterozygosity at individual loci typically fixed for species-diagnostic alleles or by unusual combinations of alleles over two such loci.

Frequencies of six informative alleles for ants from the zone of parapatry are pre-

TABLE 8. Frequencies of six informative alleles in the area of contact between *S. invicta* and *S. richteri* in southern Santa Fe Province, Argentina. 'N' is the number of diploid genotypes studied (one genotype per nest).

	<i>Est-2¹¹⁰</i>	<i>Odh⁸⁶</i>	<i>Est-4¹⁵⁵</i>	<i>Pgm¹⁰⁵</i>	<i>Pgm⁸⁵</i>	<i>Gpi¹⁰⁷</i>
<i>S. invicta</i> (N = 13)	0	0	0.269	0.154	0.193	0.038
<i>S. richteri</i> * (N = 26)	0.731	0.673	0	0	0.019	0.942
<i>S. richteri</i> (N = 23)	0.783	0.717	0	0	0	1

* Includes three colonies considered hybrid *S. richteri/invicta* or *S. richteri/quinguecupis* on the basis of morphology.

sented in Table 8, both with the three putative hybrid colonies included with *S. richteri* and with these colonies excluded. In either case four of the six alleles occur at moderate to high frequencies in colonies of one of the species, yet they are absent from colonies of the other. On the other hand, the *Pgm⁸⁵* allele (which accounts for one-fifth of the alleles at the locus *Pgm* in *S. invicta* in Rosario but is absent from all of the other species) is present in heterozygous condition in one of the putative hybrids, which bears *S. richteri* alleles at two other loci. Also, in one suspected hybrid colony and one *S. invicta* colony from the zone of parapatry are individuals heterozygous for *Gpi¹⁰⁷*, an allele effectively fixed in *S. richteri* and absent from *S. invicta* throughout their ranges in Argentina and in the United States (e.g., Tables 1 and 4). From these allelic data it appears that reproductive isolation between *S. invicta* and *S. richteri* in Argentina is not absolute in that a small amount of gene exchange most likely occurs between these species where their ranges overlap.

The range of *S. invicta* also appears to abut that of *S. quinquecupis* in southern Santa Fe Province, and the latter species is frequently associated with *S. richteri* in east-central Argentina (the two were collected together at six sites; Fig. 2). Thus it is of interest to know how strong the barrier to gene flow is between *S. quinquecupis* and the other species. The available data indicate a situation similar to that for *S. invicta* and *S. richteri*. For instance, *S. quinquecupis* is monomorphic for the allele *Est-2¹¹⁰*, an allele that is absent from *S. invicta* collected near Rosario, while the allele *Est-4¹⁵⁵*, present at moderate frequencies in all *S. invicta* populations, is absent from *S. quinquecupis* (see Table 8 for *S. invicta*, also Table 4). Similarly, the allele *Gpi¹⁰⁷*,

which is diagnostic for *S. richteri*, is absent from *S. quinquecupis* (Table 4). However, one colony collected near Rosario was identified morphologically as a probable *S. richteri/quinquecupis* hybrid, and the genotypes observed in this colony are consistent with this interpretation.

Of what significance is the limited amount of interspecific gene flow detected between *S. invicta*, *S. richteri*, and *S. quinquecupis*? To answer this question, F_{ST} was estimated as above by treating samples of each of these species collected within 30 km of Rosario as populations, with the putative hybrid colonies included with *S. richteri*. Assuming neutrality and an infinite-island model of gene flow (Wright, 1931; Slatkin, 1985, 1987), F_{ST} can be used to estimate Nm , where N is the effective population size and m is the rate of genetic migration, from the following formula:

$$Nm = (1/F_{ST} - 1)/4.$$

An island model is appropriate for such analysis because the small number of species and their adjacent or overlapping distributions mean that each species is probably equally accessible to immigrants from either of the other species.

An unbiased estimator of Nm (Nm^*) and its variance can be obtained using a jackknife procedure over loci (Johnson et al., 1988). Taking 0.5 to be a threshold value for Nm below which gene flow is insufficient to prevent the differentiation of populations by drift alone (Wright, 1931; Slatkin, 1985), the estimate of $Nm^* = 0.046 \pm 0.068$ (SE) we obtain for these fire ant species indicates that gene flow occurring among them apparently is insignificant in terms of influencing population gene dynamics. Essentially identical results are obtained if only *S. invicta* and *S. richteri* are included in the

TABLE 9. Matrix of genetic distances (Nei's *D*) for seven species of *Solenopsis* from Argentina.

	<i>S. quinquecupis</i>	<i>S. invicta</i>	<i>S. macdonaghi</i>	<i>S. interrupta</i>	'Species x'	<i>S. electra</i>
<i>S. richteri</i>	0.061	0.105	0.162	0.237	0.189	0.413
<i>S. quinquecupis</i>		0.042	0.120	0.165	0.120	0.359
<i>S. invicta</i>			0.094	0.149	0.083	0.351
<i>S. macdonaghi</i>				0.165	0.176	0.414
<i>S. interrupta</i>					0.211	0.414
'Species x'						0.414

analysis. These estimates of gene flow may be high given the small number (*n*) of populations and the fact that *Nm* should be devalued by the factor $[(n - 1)/n]^2$ in the *n*-island model (Slatkin, 1985). In any case such estimates have a substantial degree of uncertainty when only a few populations are involved (Nei et al., 1977).

*Extent of Genetic Differentiation and
Putative Phylogenetic Relationships of
Argentine Fire Ant Species*

Pairwise genetic distances for all of the fire ant species studied are presented in Table 9, while those for all species except *S. electra* are graphed in relation to genetic distances between conspecific populations in Figure 5.

For the phylogenetic distance analyses, no pronounced gap in goodness-of-fit of the 50 trees identified by the distance Wagner procedure is evident, and a strict (Nelson) consensus tree for these is completely unresolved. The best fitting of the 50 trees, which is separated from the second best tree by only 0.4 percent standard deviation, is shown as Figure 8a. The first phylogenetic character analysis, that in which allele frequencies constitute continuous character states, leads to the single tree shown as Figure 8b. The second character analysis, that in which allelic combinations are treated as discrete character states, results in three trees of minimal length; the single trees produced by applying Nelson consensus procedures and successive approximations weighting appear as Figures 8c and 8d, respectively. Placement of the roots on the trees derived from phylogenetic character analysis (Figs. 8b–d) was done using *S. electra* as the outgroup, in accord with its formal placement in a different subcomplex than the other species on the basis of morphology (Trager, 1990).

All of the trees generated from the genetic data agree in recognizing the broadly sympatric *S. richteri* and *S. quinquecupis* as sister species. An interesting additional feature of the trees based on character state analysis is that *S. richteri* and *S. invicta* consistently are placed in different sister lineages, the common ancestor of which occurs at the base of the *S. saevissima* subcomplex (= *S. saevissima* complex without *S. electra*). Thus these two species apparently are not closely related within the complex even though they hybridize readily in the southern United States.

DISCUSSION

Statements regarding the systematics and evolution of fire ants of the *Solenopsis saevissima* complex have been fraught with uncertainty, both because of the absence of a thorough taxonomic revision of the group based on extensive samples from native populations, and because of the absence of comparative population genetic data from native and introduced populations. In conjunction with a recent revision based on the morphology of the ants (Trager, 1990), the genetic data presented here establish a foundation for understanding evolutionary patterns in the *S. saevissima* complex and for relating these to population-level processes occurring in areas where these ants are newly introduced.

An important conclusion of this study is that there is substantial concordance between the genetic data and results derived from analysis of traditional taxonomic characters. All of the species previously recognized by virtue of their morphological distinctiveness (Trager, 1990) can also be identified on the basis of unique single- or multi-locus genotypic profiles. Furthermore, the clinally structured intraspecific genetic variability characterizing *S. invicta*

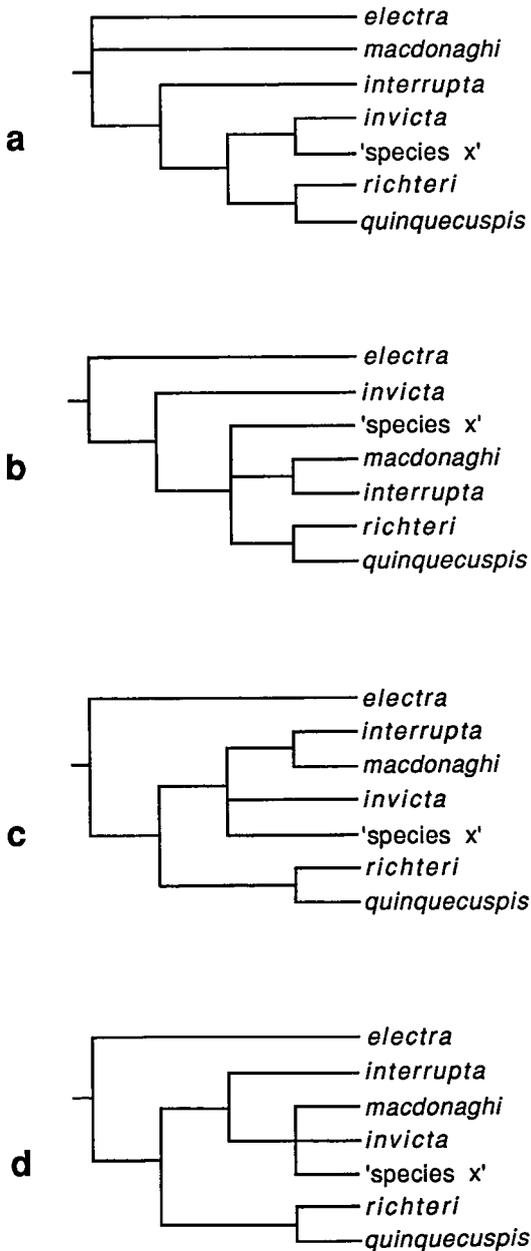


FIG. 8. Trees of putative phylogenetic relationships for Argentine fire ants (*Solenopsis saevissima* complex) generated using a distance Wagner procedure (a) or using parsimony analyses with loci treated as characters (b–d). The parsimony analyses involve direct use of allele frequency data (b) or the encoding of these as discrete character states (c, d). The tree in (a) has a percent standard deviation of 3.072 and two negative branch lengths. The tree in (b) has a length of 34.4. The tree in (c) is the consensus tree for three equally parsimonious trees with consistency indices of 77, retention indices of 50, and lengths of 2,350 (calculated according to the weights shown in Table 3). The tree in (d) is generated from successive approximations

in Argentina is paralleled by a cline in morphological character states (Trager, 1990), with the northernmost Argentine samples most closely resembling U.S. populations in both their morphology and allozymes. A significant aspect of this concordance of the character sets is that it suggests that genetic and morphological variability in this complex of ants can be effectively partitioned into intra- and interspecific components on the basis of either type of data, and thus the morphological characters are validated as suitable markers of genetic continuity among populations in most situations. As is often the case in taxonomically difficult groups, however, the electrophoretic markers are in some instances capable of distinguishing reproductively isolated populations where the morphological characters cannot.

Geographic populations of *S. invicta* in Argentina exhibit only modest genetic differentiation (as apparently does *S. richteri*), with this genetic structure similar in magnitude to that found in introduced *S. invicta* in the United States. This result is perhaps surprising in view of the presumed long history of occupation of the South American range, contrasted with the recent explosive colonization of North America. Nevertheless, average levels of gene flow appear sufficiently high on both continents to overcome effects of local adaptation or genetic drift, a conclusion that is consistent with the reported high vagility of these ants. Winged adult reproductives of both sexes take part in mass mating flights during which they may ascend to heights greater than 300 m. Mated females typically disperse less than two km from their natal nests but occasionally are transported far greater distances on air currents (Markin et al., 1971). This effective natural means of dispersal, combined with human-aided transport, has resulted in rates of spread of 10–50 km per year (generation) by *S. invicta* into uninfested regions in the United States (Vinson and Greenberg, 1986; K. Ross and D. Fletcher, unpubl.).

← character weighting of these three most parsimonious trees; the consistency index for this tree is 92, the retention index is 92, and the length is 170.

Although geographically based genetic variability in Argentine *S. invicta* is modest in extent, the variability that exists at several loci is clinally distributed along a latitudinal gradient in the area we sampled. This gradual transition in the genetic composition of *S. invicta* populations is of special note in that the more northerly populations most closely resemble *S. invicta* in the United States. Northern Argentina populations often lack alleles present further south, alleles that are also absent in the United States. Most importantly, however, a common allele in U.S. populations (*Agp-1⁴¹*) is present only in samples taken near the Paraguayan border in the north. Evidence from the morphology indicates that, among specimens collected from across the range of *S. invicta* in South America, those found along the Río Paraguay drainage near the Argentina/Paraguay frontier bear the strongest resemblance to the ants in the United States (Trager, 1990). Buren (1972) earlier placed the possible source area for the North American colonists further north along this drainage, in central Brazil, on the basis of his morphological studies. Future collections for genetic analysis from the northern sections of the drainage will be required to more accurately pinpoint the location of the source population(s).

This differential resemblance of regional populations aside, introduced *S. invicta* and introduced *S. richteri* are very similar genetically to their native conspecific populations. In both species some rare alleles present in native populations have been lost in the colonizing populations (as expected with finite founder population size), and in *S. richteri* one allele common in Argentina is all but absent in the United States (*Odh¹⁰⁰*). Nonetheless, estimates of genetic distance between native and introduced populations of both species fall close to or within the range of expected intraspecific distances based on population surveys in both countries.

There is little evidence from our study of any meaningful loss of allozymic diversity (in terms of measures of allelic evenness) associated with colonization of the United States. Values for expected heterozygosity are virtually identical between native and introduced populations of *S. invicta*, where-

as there is only a modest (not statistically significant) drop in heterozygosity in introduced *S. richteri*. Distributions of numbers of effective alleles are similar between native and introduced populations of both species. Our finding of minimal effects of the founding events on heterozygosity is in keeping with the theoretical results of Nei et al. (1975), who demonstrated that loss of heterozygosity in such situations is greatly ameliorated if there is rapid population growth following a bottleneck. Given the high intrinsic reproductive rates of fire ants and their rapid spread throughout the southern United States, there can be little doubt that the original founding populations experienced growth of the sort that would preserve most of the variability at allozyme loci, with only relatively rare alleles being lost during this process. The apparent greater loss of heterozygosity in *S. richteri*, if real, may reflect a lower reproductive rate and/or reduced effective population size compared to *S. invicta*, the far more successful of the two colonizing species (e.g., Fig. 1). This apparent difference in loss of heterozygosity between the two species could also explain why the genetic distance between native and introduced *S. richteri* falls toward the high end of the range of intraspecific differentiation (Fig. 5), as one effect of a loss of heterozygosity following a bottleneck is to increase genetic distance between populations (Chakraborty and Nei, 1977).

While the effects of founding of the U.S. populations appear to be modest or insignificant with regard to the simple enzyme-encoding loci used as markers here, effects of the same events on more complex genetic systems, such as those involving overdominance or epistasis, may be quite different (e.g., Yokoyama and Nei, 1979; Goodnight, 1987). A relevant example here is the hypothesized perturbation of the genetic sex-determining system of *S. invicta* following introduction of this species to the United States. Sex determination in *S. invicta* and other social Hymenoptera appears to be mediated by heterozygosity at one or more major sex loci, such that individuals hemizygous or homozygous at the sex locus (loci) develop into males (reviewed in Crozier, 1977b). Although the occurrence of male

diploidy in native *S. invicta* has not been studied, the unexpectedly high frequency of mated queens in the United States that produce diploid male progeny (15–20%) has been viewed as evidence of a substantial loss of allelic diversity (and concomitant heterozygosity) at the sex locus (loci) in introduced populations (Ross and Fletcher, 1985a). This hypothesis is not necessarily contradicted by the above data for enzyme markers because of the differential effects of bottlenecks on the two types of loci. For highly polymorphic loci such as the sex loci in Hymenoptera (at which 10 or more alleles are routinely present at roughly equal frequencies [Yokoyama and Nei, 1979; Ross and Fletcher, 1985a]), loss of allelic diversity is critically dependent on bottleneck size, whereas at less robustly polymorphic loci, such as those we have studied electrophoretically, the loss of variability may be more dependent on the nature of subsequent population growth (Nei et al., 1975; also Maruyama and Fuerst, 1985). Thus a scenario in which relatively few colonists established a rapidly growing population presumably could explain differing effects of the founding event on the two types of genes.

These remarks are pertinent also with regard to other features of introduced fire ant populations suggested to have arisen as a result of loss of genetic variability during colonization, including polygyny (multiple queens per colony) (Tschinkel and Nierenberg, 1983) and interspecific hybridization (Ross et al., 1987b). Depending on the nature of the genetic architectures involved, the nestmate- and mate-recognition systems of these ants (which mediate social organization and premating isolation) may or may not have survived the founding events relatively intact (see Crozier [1987] for a review of the genetic bases of nestmate recognition and Templeton [1980] and Giddings and Templeton [1983] for discussion of bottleneck effects on mate-recognition systems). That polygynous social organization in *S. invicta* in the United States did not arise solely as a result of founder-induced disruption of recognition capabilities is suggested by the discovery of polygynous populations of *S. invicta*, *S. richteri*, and *S. quinquecupis* in Argentina (Jouvenaz et al., 1989; Ross and Trager, unpubl.).

Demonstration of similar values for heterozygosity at enzyme genes in native and introduced *S. invicta* and *S. richteri* is of further significance as it bears on the more general issue of levels of enzyme-gene variability in eusocial Hymenoptera. Native populations of all four fire ant species for which we have adequate samples are characterized by values of H_{exp} of 0.05 or less, with *S. quinquecupis* essentially monomorphic at all 26 loci studied. The mean value for these four species (0.034) is identical to the mean value for all other ant species for which similar surveys have been conducted (Graur, 1985), but is three to four times lower than typical values estimated for non-hymenopterous insects ($\bar{H}_{exp} = 0.116$ for 127 species excluding *Drosophila*; Graur, 1985). Thus the suggestion that eusocial Hymenoptera are relatively deficient in genetic variability as determined from electrophoresis (e.g., Metcalf et al., 1975; Pamilo and Crozier, 1981; Graur, 1985) receives further support from this study.

Our data indicate that *S. saevissima* complex species are discrete, recognizable entities that maintain their genetic integrity even though the ranges of many of the species are more or less broadly overlapping. Close examination of the strength of barriers to gene flow between *S. invicta* and *S. richteri* in a zone of parapatry and between *S. richteri* and *S. quinquecupis* over their broadly sympatric ranges reveals that these barriers are not absolute; rather, they appear to be slightly 'leaky' boundaries, permitting small amounts of interspecific nuclear gene flow. Nonetheless, the extent of such gene flow appears insufficient to degrade the internal cohesion of these species or to prevent their further differentiation in the face of genetic drift or divergent selective regimes (e.g., Templeton, 1989). Thus the members of this complex are effectively independent evolutionary entities that warrant species status (cf. Szymura and Barton, 1986).

This finding of insignificant levels of interspecific gene flow in native fire ant populations is especially important in view of the extensive hybridization that occurs between *S. invicta* and *S. richteri* populations introduced to the United States. Hybrid populations of these species feature a full

array of recombinant genotypes occurring in proportions approaching Hardy-Weinberg expectations (Ross et al., 1987b), and hybrid individuals experience only slight disruption of developmental regulation relative to the parental species, as determined from analyses of fluctuating bilateral asymmetry (Ross and Robertson, 1990). Thus postzygotic barriers to gene flow between these two species clearly are poorly developed. Our phylogenetic character analyses suggest that *S. invicta* and *S. richteri* are members of two different lineages constituting the *S. saevissima* subcomplex, so that the time since their divergence from a common ancestor (during which genetic incompatibilities have accumulated [e.g., Coyne and Orr, 1989]) is likely to be as great as that for any species pair in the subcomplex. Postzygotic barriers may thus be minimal between many of the species. From this it follows that reproductive isolation of native sympatric populations may typically be enforced by prezygotic mechanisms which, in the case of *S. invicta* and *S. richteri*, appear to have been compromised in some way during colonization of the United States. One possibility here is that such prezygotic isolating mechanisms are well developed in areas of natural parapatry between these two species, such as in central Argentina, but poorly developed in areas where they do not come into contact (presuming continuing selection for reinforcement occurs in parapatry and intraspecific gene flow is not overwhelming [e.g., Butlin, 1987]). This possibility attains significance because of the probable origin of introduced *S. invicta* from the more northern areas of its range, where *S. richteri* does not occur. Prezygotic barriers operating in this group of ants could be as simple as differences in the daily timing of mating flights or in the phenology of production of sexual forms, both of which appear to vary among conspecific and heterospecific populations (Trager and Ross, unpubl.), but our ignorance of the basic biology of native fire ants precludes any further speculation as to the nature of the forces responsible for reproductive isolation.

Our conclusions regarding the relative importance of prezygotic versus postzygotic isolating mechanisms in the *S. saevissima* complex parallel recent findings from *Dro-*

sophila, which suggest that prezygotic barriers typically develop in advance of postzygotic barriers in parapatric or sympatric species pairs (Coyne and Orr, 1989). If it is assumed that hybrid viability is likely to decrease monotonically with time since divergence, the formation of viable fire ant hybrids between two of the more distantly related members of the complex, taken together with the modest genetic distances between species ($\bar{D} = 0.212$), suggest that this is a youthful group in a phase of active radiation. Future studies of the ecology, breeding habits, and biogeography in South America may shed light on the nature and origin of prezygotic isolating mechanisms and the possibility that their reinforcement is involved in the process of speciation in these ants.

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