Evolution, 41(2), 1987, pp. 280–293

BIOCHEMICAL PHENOTYPIC AND GENETIC STUDIES OF TWO INTRODUCED FIRE ANTS AND THEIR HYBRID (HYMENOPTERA: FORMICIDAE)

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Abstract.—Two introduced fire ants, *Solenopsis invicta* and *S. richteri*, and their hybrid were studied using phenotypic markers from gas chromatographic analysis of hydrocarbons and venom alkaloids, as well as genetic markers from enzyme electrophoresis. Both methods show that extensive gene introgression is occurring over a distance of at least 120 km at the contact zone between the two forms in eastern Mississippi. Genetic analyses suggest that the hybrid population does not depart dramatically from panmixia. Also, recombinant genotypes predominate in the hybrid zone, indicating that F_1 hybrids are viable. Allele frequency clines through the hybrid zone are apparent for four polymorphic loci. Data sets generated by the chromatographic and electrophoretic methods are highly concordant in that they differentiate completely between the two forms and agree in designating colonies from the contact zone as hybrid or parental in a high proportion (90%) of cases. The two methods can serve as complementary tools for studying closely related but genetically distinct populations in this, and perhaps other, groups of insects.

Received May 5, 1986. Accepted December 4, 1986

Central problems in evolutionary biology and systematics include defining the level of reproductive isolation among related populations, assessing the evolutionary significance of this isolation, and reaching a consensus as to the taxonomic status of the populations. These problems are particularly acute in poorly known or extremely diverse groups, such as many insects, and may often be compounded by human interference with the natural ecology and dispersal of the organisms. At the heart of these difficulties lie our current concepts of species (see Hull, 1974; Ghiselin, 1975; Wiley, 1981; Mayr, 1982), none of which seems sufficient to embrace all situations or all manner of data collected by taxonomists, geneticists, and ecologists. With the advent of the newer molecular tools for studying organic diversity, there is reason to believe that fresh insights into the origin and subsequent interactions of genetically distinct populations may be gained, even if absolute consensus on taxonomic issues is unlikely to emerge.

Fire ants in the subgenus *Solenopsis* (Hymenoptera: Formicidae) are new world species that occur primarily in tropical and subtropical [illegible] 1920). Due to the large size of their nests and aggressive habits of the workers, many species are exceptionally conspicuous elements of local ant faunas. In the early part of this century a heterogeneous group of fire ants native to South America was introduced to North America through the port of Mobile, Alabama (Wilson, 1958; Lofgren et al., 1975), and these ants have subsequently emerged as significant pests in many parts of the southern U.S. Two distinctive forms comprising the introduced fauna were originally recognized as subspecific variants (Wilson, 1953, 1958), but later were accorded species status by Buren (1972), primarily on the basis of external morphological characters and lack of hybridization. *Solenopsis richteri*, introduced prior to 1920, apparently succeeded in colonizing southern Alabama and some areas of central Mississippi, however, the subsequent introduction of *S. invicta* in the 1930s resulted in a rapid expansion of this species and exclusion of *S. richteri* from the southernmost areas of its range (Buren, 1972). Presently, *S. richteri* is confined to a very limited area in northern Mississippi and Alabama (Buren et al., 1974; Vander Meer et al., 1985), while *S. invicta* is distributed throughout the southeast and south-central U.S. (see Fig. 1). The distribution of [illegible] poorly known, although they are reported to be allopatric or parapatric (Buren et al.,

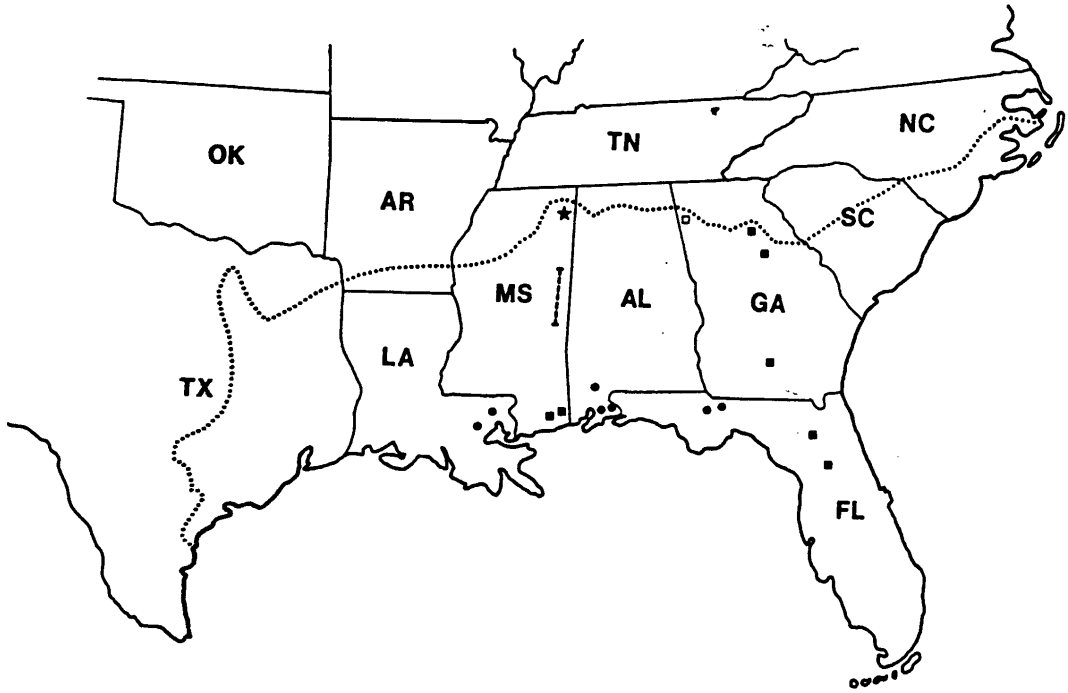

FIG. 1. Approximate limit of distribution of introduced fire ants in North America and locations of samples of *S. invicta*, *S. richteri*, and their hybrid for biochemical phenotypic and genetic analyses. (●) *S. invicta* for GC analyses; (■) *S. invicta* for electrophoretic analyses; (★) *S. richteri* for all analyses. Dashed line indicates sampling transect for east-central Mississippi hybrid zone. (□) Additional hybrids used for inheritance studies.

1974), with *S. richteri* occupying a more southerly (temperate) area than *S. invicta*. Thus, displacement of *S. richteri* to the northern limit of the range of introduced fire ants may be related to the relative competitiveness of the two forms under different climatic regimes (Buren, 1972).

Recent gas chromatographic (GC) studies of ants collected near the contact zone of the two forms in Mississippi revealed intermediate expression of what were considered to be species-specific phenotypic patterns (Vander Meer et al., 1985), suggesting that gene introgression (hybridization) occurs in this area, or alternatively, that the two forms do indeed represent variants from a single reproductive community. These findings prompted the present investigation for several reasons. First, it is desirable to apply an independent method (gel electrophoresis) to determine the extent of genetic differentiation between the two forms and to examine more closely the possibility of gene introgression. Such data presumably would aid in deciding the taxonomic status of the groups, with interpretations being all the more compelling if the morphological, electrophoretic, and GC data are highly concordant. Furthermore, the degree of concordance between the biochemical genetic (electrophoretic) and biochemical phenotypic (GC) data could be used to assess the value of the latter type of data for studying systematic problems in insects, an unresolved issue because of their complex and poorly understood genetic bases (e.g., Averhoff and Richardson, 1976; Grula and Taylor, 1979) and the possibility that their expression may be environmentally affected (Nelson, 1978; Howard and Blomquist, 1982). To our knowledge, the utility of biochemical phenotypic markers for insect taxonomic studies has not previously been examined by reference to genetic markers. Finally, confirmation of hybridization between the two introduced fire ants is significant in that hybrid zones constitute interesting evolutionary phenomena that may provide insights into the nature of genetic divergence between species as well as the

factors which normally act to maintain their distinctiveness (Barton and Hewitt, 1985; Littlejohn and Watson, 1985; Kocher and Sage, 1986).

Materials and Methods

For purposes of ordering the experimental work and facilitating discussion in this paper, we adhere to the currently accepted taxonomy of the *Solenopsis saevissima* species complex (Buren, 1972), although the validity of this phylogenetic framework is currently controversial (see Discussion; Trager, unpubl.). The term "hybrid" is used to refer to the array of recombinant genotypes containing genetic elements from both of the parental forms (cf. Barton and Hewitt, 1985) and detectable as such by chromatographic and/or electrophoretic analysis.

Collection of Samples

Our first objective was to characterize the parental forms for both types of biochemical markers (phenotypic and genetic) by sampling in areas where only the parental forms were likely to occur. Samples of *S. invicta* for the GC analyses were collected from seven sites: Gadsden Co., FL (2 sites); Baldwin Co., AL (3); and Tangipahoa Parish, LA (2) (Fig. 1). Fifty worker ants from each of five colonies were collected at each site. Ants from each colony were transferred to glass vials containing 5–10 ml hexane. The solvent was removed after 2–7 days and utilized for the chromatographic analyses. Samples of *S. invicta* for electrophoresis were also collected from seven sites: Marion Co., FL; Alachua Co., FL; Turner Co., GA; Walton Co., GA; Morgan Co., GA; and Jackson Co., MS (2 sites) (Fig. 1). Several mature winged queens were collected from each of 30 colonies at each site. These were held in liquid nitrogen until returned to the laboratory, then held in a freezer at −60°C until electrophoresis.

Samples of *S. richteri* and samples from the putative hybrid zone in Mississippi were collected in concert for GC and electrophoretic analyses. For *S. richteri*, 10–30 winged queens and 7–25 males were collected from each of 59 colonies in northeastern Mississippi (Prentiss, Itawamba, and Tishomingo Cos.; Fig. 1), as described above for electrophoretic samples. Worker ants from 49 of these colonies were collected in hexane for chromatography. That only *S. richteri* colonies are likely to occur in this area is indicated by Buren et al. (1974) and Vander Meer et al. (1985). (Independent morphological confirmation that the samples were *S. richteri* was provided by J. C. Trager, Univ. Florida, who is currently revising the species complex.)

Winged queens and males (sample sizes as above) were also collected from each of 59 colonies located on a 120 km north-south transect through the putative hybrid zone in east-central Mississippi (Lauderdale, Kemper, Noxubee, and Lowndes Cos.; Fig. 1). The boundaries of this zone have been tentatively defined by Vander Meer et al. (1985) on the basis of preliminary GC data. Worker ants from 50 of the colonies were collected for chromatography. Colonies were grouped according to relative position along the transect for some of the analyses below. Winged queens and males were sampled from an additional 15 colonies from Floyd Co., GA (in another putative hybrid population [Diffie and Vander Meer, unpubl.]; Fig. 1) for inclusion in the inheritance studies. Because of the high genotypic correlations for female nestmates ($r = 0.75$; Ross and Fletcher, 1985*a*; Ross, unpubl.), only one queen genotype per colony was used in most of the genetic analyses.

Gas Chromatographic Analysis of Venom Alkaloids and Hydrocarbons

GC analyses were carried out on a Varian 3700 gas chromatograph (Walnut Creek, CA) equipped with a flame ionization detector and a 30 m × 0.032 mm ID DB-1 fused silica capillary column (J & W Scientific, Inc., Rancho Cordova, CA). Helium was used as the column carrier gas, and detector sensitivity was increased by using nitrogen as the make-up gas. Venom alkaloids and hydrocarbons were analyzed simultaneously. The oven temperature was held at 150°C for one min, then programmed to 285°C at 4°C per min. The temperature was held at 285°C until the components of interest had eluted from the column. Venom peak assignments were based on GC and GC-mass spectrometry of *S. invicta* venom alkaloids from extirpated poison sacs. Gas chromatograms of purified hydrocarbons

from the two parental forms showed patterns identical to those obtained from the hexane soak. The venom alkaloids and hydrocarbons are present in multiple microgram quantities and therefore swamp out any potential interfering components. The data were analyzed on a Varian Vista 401 Data Processor.

We developed numerical indices for the GC data to define the range of variability present in *S. richteri* and *S. invicta* and to specify quantitatively the biochemical similarity of hybrid specimens to either of the parental forms. For the hydrocarbons this was done by first defining characteristic hydrocarbon peaks for the two parental forms. Since each form has in minor amounts components diagnostic for the other form (see Fig. 2; Nelson et al., 1980; Vander Meer et al., 1985), it was necessary to introduce corrective factors. Of the hydrocarbon peaks found between a retention time of 17 to 24 min, *S. richteri* contributed a mean of 11.6% to the *S. invicta* designated region and *S. invicta* contributed a mean of 7.2% to the *S. richteri* designated region. Thus a hydrocarbon index (I_{HC}) was calculated from the formula:

$$I_{HC} = \frac{P_{SI} - (P_{SR} \cdot 0.116)}{[P_{SI} - (P_{SR} \cdot 0.116)] + [P_{SR} - (P_{SI} \cdot 0.072)]},$$

where P_{SI} and P_{SR} are the proportional hydrocarbon peak areas found within the retention times assigned to *S. invicta* and *S. richteri*, respectively ($P_{SI} + P_{SR} = 1.0$).

The venom alkaloids of both *S. richteri* and *S. invicta* are 6-methyl piperidines with 2-substituted alkyl or alkenyl side chains (Brand et al., 1972). Since the differences in structure are in the 2-substituted side chain, the various homologs are simply referred to by the length of the side chain and whether or not it has a double bond, e.g., $C_{13:0}$ refers to a 13-carbon side chain with no double bonds, and $C_{15:1}$ refers to a 15-carbon side chain with one double bond. The proportion of $C_{15:1}$ venom alkaloid is rather invariable in *S. invicta* (0.423 ± 0.026, mean ± SD; $N = 35$) while the proportion in *S. richteri* is zero (Vander Meer, unpubl.). Thus an alkaloid index (I_{ALK}) was defined by the formula:

$$I_{ALK} = (P_{15:1})(2.36),$$

where $P_{15:1}$ is the proportion of $C_{15:1}$ alkaloid. A combined index for the GC characters (I) is the mean of the values for the hydrocarbon (I_{HC}) and venom alkaloid (I_{ALK}) indices, rescaled to vary between 0 and 1.0. Values of I for parental *S. richteri* are close to 0 while values for parental *S. invicta* approach 1.0 (see Results). The combined index is used in this study, since no additional information was obtained by using the hydrocarbon and alkaloid indices separately.

Gel Electrophoresis of Isoenzymes

A preliminary screen of enzyme systems in *S. invicta* (Ross et al., 1985) indicated that the products of 26 presumptive loci could be adequately resolved for study. These markers, and others developed subsequently (Ross, unpubl.), were used to study the comparative biochemical genetics of *S. invicta* and *S. richteri*. Four loci: *Alpha-glycerophosphate dehydrogenase-1* (*Agp-1*, EC 1.1.1.8); *Octanol dehydrogenase* (*Odh*, EC 1.1.1.73); *Esterase-2* and *Esterase-4* (*Est-2,4*; EC 3.1.1.1), were found to be informative with regard to distinguishing between the two forms and detecting gene introgression, and thus were used as genetic markers in the present study. Complete comparative data on the biochemical genetics and population structures of the two species will be reported separately.

Electrophoresis was carried out on 12% horizontal starch gels using standard techniques (see Harris and Hopkinson, 1976; May et al., 1979). All four loci are strongly expressed in the alitrunk, so only this body part was used. The enzyme products were best resolved using the amine-citrate buffer of Clayton and Tretiak (1972; "C" buffer of Ross et al., 1985). Staining followed Shaw and Prasad (1970) and Ross and Fletcher (1985*a*).

Mendelian inheritance of the electromorphs of *Agp-1* and *Est-4* was previously confirmed by Ross and Fletcher (1985*a*, 1985*b*). To show that the products of *Est-2* and *Odh* are also encoded by single Mendelian loci, genotype distributions for 14.8 ± 4.62 (mean ± SD) females from each of 132 colonies, and 13.0 ± 5.66 females from each

TABLE 1. Genotype distributions for virgin queens, males, foundresses, and the male mates of foundresses at two electrophoretic loci in eight representative colonies collected in a *S. invicta/richteri* hybrid zone. Genotypes of foundresses and their single mates are inferred from offspring genotypes.

	Est-2						*Odh*		
Colony	*F/F**	*F/M*	*F/S*	*M/M**	*M/S*	*S/S**	*F/F**	*F/S*	*S/S**
65-1O									
Queens		13	5					13	
Males				8		11	12		
Foundress					1		1		
Mate	1								1
65-1S									
Queens					8	10	12	6	
Males				9		7	6		4
Foundress					1			1	
Mate						1	1		
65-2Q									
Queens					15	9	22		
Males				7		4	8		
Foundress					1		1		
Mate						1	1		
65-1L									
Queens		4		9				18	
Males	8			10			19		
Foundress		1					1		
Mate				1					1
65-2G									
Queens					6	11		10	7
Males				5		7	2		7
Foundress					1			1	
Mate						1			1
65-2B									
Queens				19					19
Males				11					11
Foundress				1					1
Mate				1					1
65-2O									
Queens			13		5		11	6	
Males	6			5			4		6
Foundress		1						1	
Mate						1	1		
919-1E									
Queens					9	11		8	12
Males				7		8	6		9
Foundress					1			1	
Mate						1			1

* For males, these columns represent the corresponding hemizygote genotypes.

of 70 colonies were studied for *Est-2* and *Odh*, respectively. Females collected from each colony were the progeny of single foundress queens which were not, however, collected with the samples. This means that neither male nor female parents were available for genetic analyses. However, because males are haploid and queens are singly inseminated (Ross and Fletcher, 1985*a*; Ross, unpubl.), colony genotype distributions can be readily interpreted (Table 1). One of the following patterns was observed for females in every colony at each marker locus: 1) a single genotypic class was present, or 2) two genotypic classes were present in a 1:1 ratio, with at least one of the classes (one only at

Odh) being the heterozygote class. These data, and the fact that males exhibited only banding patterns expected from haploid progeny of the foundresses (Table 1), indicate that these products are encoded by single Mendelian loci.

Results

GC Phenotypes

Gas chromatographic analyses of hydrocarbons and venom alkaloids of *S. invicta* and *S. richteri* indicate that their biochemical phenotypes are sufficiently distinct to be used as diagnostic characters, substantiating the results of earlier studies (e.g., Brand et al., 1972; Lok et al., 1975; Barlin et al., 1976; MacConnell et al., 1976; Nelson et al., 1980; Vander Meer and Wojcik, 1982; Vander Meer et al., 1985). Under the GC conditions used, hydrocarbon peaks eluting from 17 to 21 min were characteristic of *S. richteri* and peaks eluting from 21 to 24 min were characteristic of *S. invicta* (Fig. 2), although minor amounts of components diagnostic of the other form were always present (see Materials and Methods). For the venom alkaloids, *S. invicta* was characterized by an abundance of C_{15} alkaloids absent from *S. richteri* venom, while possessing in low amounts the C_{11} alkaloid abundant in *S. richteri* venom (Fig. 2). For the 35 colonies sampled from the *S. invicta* range, the combined GC index (*I*) based on hydrocarbons and venom alkaloids varied from 0.85 to 1.00 (0.93 ± 0.03, mean ± SD), while for the 49 colonies from the *S. richteri* range this index varied from 0 to 0.06 (0.03 ± 0.01).

A colony was considered to be hybrid when its combined index fell outside the ranges characterizing the parental forms (i.e., $0.06 < I < 0.85$). Forty-four of the 50 (88.0%) colonies sampled from the putative hybrid zone possessed such hybrid phenotypes. The frequency distribution for the combined GC indices of these 44 colonies (Fig. 3) shows that most of them possessed biochemical phenotypes clearly intermediate between those of the parental forms (mean *I* ± SD for these colonies: 0.53 ± 0.13). A GC trace of hydrocarbons and venom alkaloids from a representative hybrid colony is shown in Figure 2.

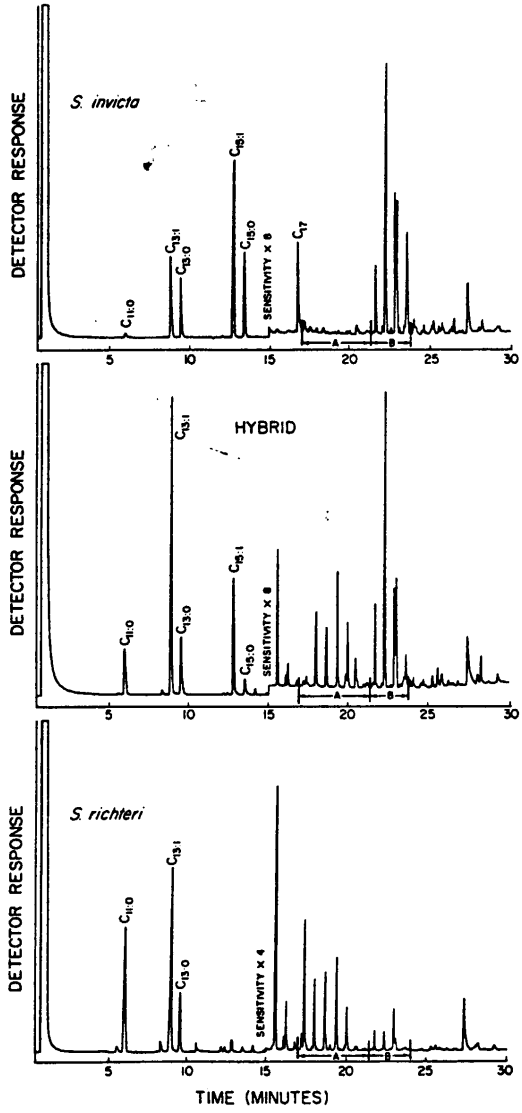

FIG. 2. Representative gas chromatograph traces of venom alkaloids and hydrocarbons from *S. invicta*, *S. richteri*, and their hybrid. The 2-alkyl or alkenyl side chains on the piperidine alkaloids are labeled on each chromatogram (retention times to 17 min). The hydrocarbon sections marked (A) and (B) define, respectively, the *S. richteri* and *S. invicta* components used to calculate the hydrocarbon index (I_{HC}) (see Materials and Methods).

Electrophoretic Markers

Four loci were found to be informative for distinguishing between ants collected from the parental ranges. Two of these (*Agp-1, Est-4*) are diallelic loci in *S. invicta*, while in *S. richteri* each is fixed for the more com-

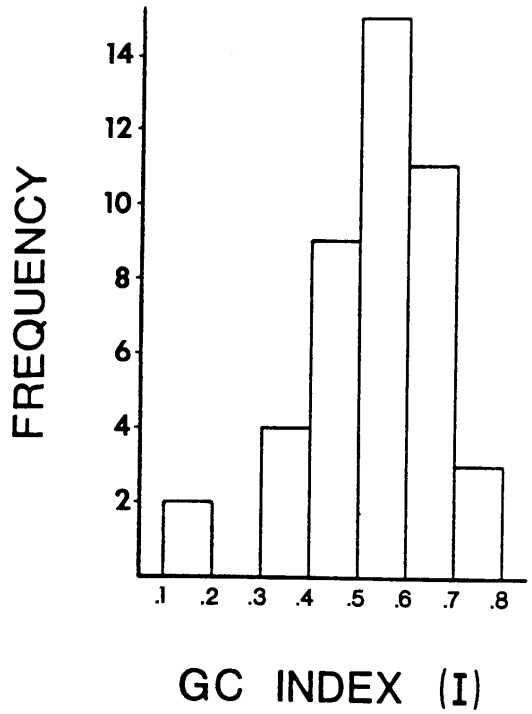

FIG. 3. Frequency distribution for combined GC indices (I) of 44 hybrid *S. invicta/richteri* colonies sampled from the hybrid zone in Mississippi. Values for parental *S. richteri* range between 0 and 0.06, while values for *S. invicta* range between 0.85 and 1.0.

mon *S. invicta* allele (see also Ross and Fletcher, 1985*a*, 1985*b*). At the other two loci (*Est-2, Odh*), the two forms apparently share no alleles in common and thus are completely differentiated. *S. richteri* is polymorphic at *Est-2* (two alleles), while *S. invicta* is fixed for a third distinct allele. The product of the locus *Odh* stains as a double-banded electromorph in all specimens of *S. invicta*; thus the ant is monomorphic at this locus. *S. richteri* is also monomorphic at *Odh*, but the enzyme product stains as a distinct single band of lower mobility than the *S. invicta* isoenzyme. One colony collected from the parental *S. richteri* area had 50% of females heterozygous for the slow (*S. richteri*) allele and another, faster allele at *Odh*, suggesting that an alternate allele occurs at an extremely low frequency (0.008) in *S. richteri*. Because a homozygote for this allele was not observed and because of poor resolution of the banding patterns of heterozygotes, we cannot at present say whether the product of this alternate allele has a mobility identical to the *S. invicta* electromorph.

Genetic distances (Nei's D^*: Nei, 1972; Hillis, 1984) between the seven sampled *S. invicta* populations and the *S. richteri* population were calculated, utilizing information from the four polymorphic and 22 monomorphic loci. The mean value of D^* ($\pm$SE) between the two forms is 0.183 ± 0.003. Similar genetic distances have been estimated between sibling species in the ant genera *Aphaenogaster* (Crozier, 1977: minimum $D = 0.19$), *Rhytidoponera* (Ward, 1980: mean $D = 0.136$), and *Formica* (Pamilo et al., 1979: mean $D = 0.123$ and 0.353 for two species complexes).

Electrophoretic evidence for hybrid ancestry of a fire ant colony exists when: 1) individuals are heterozygotes possessing one allele from each of the parental forms at either *Est-2* or *Odh*; or, 2) individuals possess alleles characteristic of one of the parental forms at one marker locus and alleles of the other form at a different locus. Considering our minimum sample size of 10 females per colony and the fact that queens are singly inseminated, the probability of not detecting hybrid genotypes at either *Est-2* or *Odh* in any colony where they occur is less than 0.01 (binomial probability).

Electrophoretic data for colonies from the Mississippi hybrid zone confirm that gene introgression between the two forms is occurring here. Forty-seven of 59 (79.7%) colonies sampled possessed female genotypes indicative of hybridization. At the locus *Est-2* all six possible recombinant genotypes were observed in the hybrid zone, as were all three recombinant genotypes at the locus *Odh* (see Table 1). Individuals heterozygous for the two parental alleles at *Odh* exhibited a poorly resolved band(s) upon staining, with mobility intermediate to that of the parental electromorphs. As expected, no males exhibited this banding pattern. Allele frequencies at the four marker loci for the two parental forms and Mississippi hybrid population are presented in Table 2.

Of the 47 hybrid colonies detected by electrophoresis, none exhibited multilocus genotype arrays for females consistent with their being F_1 hybrids (i.e., colonies founded by a queen of one form mated to a male of the other form). For 16 of the 47 colonies

TABLE 2. Allele frequencies at four electrophoretic loci for *S. invicta*, *S. richteri*, and a hybrid population in east-central Mississippi. One female genotype per colony is used for the estimates (sample sizes in parentheses). Colonies from the hybrid zone characterized as parental-types by both electrophoresis and GC analysis ($N = 6$) are included in the hybrid population. Subscripts denote allelic designations at each locus.

	Est-2	*Agp-1*	*Est-4*	*Odh*
S. invicta ($N = 210$)	$p_F = 0$ $q_M = 1.0$ $r_S = 0$	$p_F = 0.629$ $q_S = 0.371$	$p_A = 0.597$ $q_B = 0.403$	$p_F = 1.0$ $q_S = 0$
Hybrid ($N = 59$)	$p_F = 0.136$ $q_M = 0.5$ $r_S = 0.364$	$p_F = 0.898$ $q_S = 0.102$	$p_A = 0.856$ $q_B = 0.144$	$p_F = 0.551$ $q_S = 0.449$
S. richteri ($N = 59$)	$p_F = 0.553$ $q_M = 0$ $r_S = 0.447$	$p_F = 1.0$ $q_S = 0$	$p_A = 1.0$ $q_B = 0$	$p_X = 0.008$ $q_S = 0.992$

(34%), female genotypes were consistent with the foundresses having been F_1 hybrids, but the remaining hybrid colonies must have been founded by queens which were the products of subsequent generations of hybridization or backcrossing with the parental forms. Such a lack of parental and F_1 genotypes is the expected outcome of long-term random mating in a hybrid zone (Barton, 1982). Our data indicate a sufficiently high level of genetic compatibility between the parental fire ant taxa to preclude significant breakdown of F_1 viability, but do not rule out some reduced lifetime fitness for these or other recombination products relative to the parental forms.

Comparisons of observed frequencies of genotypes at the four polymorphic loci in the hybrid zone with frequencies expected under Hardy-Weinberg equilibrium reveal no significant differences when colonies designated as "pure" parental-types by both biochemical methods are excluded ($N = 52$ colonies; chi-square test, all $P > 0.05$). When these parental-type colonies are included in the analysis, genotype frequencies at two loci remain as expected, but frequencies at *Odh* and *Est-2* depart significantly from Hardy-Weinberg expectations ($N = 59$ colonies; chi-square test, $P < 0.02$ and $P < 0.05$, respectively), with deficiencies of heterozygotes at each locus. (Due to the limited number and resolution of samples from the hybrid zone it is not clear whether the parental-type colonies lie within the zone of introgression and should be included in the analysis.) The results would seem to suggest an essentially panmictic hybrid population with no extreme disruptive selection against hybrid genotypes, although further studies will be required to clarify this point (see also Hunt and Selander, 1973; McDonnell et al., 1978; Kocher and Sage, 1986).

Concordance of Data Sets and Structure of the Hybrid Zone

Concordance between the biochemical phenotypic and biochemical genetic data sets is virtually complete, and both data sets agree well with the taxonomy derived from external morphology. Colonies sampled from the parental ranges were unambiguously assigned to the proper (morphological) taxon using either biochemical method. In the hybrid zone, the two methods agreed on assignments of colonies as hybrid or parental for 45 of the 50 colonies (90.0%). Morphological assignment of the ants from these 45 colonies by J. C. Trager also agreed well with the biochemical designations (hybrid ants are morphologically intermediate between the parental forms; complete morphological data on the parental forms and hybrid will be published elsewhere by Trager). The high degree of concordance for the biochemical data from the hybrid zone is particularly noteworthy in light of some expected discordance because of Mendelian inheritance of the allozyme markers. With a limited number of these markers assorting independently, some hybrid colonies will by chance possess recombinant genotypes characteristic of either of the parental forms, given that hybridization has been occurring for a sufficiently long period (the genetic data

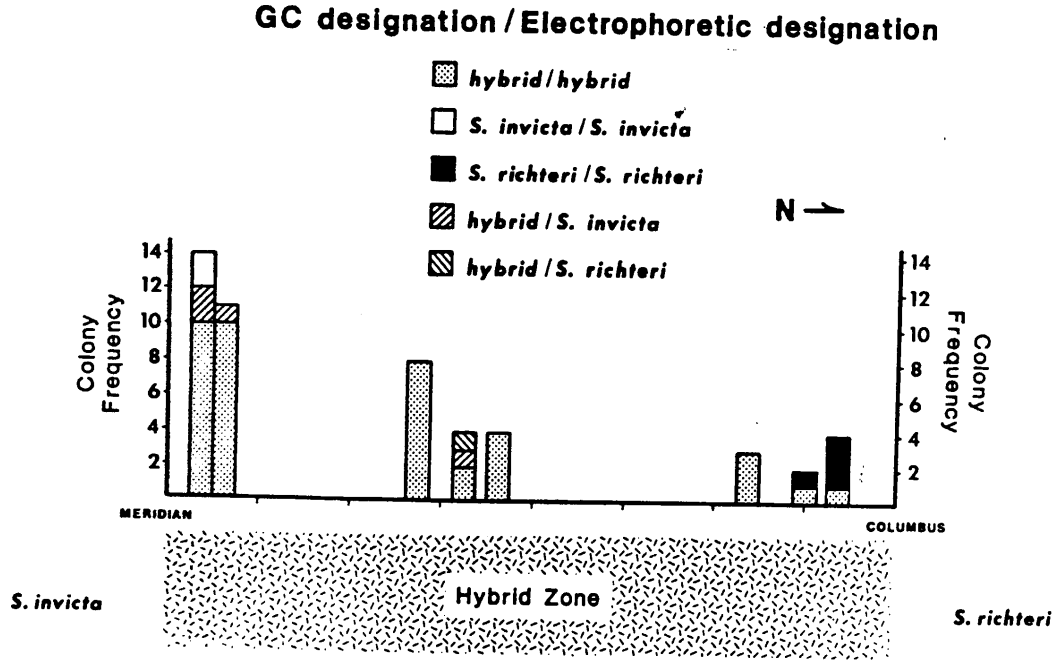

FIG. 4. GC and electrophoretic designations of colonies collected along a north-south transect in the *S. invicta/richteri* hybrid zone in Mississippi. Ticks occur on the horizontal axis every 15 km.

presented above and GC analyses of museum specimens [Vander Meer et al., unpubl.] suggest that this is the case). Indeed, all five colonies for which the biochemical results are discordant were designated as hybrid colonies by the GC method but exhibited parental genotypes at the electrophoretic loci. Discordance of a more problematic nature would exist if colonies shown to be hybrids by electrophoresis exhibited parental hydrocarbon and venom alkaloid phenotypes. Such colonies were not found.

The GC and electrophoretic designations of the 50 study colonies from the hybrid zone are depicted in relation to their position along the collecting transect in Figure 4. Only six of the 50 colonies (12.0%) from the hybrid zone exhibited both parental phenotypes and genotypes. These results and the genetic evidence for F_1 viability and random mating indicate that this zone represents a "true hybrid zone" (Littlejohn and Watson, 1985) rather than a zone of overlap with some hybridization.

Nine of 11 colonies designated as parental by either or both of the methods occurred near the margins of what appeared on the basis of previous data and field observations to be the "pure" parental ranges (Fig. 4). Five of the nine colonies were designated as *S. invicta* and were collected within 9 km of Meridian, MS, the presumed northern limit of *S. invicta* in this area. The other four colonies, designated as *S. richteri*, occurred within 16 km of Columbus, MS, the probable southern limit of this form (Vander Meer et al., 1985). Such results are to be expected if the collecting transect in the hybrid zone coincides with a north-south cline in gene frequencies, with the pure species genotypes dominating north and south of the transect. Although our sampling regime was not designed to investigate the genetic structure of the hybrid zone, when allele frequencies are plotted for groups of colonies according to their relative position along the transect, gene frequency clines are evident for the four polymorphic loci (Fig. 5). These clinal patterns persist for all loci except *Odh* even when the six colonies designated as parental-types by both methods are excluded (Fig. 5).

A suggestive trend in the mean combined GC indices for groups of colonies from the hybrid zone (Fig. 6) hints that the genetic determinants of the hydrocarbon and al-

kaloid phenotypes may exhibit similar clinal structures here. If such a pattern is confirmed by more detailed studies of this zone, then these biochemical markers would appear to be sufficiently sensitive to estimate the genetic character of individual hybrid colonies (i.e., the relative genetic contribution from each of the parental forms).

Discussion

Our biochemical phenotypic and genetic data clearly differentiate between North American *S. invicta* and *S. richteri,* in accord with the morphological studies of Buren (1972). They also confirm that extensive hybridization between the two forms is occurring at the contact zone in Mississippi, and probably in other areas of the North American range as well (Vander Meer and Diffie, unpubl.; Ross, unpubl.). The fire ant hybrid zone in Mississippi is unique among an increasing number of well-documented cases of animal hybridization (see Barton and Hewitt, 1985) in that the history of its geographical origin and subsequent development is becoming rather well known. For instance, it is clear that hybridization between these two introduced taxa has resulted from secondary contact and that hybridization was occurring several decades ago where contact was first established in southern Alabama (based on chromatographic analyses of museum specimens; Vander Meer et al., unpubl.). Furthermore, the results of the present study, taken with those of Vander Meer et al. (1985) and Vander Meer et al. (unpubl.) show conclusively that the hybrid zone has moved northward over the past several decades to occupy its present position in east-central Mississippi, since only genetically and phenotypically "pure" *S. invicta* colonies persist in southern Alabama and Mississippi at this time. These results are not consistent with the predictions of one of the major models explaining zones of hybridization, the dispersal-dependent model of neutral introgression (see Moore [1977]; Barton and Hewitt [1985]; Littlejohn and Watson [1985]; and Moore and Buchanan [1985] for discussions of models of hybridization). In this model, neither a barrier to gene flow nor competition between the parental forms exists, so that for organisms with high reproductive

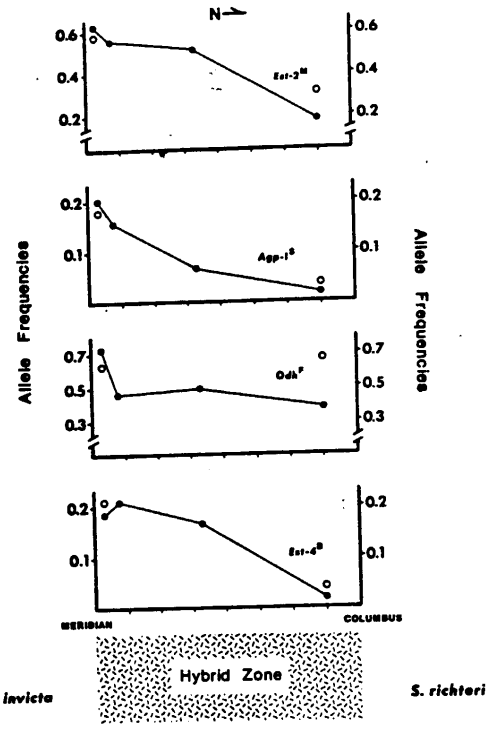

FIG. 5. Frequencies of diagnostic *S. invicta* alleles at four loci for four groups of colonies ($N = 12$–16 colonies/group, total $N = 59$ colonies) collected along a north-south transect through the *S. invicta/richteri* hybrid zone in Mississippi. Open circles represent allele frequencies in the two terminal groups when colonies designated as parental types by both biochemical methods are excluded. Ticks occur on the horizontal axis every 15 km.

rates and considerable vagility, such as fire ants (Markin et al., 1971; Lofgren et al., 1975), secondary contact and introgression would result in the appearance of rapidly decaying clines (expanding hybrid zones) at the site where contact was first established.

Two other models of hybridization invoke selection favoring or disfavoring hybrid individuals as important forces maintaining hybrid zones (the bounded hybrid superiority and dynamic equilibrium models). Selection on hybrids constitutes a substantial barrier to gene flow in these models and leads to the appearance of temporally stable, but not necessarily spatially stable zones of introgression. In the dispersal-dependent model of dynamic equilibrium, selection against hybrids is balanced by gene flow from the parental populations (Barton and Hewitt, 1981,

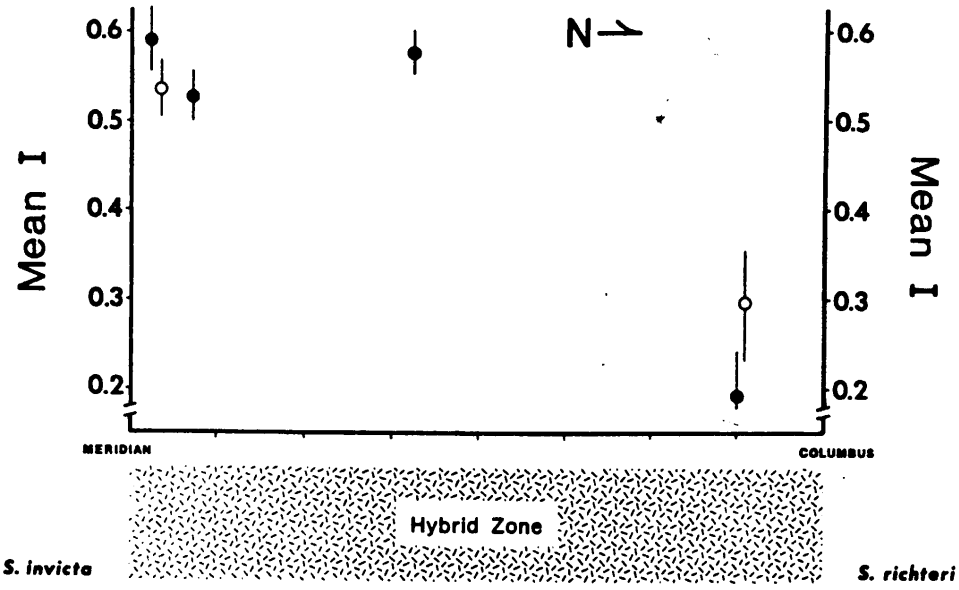

FIG. 6. Mean combined GC indices (I) for four groups of colonies (N = 9–16 colonies/group, total N = 50 colonies) collected along a north-south transect through the *S. invicta/richteri* hybrid zone in Mississippi. Open circles represent mean combined GC indices for the two terminal groups when colonies designated as parental types by both biochemical methods are excluded. Bars represent one standard error above and one below the mean. Similar results were obtained when the venom alkaloid (I_{ALK}) and hydrocarbon (I_{HC}) indices were plotted separately. Ticks occur on the horizontal axis every 15 km.

1985). Although no obvious reduction in fitness of F_1 hybrids is apparent from our data, some degree of dysgenesis could occur in subsequent hybrid or backcross generations (e.g., Moran, 1981), so that dynamic equilibrium cannot be discounted as an explanation for the *S. invicta/richteri* hybrid zone in Mississippi. The dispersal-independent model of hybrid superiority invokes a fitness advantage to recombinant genotypes in habitats intermediate to those favored by the parental forms (Moore, 1977). The probable occurrence of hybrid swarms in northern Alabama and Georgia which are not bounded to the north by *S. richteri* (Diffie and Vander Meer, unpubl.) would appear to support this model (Moore, 1977; Barton and Hewitt, 1985), although the recent dramatic movement of the Mississippi hybrid zone may be problematic. An intriguing notion here is that hybrid superiority in some habitats may be related to an increase in genetic diversity relative to the parental forms, both of which may have suffered a loss of diversity during colonization of North America (as manifested in increased frequencies of diploid male production and colony mortality; see Ross and Fletcher, 1985*b*, 1986).

A final explanation for the Mississippi hybrid zone invokes a wave of advance of a competitively superior genotype, with the hybrid zone moving in the direction of the inferior competitor (Endler, 1977; Barton and Hewitt, 1985). In view of the rapid spread and ecological success of *S. invicta* in North America relative to *S. richteri*, the appeal of this model is obvious, although, in common with other dispersal-dependent mechanisms, it cannot explain persistent hybrid populations isolated from either of the parental forms. Future long-term genetic, distributional, and fitness studies of introduced fire ants will be necessary to identify the forces regulating the dynamics of the parental and hybrid populations.

As to the taxonomic status of *S. invicta* and *S. richteri*, opinion varies, and any decision is rendered more difficult by the fact that these are introduced organisms. According to Barton and Hewitt (1985), these forms would not be considered biological

species since reproductive isolation is not complete. On the other hand, Bigelow (1965) and Mayr (1982) suggest that the gene pools of different species need not be completely isolated, so long as "one well-integrated and harmoniously coadapted gene pool is protected from swamping by another" (Bigelow, 1965). An important conclusion of the present study is that this latter situation appears to hold for North American *S. invicta* and *S. richteri*. Genetically "pure" *S. richteri* populations persist to the north of the Mississippi hybrid zone, and more importantly, pure *S. invicta* is found to the south of the zone, in areas where hybrids previously occurred (Vander Meer et al., unpubl.). Thus, substantial barriers to gene introgression probably exist, and this stability of the parental forms suggests that the taxa have embarked on independent evolutionary paths.

The situation in South America is less than clear, however, because of the large diversity of forms within the subgenus *Solenopsis* and the occurrence of many intermediates between these (Creighton, 1930; Wilson, 1952; Trager, unpubl.). It is certainly conceivable that the two taxa represent ecotypes characterized by limited genetic exchange in South America, and that possible bottlenecks experienced by both forms upon introduction further crystallized their genetic distinctiveness. Alternatively, they may be fully reproductively isolated in South America, but colonization of a novel environment has effectively compromised the reproductive barriers. Unfortunately, anthropogenic habitat alteration is further confusing the situation in South America by breaking down presumed ecological barriers to gene flow among fire ant populations, as also appears to be the case for a diversity of other organisms forming stable hybrid zones (Anderson, 1948; Hubbs, 1955; Wasserman, 1957; McDonnell et al., 1978; Littlejohn and Watson, 1985). Detailed distributional, ecological, and biochemical studies of the *S. saevissima* species complex in South America may help clarify the situation by ascertaining whether gene introgression among genetically distinct populations is commonplace.

Finally, one clear outcome of our study is that the GC methods utilized appear to be appropriate and sensitive tools for systematic study of at least this group of insects, and may be widely applicable in arthropods. The hydrocarbon and venom alkaloid data were fully concordant with the electrophoretic data and also agreed well with morphological assignment of the specimens. Electrophoresis and chromatography can be complementary molecular techniques in the sense that they investigate traits with fundamentally different genetic bases, those observed with electrophoresis being discrete Mendelian traits and those observed with chromatography being continuous traits determined polygenically. Both techniques should be regarded as potentially valuable independent sources of data for systematics and population genetics.

Acknowledgments

We thank R. H. Crozier, E. O. Wilson, J. M. Carpenter, and J. C. Trager for helpful comments on the manuscript. J. T. Brooks, R. Weeks, and S. U. Hall assisted in the laboratory. Special thanks to T. D. Canerday for support throughout the project. This research was funded in part by NSF grant PCM 82 09097 to M. S. Blum and D. J. C. Fletcher.

Literature cited

Anderson, E. 1948. Hybridization of the habitat. Evolution 2:1–9.

Averhoff, W. W., and R. H. Richardson. 1976. Multiple pheromone system controlling mating in *Drosophila*. Proc. Nat. Acad. Sci. USA 73:591–593.

Barlin, M. R., M. S. Blum, and J. M. Brand. 1976. Fire ant trail pheromones: Analysis of species specificity after gas chromatographic fractionation. J. Insect Physiol. 22:839–844.

Barton, N. H. 1982. The structure of the hybrid zone in *Uroderma bilobatum* (Chiroptera:Phyllostomatidae). Evolution 36:863–866.

Barton, N. H., and G. M. Hewitt. 1981. Hybrid zones and speciation, pp. 109–145. *In* W. R. Atchley and D. S. Woodruff (eds.), Evolution and Speciation: Essays in Honour of M. J. D. White. Cambridge Univ. Press, London, U.K.

———. 1985. Analysis of hybrid zones. Ann. Rev. Ecol. Syst. 16:113–148.

Bigelow, R. S. 1965. Hybrid zones and reproductive isolation. Evolution 19:449–458.

Brand, J. M., M. S. Blum, H. M. Fales, and J. G. MacConnell. 1972. Fire ant venoms: Comparative analyses of alkaloidal constituents. Toxicon 10:259–271.

BUREN, W. F. 1972. Revisionary studies on the taxonomy of the imported fire ants. J. Georgia Entomol. Soc. 7:1–26.

BUREN, W. F., G. E. ALLEN, W. H. WHITCOMB, F. E. LENNARTZ, AND R. N. WILLIAMS. 1974. Zoogeography of the imported fire ants. J. N.Y. Entomol. Soc. 82:113–124.

CLAYTON, J. W., AND D. N. TRETIAK. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29:1169–1172.

CREIGHTON, W. S. 1930. The new world species of the genus *Solenopsis* (Hymenoptera:Formicidae). Proc. Amer. Acad. Arts & Sci. 66:39–151.

CROZIER, R. H. 1977. Genetic differentiation between populations of the ant *Aphaenogaster rudis* in the southeastern United States. Genetica 47:17–36.

ENDLER, J. A. 1977. Geographic Variation, Speciation, and Clines. Princeton Univ. Press, Princeton, NJ.

GHISELIN, M. T. 1975. A radical solution to the species problem. Syst. Zool. 23:536–544.

GRULA, J. W., AND O. R. TAYLOR. 1979. The inheritance of pheromone production in the sulphur butterflies *Colias eurytheme* and *C. philodice*. Heredity 42:359–371.

HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland, N.Y.

HILLIS, D. M. 1984. Misuse and modification of Nei's genetic distance. Syst. Zool. 33:238–240.

HOWARD, R. W., AND G. J. BLOMQUIST. 1982. Chemical ecology and biochemistry of insect hydrocarbons. Ann. Rev. Entomol. 27:149–172.

HUBBS, C. L. 1955. Hybridization between fish species in nature. Syst. Zool. 4:1–20.

HULL, D. L. 1974. Philosophy of Biological Science. Prentice-Hall, Englewood Cliffs, NJ.

HUNT, W. G., AND R. K. SELANDER. 1973. Biochemical genetics of hybridisation in European house mice. Heredity 31:11–33.

KOCHER, T. D., AND R. D. SAGE. 1986. Further genetic analyses of a hybrid zone between leopard frogs (*Rana pipiens* complex) in central Texas. Evolution 40:21–33.

LITTLEJOHN, M. J., AND G. F. WATSON. 1985. Hybrid zones and homogamy in Australian frogs. Ann. Rev. Ecol. Syst. 16:85–112.

LOFGREN, C. S., W. A. BANKS, AND B. M. GLANCEY. 1975. Biology and control of imported fire ants. Ann. Rev. Entomol. 20:1–30.

LOK, J. B., E. W. CUPP, AND G. J. BLOMQUIST. 1975. Cuticular lipids of the imported fire ants, *Solenopsis invicta* and *richteri*. Insect Biochem. 5:821–829.

MACCONNELL, J. G., M. S. BLUM, W. F. BUREN, R. N. WILLIAMS, AND H. M. FALES. 1976. Fire ant venoms: Chemotaxonomic correlations with alkaloidal compositions. Toxicon 14:69–78.

MARKIN, G. P., J. H. DILLIER, S. O. HILL, M. S. BLUM, AND H. R. HERMANN. 1971. Nuptial flight and flight ranges of the imported fire ant, *Solenopsis saevissima richteri*. J. Georgia Entomol. Soc. 6:145–156.

MAY, B., J. E. WRIGHT, AND M. STONEKING. 1979. Joint segregation of biochemical loci in Salmonidae: Results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Board Can. 36:1114–1128.

MAYR, E. 1982. The Growth of Biological Thought: Diversity, Evolution, and Inheritance. Belknap, Cambridge, MA.

MCDONNELL, L. J., D. F. GARTSIDE, AND M. J. LITTLEJOHN. 1978. Analysis of a narrow hybrid zone between two species of *Pseudophryne* (Anura: Leptodactylidae) in south-eastern Australia. Evolution 32:602–612.

MOORE, W. S. 1977. An evaluation of narrow hybrid zones in vertebrates. Quart. Rev. Biol. 52:263–277.

MOORE, W. S., AND D. B. BUCHANAN. 1985. Stability of the Northern Flicker hybrid zone in historical times: Implications for adaptive speciation theory. Evolution 39:135–151.

MORAN, C. 1981. Genetic demarcation of geographic distribution by hybrid zones. Proc. Ecol. Soc. Austral. 11:67–73.

NEI, M. 1972. Genetic distance between populations. Amer. Natur. 106:283–292.

NELSON, D. R. 1978. Long-chain methyl-branched hydrocarbons: Occurrence, biosynthesis, and function. Adv. Insect Physiol. 13:1–33.

NELSON, D. R., C. L. FATLAND, R. W. HOWARD, C. A. MCDANIEL, AND G. J. BLOMQUIST. 1980. Re-analysis of the cuticular methylalkanes of *Solenopsis invicta* and *S. richteri*. Insect Biochem. 10:409–418.

PAMILO, P., K. VEPSALAINEN, R. ROSENGREN, S-L. VARVIO-AHO, AND B. PISARSKI. 1979. Population genetics of *Formica* ants. II. Genic differentiation between species. Ann. Entomol. Fenn. 45:65–76.

ROSS, K. G., AND D. J. C. FLETCHER. 1985*a*. Comparative study of genetic and social structure in two forms of the fire ant, *Solenopsis invicta* (Hymenoptera:Formicidae). Behav. Ecol. Sociobiol. 17:349–356.

———. 1985*b*. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera:Formicidae), and its evolutionary significance. Evolution 39:888–903.

———. 1986. Diploid male production—A significant colony mortality factor in the fire ant, *Solenopsis invicta* (Hymenoptera:Formicidae). Behav. Ecol. Sociobiol. 19:283–291.

ROSS, K. G., D. J. C. FLETCHER, AND B. MAY. 1985. Enzyme polymorphisms in the fire ant, *Solenopsis invicta* (Hymenoptera:Formicidae). Biochem. Syst. Ecol. 13:29–33.

SHAW, C. R., AND R. PRASAD. 1970. Starch gel electrophoresis of enzymes—A compilation of recipes. Biochem. Genet. 4:297–320.

VANDER MEER, R. K., C. S. LOFGREN, AND F. M. ALVAREZ. 1985. Biochemical evidence for hybridization in fire ants. Florida Entomol. 68:501–506.

VANDER MEER, R. K., AND D. P. WOJCIK. 1982. Chemical mimicry in the myrmecophilous beetle *Myrmecaphodius excavaticollis*. Science 218:806–808.

WARD, P. S. 1980. Genetic variation and population differentiation in the *Rhytidoponera impressa* group, a species complex of ponerine ants (Hymenoptera: Formicidae). Evolution 34:1060–1076.

WASSERMAN, A. O. 1957. Factors affecting inter-

breeding in sympatric species of spadefoots (genus *Scaphiopus*). Evolution 11:320–338.

WILEY, E. O. 1981. Phylogenetics: The Theory and Practice of Phylogenetic Systematics. Wiley, N.Y.

WILSON, E. O. 1952. O complexo *Solenopsis saevissima* na America do Sul (Hymenoptera:Formicidae). Mem. Inst. Oswaldo Cruz 50:49–68.

———. 1953. Origin of the variation in the imported fire ant. Evolution 7:262–263.

———. 1958. The fire ant. Sci. Amer. 198:36–41.

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