

# Development of RAPD markers in two introduced fire ants, *Solenopsis invicta* and *S. richteri*, and their application to the study of a hybrid zone

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## Abstract

We developed RAPD DNA markers useful in distinguishing the fire ants *Solenopsis invicta* and *S. richteri*. An initial survey of 200 primers revealed seven informative markers; family studies allowed us to determine expression patterns and to confirm Mendelian inheritance of these markers. The seven RAPD markers, one of which is inherited as a codominant marker, were employed along with three allozyme markers to describe the structure of a hybrid zone that has formed between the two species in the USA, where they have been introduced. The data suggest minor introgression of alleles from one parental species (*S. richteri*) into the other (*S. invicta*), which most likely reflects the documented recent movement of this hybrid zone. This pattern is interpreted as interspecific introgression rather than shared intraspecific polymorphism on the basis of comparisons with samples from native, non-hybridizing populations in South America that lack such polymorphism. The data further reveal that the structure of the hybrid zone in the USA varies geographically. One parental species (*S. invicta*) and the hybrids exhibit a mosaic distribution in the east; a gradual transition between the parental species occurs in the centre, with a large intervening area of hybrid genotypes only; and there is apparent contact between parental populations with a small or no intervening zone of hybridization in the west. These differing patterns in the structure of the hybrid zone presumably reflect the unique histories of colonization in different parts of the range of introduced fire ants.

**Keywords:** fire ants, hybridization, hybrid zone, random amplified polymorphic DNA (RAPD), *Solenopsis invicta*, *Solenopsis richteri*

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## Introduction

Random amplified polymorphic DNA (RAPD) markers have become increasingly important in their applications to population and molecular biology. These markers, first developed by Williams *et al.* (1990), have been used effectively in constructing genetic linkage maps, in assessing patterns of paternity and kinship, in determining the taxonomic identity of strains or varieties, in resolving the phylogenetic relationships of taxa, and in analysing patterns of interspecific gene flow (Williams *et al.* 1990; Welsh & McClelland 1990; Chapco *et al.* 1990; Arnold

*et al.* 1991; Hadrys 1991; Hadrys *et al.* 1992; Kazan *et al.* 1993; Fondrk *et al.* 1993). RAPD markers offer several advantages over conventional molecular markers such as RFLPs, including significant reduction in expense of their development and use, relative ease of the technical methods involved, and the ability to generate both large sample sizes and large numbers of markers. Furthermore, development of these markers requires no prior knowledge of the DNA sequences of the organism under study and the markers usually seem to be distributed randomly throughout the genome (but see Tinker *et al.* 1993), making them ideal markers for population studies of gene flow and mating structure (Hadrys *et al.* 1992; Bowditch *et al.* 1993). However, there are some disadvantages of RAPD markers, the most important of which is non-genetic artefactual variation that has been reported in

some studies (Riedy *et al.* 1992; Ellsworth *et al.* 1993). The existence of such nongenetic variation emphasizes the importance of confirming the mode of inheritance of all such markers early in a project.

The purpose of this study was two-fold. First, we wished to develop RAPD markers that are of use in distinguishing two fire ant species that have been introduced into the USA, *Solenopsis invicta* and *S. richteri*. Such markers ideally would be ones in which alternate alleles or phenotypes are fixed in each of the two species (hereafter termed diagnostic markers; cf. Ayala & Powell 1972). Secondly, we wished to use these new markers, along with existing markers of the nuclear genome generated by protein electrophoresis, to describe with greater resolution than previous studies the macrogeographic structure of a hybrid zone that has formed between the two species in their introduced range. Such information on patterns of introgression on a macrogeographic scale could then be used to decide where future genetic analyses of hybridization at microgeographic scales should be conducted.

Hybridization between *S. invicta* and *S. richteri* was first reported by Vander Meer *et al.* (1985), and subsequent study has suggested that the hybrid zone is rather extensive, spanning an area from western Georgia through Alabama to central Mississippi (Diffie *et al.* 1988). However, these earlier descriptions of the distributions of the two species and their hybrids were based on relatively few samples collected intermittently throughout the introduced range. Furthermore, taxonomic characterization of the collected ants was based on biochemical markers with an unknown genetic basis (venom alkaloid and cuticular hydrocarbon patterns generated from gas chromatography). Thus, it was of interest to describe in finer detail the macrogeographic structure of this hybrid zone using a large array of genetic markers with well understood patterns of inheritance.

Hybrid zones – regions in which interactions between genetically distinct groups result in some offspring of mixed ancestry – are rich sources of information for evolutionary genetic studies (Harrison 1990). Such studies can provide insights into the effects of introgression on the maintenance of genetic distinctiveness, into the genetic architecture and functioning of reproductive barriers to gene exchange, and into the genetic and ecological nature of species differences (e.g. Barton & Hewitt 1985; Kocher & Sage 1986; Harrison 1990). Studies of the hybrid zone formed between *S. invicta* and *S. richteri* promise to be particularly fruitful because the history of formation of the zone is known (i.e. it formed through secondary contact earlier in this century), the reproductive and dispersal biology of these species is well understood, and information on the population and molecular genetics of these ants is accumulating rapidly (Markin *et al.* 1971; Lofgren 1986; Ross & Trager 1990; Ross *et al.* 1987b, 1988, 1993).

The most useful evolutionary genetic studies of hybrid zones employ detailed genetic analyses of selected regions of the zones (Szymura & Barton 1986, 1991; Kocher & Sage 1986; Harrison 1990, 1993; Cruzan & Arnold 1993). Areas most appropriate for such detailed analyses of fire ant hybridization can only be identified from a complete description of the macrogeographic structure of the hybrid zone, as is accomplished here.

## Materials and methods

### Collection of samples

Seven to ten adult winged fire ant queens were collected from each of 550 colonies at 106 sites distributed throughout Mississippi, Alabama, and western Georgia, USA. Sites were chosen so that we could characterize the distributions of *Solenopsis invicta* and *S. richteri* and resolve the macrogeographic structure of the hybrid zone formed between them on a finer scale than in previous studies. Ants from an additional ten colonies known to be headed by a single egg-laying queen (i.e. monogyne colonies) were collected for the purpose of conducting family studies to determine the mode of inheritance of the RAPD markers. These samples were collected within the hybrid zone in central Mississippi and included the wingless queen heading each colony and seven to ten of her adult male or adult female (winged queen) offspring. Ants from the USA were identified taxonomically on the basis of their genotypes at three allozyme loci (see below). Fire ants also were collected from their native ranges in northern and central Argentina (Corrientes, Formosa, and Sante Fe Provinces), where we collected one adult winged queen from each of 20 colonies for each of the two species (Trager 1991). In contrast to the extensive hybridization that occurs in the USA, *S. invicta* and *S. richteri* appear to be reproductively isolated in South America (Ross & Trager 1990). Thus, these Argentine samples were important for determining whether any shared variation observed in the USA is due to ancestral intraspecific polymorphism or to introgression that has occurred between the species following their recent colonization of the USA. Ants from Argentina were identified taxonomically on the basis of morphological characters. All collected samples were stored on liquid nitrogen in the field until returned to the laboratory, where they were stored in a freezer at  $-70^{\circ}\text{C}$ .

### Allozyme analyses

The genotypes of a single adult winged queen from each sampled colony were determined at three allozyme loci (*Gpi*, *Est-2*, and *Odh*) that previously have been shown to be diagnostic for the two species in the USA and to exhibit

Mendelian patterns of inheritance (Ross *et al.* 1987a,b). Thus, a colony could be characterized as 'hybrid' if an individual was heterozygous for one allele from each parental species at one or more of these loci or if an individual was homozygous for alleles characteristic of one species at one locus and for alleles of the other species at a different locus.

The genotypes of an additional seven winged queens per colony were determined at the three diagnostic allozyme loci for the 240 colonies that were collected from 49 of our sampling sites in central and eastern Mississippi. We determined this area to be of special interest because our initial survey suggested that the ranges of the two parental species may come into contact here without an intervening zone of hybridization. By examining this greater number of individuals per colony, we were more likely to uncover the entire spectrum of genotypes that exists in each colony and thus to correctly identify colonies as having genotypic profiles characteristic of the parental species or hybrids. Indeed, by scoring the genotypes of eight offspring per colony we could be more than 99% confident that all genotypes present at a given locus in the colony were found (assuming that all of these colonies were headed by a single egg-laying queen mated to a single haploid male; see Ross & Fletcher 1985; Ross *et al.* 1988). Correct identification of the 240 colonies composing this subset of samples also was important because these same colonies were used for developing our RAPD markers. Thus, it was desirable to avoid misclassifying any colonies of recent mixed ancestry as parentals because this might have resulted in the early rejection of a potentially informative new marker.

#### DNA isolations

Total DNA was isolated from single gasters (abdomens) of adult winged queens using a modification of Protocol 48 of Ashburner (1989). Each gaster was homogenized in 400  $\mu$ L of grinding buffer [4:1 ratio of homogenization buffer (0.1-M NaCl; 0.2-M sucrose; 10-mM EDTA; 30-mM Tris-HCl pH 8.0) to OLB/phage lysis buffer (25-mM EDTA; 0.5-M Tris-HCl pH 9.2 with 2.5% SDS)] and incubated at 65 °C for 30 min. Sixty microlitres of 8-M KAc was added to each sample and these were placed on ice for 30 min, followed by centrifugation at 4 °C for 10 min. Six-hundred microlitres of 100% ethanol was then added to the supernatant. The samples were immediately centrifuged and the pellet resuspended in 200  $\mu$ L of Tris-EDTA (TE). Samples were then treated with RNAase for 30 min at 37 °C and extracted with phenol/chloroform/isoamyl alcohol (25:24:1). After extraction, phases were separated by centrifugation for ten min. DNA was precipitated overnight at -20 °C using 1/10 volume (20  $\mu$ L) of 3-M NaAc and two volumes (400  $\mu$ L) of 100% ethanol. Sam-

ples were then centrifuged for five min and the pellet resuspended in 100  $\mu$ L of TE. Five microlitres of each sample was electrophoresed in 1% agarose minigels in the presence of ethidium bromide and was visualized under UV light to test for DNA degradation and completion of RNA digestion.

#### Screening of RAPD primers

We used RAPD analysis to develop diagnostic nuclear DNA markers that distinguish *S. invicta* and *S. richteri*. This technique entails amplifying DNA by using the polymerase chain reaction and single oligonucleotide primers of arbitrary sequence ('random amplified polymorphic DNA' or 'RAPD' analysis; Williams *et al.* 1990; Welsh & McClelland 1990). For the initial screens, we amplified DNA from a single individual of each species collected in the USA using 200 RAPD primers obtained from the University of British Columbia Biotechnology Laboratory (primer sets UBC 101-200 and UBC 401-500). Reaction mixtures (25  $\mu$ L) for the RAPD analysis contained 10-mM Tris-HCl (pH 8.3); 50-mM KCl; 1.9-mM MgCl<sub>2</sub>; 0.001% gelatin; 0.1 mM each of dATP, dGTP, dCTP and dTTP; 5 pmol of a single, unique primer; 125 ng of total genomic DNA; and 0.5 units of *Taq* DNA polymerase (Promega). Amplification reactions were carried out using a Perkin-Elmer-Cetus DNA thermal cycler, programmed for 45 cycles of 1 min at 92 °C, one minute at 35 °C, and 2 min at 72 °C. Amplification products were run in 2.0% agarose gels in the presence of ethidium bromide at 30 V for 12-18 h. Gels were then photographed using a UV light source.

Banding patterns generated from these 200 primers that appeared to distinguish the two species were further screened by amplifying DNA from ten individuals of each species collected in the USA. From these candidates, we chose for further analysis only the primers that yielded consistent banding patterns and that appeared to be diagnostic. These further analyses involved amplifying DNA from single winged queens (one per colony) representing 16-43 *S. invicta* colonies, 14-36 *S. richteri* colonies, and 14-39 hybrid colonies, all collected in the USA (the taxonomic identity of these colonies was established previously using the three diagnostic allozyme markers). From these studies we established that the banding patterns for each primer were consistently produced over different experiments and individuals. We estimated the frequencies of each band of interest in each species and determined whether the RAPD markers were concordant with the diagnostic allozyme markers in classifying individual colonies as hybrids or as one of the parental species. This latter task was important because a high level of concordance would suggest that our new RAPD markers are diagnostic, as well as provide evi-

dence that the banding phenotypes scored for each primer have a simple genetic basis.

Finally, we conducted family studies of the seven RAPD markers that were judged to be useful on the basis of the foregoing analyses. Ten families of hybrid fire ants were studied for each marker. DNA was amplified from the single mother queen heading each of these colonies and from seven to nine of her adult male or female (winged queen) offspring. These studies were important for determining the expression patterns (dominance) of each marker and for confirming that each was inherited as a simple Mendelian marker.

## Results and discussion

### *Genetic basis of nuclear DNA (RAPD) markers*

Our screen of 200 RAPD primers resulted in the discovery of seven potentially informative markers. The data from the family studies of these markers used to infer the inheritance patterns are summarized in Table 1. Previous studies have demonstrated that monogyne fire ant colonies represent simple families, that is, families in which all the offspring in a colony are the progeny of a single female mated to a single haploid male (Ross & Fletcher 1985; Ross *et al.* 1988). Thus, we can predict the expected phe-

notype distributions of offspring in these colonies for Mendelian markers. Furthermore, because males are normally haploid in fire ants (as in other hymenopteran insects), there are two additional sources of information on the inheritance of the markers that can be obtained from males. First, males represent the haplotypes of their mother's gametes so that we can predict her genotype from her sons' haplotypes regardless of the particular expression patterns. Second, we can predict a set of phenotype distributions for the haploid male offspring of a single queen that is independent of the set expected for her diploid female offspring.

Only two classes of phenotype distributions are expected for both male and female offspring for dominant loci represented by a single band. We should observe either a single phenotype within a colony (either the band is invariably present or invariably absent) or we should see both phenotypes segregating in a 1:1 ratio. In colonies that have mother queens with the band present, offspring phenotypes should be either band presence or equal segregation of band presence and band absence. In colonies that have mother queens with the band absent, all daughter phenotypes should be either band presence or band absence, depending on the type of male with which the queen mated, whereas all son phenotypes should be band absence. Six of the seven RAPD markers we have devel-

**Table 1** Genotype/phenotype distributions for the egg-laying mother queen (Q) and her male progeny (MP) or female progeny (FP) from ten hybrid fire-ant colonies. Each value indicates the number of individuals possessing a given genotype or phenotype. For the dominant markers, '+' refers to band presence and '-' to band absence. In the case of the codominant marker (UBC 105), the first and third columns refer to homozygous genotypes in the case of females and hemizygous genotypes in the case of the (haploid) males

Colony	UBC 430		UBC 153		UBC 499		UBC 174		UBC 182		UBC 106		UBC 105		
	+	-	+	-	+	-	+	-	+	-	+	-	aa(a)	ab	bb (b)
16C Q		1		1		1		1		1		1			1
16C MP		8		8		7		9		8		7			8
4C Q	1		1		1		1		1		1		1		
4C MP	4	4	5	3	9		8		7		8		8		
20C Q		1		1		1		1		1		1			1
20C MP		9		8		9		7		8		8			8
29C Q		1		1		1		1		1		1			1
29C MP		9		8		7		7		7		8			8
23C Q	1		1		1		1		1		1			1	
23C MP	3	5	8	5	3	3	5	3	5	4	4	5	5		3
24C Q		1		1		1		1		1		1			1
24C MP		8		8		8		8		8		7			8
13D Q		1	1			1	1		1			1		1	
13D FP		8	2	6		8	2	6	8		8		8	5	3
13C Q		1	1			1		1		1		1		1	
13C FP		8	4	4		8		8		8		8	3	5	
28C Q		1		1		1		1		1		1		1	
28C FP		8		8		8		8	7	1		8		8	
36F Q	1		1		1		1		1		1		1		1
36F FP	5	4	8	7	4	4	4	4	4	4	8		6		

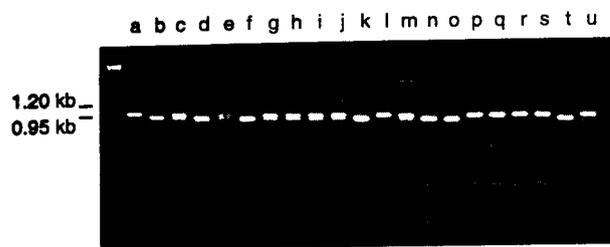


Fig. 1 Segregation of codominant RAPD marker *UBC 105* (bright bands of around 0.95 kb) in two families of hybrid fire ants. Family 13D: lane c, mother queen; lanes d–k, female progeny. Family 23C: lane m, mother queen; lanes n–u, male progeny. Lanes a and l represent *S. invicta* standard and lane b represents *S. richteri* standard. The first lane contains size markers (kb).

oped were consistent with these expectations for dominant markers that display Mendelian inheritance (Table 1).

One marker (*UBC 105*) was inconsistent with these expectations and seems to be inherited as a codominant marker with two alleles, each represented by a band of different mobility. This conclusion is inferred from several lines of evidence. First, we always observed one of two classes of phenotype distributions in the female offspring of single mothers: either one phenotype was present (all individuals with one of the two bands or with both bands), or two phenotypes were present in a 1:1 ratio, one of which was always the putative heterozygote recognizable by the presence of both bands (Table 1; Fig. 1). These offspring phenotype distributions typically were as expected given the phenotype of the mother queens and assuming codominance (e.g. Fig. 1). Males always exhibited only a single band, and the identities and distributions of these male offspring phenotypes also were as expected from their mothers' phenotypes, assuming codominance. The final bit of evidence for codominance is that if each putative allelic band in fact represents a separate locus, then we should have observed some females lacking bands altogether (i.e. females doubly homozygous for the null alleles) and some males possessing both bands or lacking both bands. These patterns were not observed. Although this is not the first report of a codominant RAPD marker, such a pattern of inheritance clearly is the exception rather than the rule for this class of DNA markers (Williams *et al.* 1990; Carlson *et al.* 1991; Hunt & Page 1992; Hadrys *et al.* 1992; Tinker *et al.* 1993; M. Cruzan & M. Arnold unpubl. data).

*Distribution of nuclear DNA (RAPD) markers in S. invicta and S. richteri*

The frequencies of the alleles (codominant markers) or phenotypes (dominant markers) for the seven RAPD markers we have developed and for three diagnostic

Table 2 Frequencies of alleles (codominant markers) or phenotypes (dominant markers) for each allozyme and RAPD DNA marker studied in introduced (USA) and native (Argentina) populations of *S. invicta* and *S. richteri*. Entries for dominant markers are for the band presence (+) and band absence (–) phenotypes. Sample sizes (numbers of individuals from separate colonies) are shown in parentheses below the frequency values. Samples for the allozymes in the USA are from colonies in Mississippi in which the genotypes of eight individuals were determined. A subset of these colonies was used for the RAPD markers. Sample sizes for Argentina are in some cases less than twenty because of repeated failure of PCR reactions

Allele/ Phenotype	USA		Argentina	
	<i>S. invicta</i>	<i>S. richteri</i>	<i>S. invicta</i>	<i>S. richteri</i>
<i>Gpi</i>				
<i>a</i>	1.0	0	1.0	0
<i>b</i>	0	1.0	0	1.0
	(50)	(32)	(20)	(20)
<i>Est-2</i>				
<i>a</i>	1.0	0	0.85	0
<i>b</i>	0	0.69	0.15	0.93
<i>c</i>	0	0.31	0	0
<i>d</i>	0	0	0	0.05
<i>e</i>	0	0	0	0.02
	(50)	(32)	(20)	(20)
<i>Odh</i>				
<i>a</i>	1.0	0	1.0	0.33
<i>b</i>	0	1.0	0	0.67
	(50)	(32)	(20)	(20)
<i>UBC 430</i>				
+	1.0	0.13	1.0	0.72
–	0	0.77	0	0.28
	(26)	(15)	(18)	(18)
<i>UBC 153</i>				
+	1.0	0	0.85	0
–	0	1.0	0.15	1.0
	(19)	(16)	(20)	(19)
<i>UBC 499</i>				
+	1.0	0	1.0	0.82
–	0	1.0	0	0.18
	(21)	(14)	(19)	(17)
<i>UBC 174</i>				
+	0.95	0	0.95	0.16
–	0.05	1.0	0.05	0.84
	(21)	(16)	(19)	(19)
<i>UBC 182</i>				
+	0.05	1.0	0	0.84
–	0.95	0	1.0	0.16
	(21)	(16)	(20)	(19)
<i>UBC 106</i>				
+	1.0	0	0.95	0.20
–	0	1.0	0.05	0.80
	(21)	(16)	(19)	(15)
<i>UBC 105</i>				
<i>a</i>	1.00	0.0	0.98	0.33
<i>b</i>	0.0	1.00	0.02	0.67
	(29)	(15)	(20)	(20)

allozyme markers are shown in Table 2 for both introduced (USA) and native (Argentina) populations of *S. invicta* and *S. richteri*. It is evident that not all of the RAPD markers are fully diagnostic (i.e. each species is not characterized by exclusive possession of alternate alleles or phenotypes), even if only the introduced populations are considered. Nonetheless, all of these new markers exhibit extreme frequency differences between the two species in the USA and thus are informative for our purposes of identifying colonies and resolving the distributions of the parentals and their hybrids over the introduced range.

We included samples of each species from native populations in Argentina to determine whether variation shared between the species at any RAPD marker in the USA was due to joint possession of an ancestral polymorphism or to recent interspecific introgression. We can in principle distinguish between these possibilities because the two species apparently do not hybridize in their native South American ranges (Ross & Trager 1990). Thus, for instance, variation in introduced *S. invicta* at marker *UBC 182* that is absent in Argentina may be most reasonably explained by introgression in the USA (Table 2), although we cannot completely rule out ancestral polymorphism because of the small sample sizes in Argentina. On the other hand, variation in introduced *S. invicta* at *UBC 174* and in *S. richteri* at *UBC 430* also occurs in South American populations and thus most likely reflects genuine intraspecific polymorphism.

We commonly observed intraspecific variation at the RAPD markers in native populations in Argentina that is absent in the USA, a pattern especially prominent for *S. richteri*. Introduced populations of both species have experienced recent genetic bottlenecks associated with their colonization of the USA (Ross & Trager 1990; Ross *et al.* 1993), which has been revealed by the loss of uncommon alleles at enzyme-encoding and sex-determining loci. Such bottlenecks could explain some loss of variation at the RAPD loci in the introduced parental populations. However, the absence of some variants in *S. richteri* in the USA that are common in Argentina (e.g. for *UBC 105*, *UBC 499*, and *UBC 106*) seems unlikely to have resulted solely from a bottleneck and suggests instead that the native population of this species that we sampled may be genetically distinct from the population that served as the source for North American colonists (the identity of which is presently unknown).

Table 3 shows the concordance of the allozyme and RAPD markers in designating colonies as hybrids or as one of the parental species. The data for all RAPD markers are shown separately from the data for marker *UBC 105* because the former come from only a subset of the colonies used for the latter. It is evident that the two classes of markers are highly concordant in identifying colonies. Eighty-two per cent of the colonies lie along the

**Table 3** Concordance of allozyme and RAPD markers in identifying colonies as *S. invicta* (I), *S. richteri* (R), or hybrids (H). Values indicate the total number of colonies for each possible combination

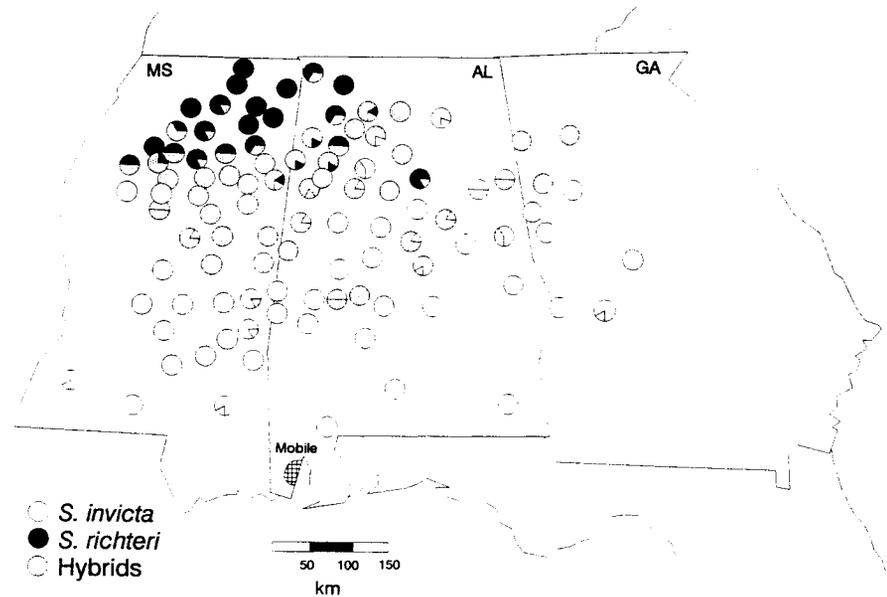
	Allozyme markers		
	I	H	R
<i>UBC105</i>			
I	43	2	0
H	4	23	2
R	0	14	36
All RAPD markers			
I	23	1	0
H	6	20	2
R	0	0	20

diagonal of complete concordance in the comparison of the allozyme markers with *UBC 105*, whereas 88% of the colonies lie along this diagonal when the allozyme markers are compared with all of the RAPD markers. Note that when comparing the allozyme markers with the single codominant RAPD marker, *UBC 105*, a rather large proportion (36%) of hybrid colonies identified from the allozymes is misclassified by the RAPD marker as belonging to one of the parental species. This finding reflects our greater ability to identify hybrid genotypes with the three allozyme loci than with a single RAPD locus. In fact, when we include all seven RAPDs in the analysis we see a reversal of this trend, with 28% of hybrid colonies identified from the RAPDs misclassified as one of the parentals using only allozyme markers. The observed overall high level of concordance between the diagnostic Mendelian allozyme markers and our new RAPD markers further confirms that the latter are diagnostic or informative in distinguishing *S. invicta* and *S. richteri* and that the banding patterns that we scored reflect underlying genetic variation rather than nongenetic artefact bands.

#### *Macrogeographic structure of the fire ant hybrid zone*

The second goal of this study was to describe the distribution of the two parental species and their hybrids in the USA at macrogeographic scales using our newly developed RAPD markers and diagnostic allozyme markers. Figure 2 summarizes the results of these distributional analyses. At the largest scale, *S. richteri* was found only in an isolated area in northern Mississippi and Alabama, whereas *S. invicta* was found throughout the southernmost regions of Mississippi, Alabama, and Georgia. A broad zone of hybridization separates the pure parental populations throughout most of the range over which the introduced populations occur. These results, while

**Fig. 2** Distribution of *S. invicta*, *S. richteri*, and hybrid colonies in Mississippi (MS), Alabama (AL), and western Georgia (GA), USA. A total of 550 colonies from 106 sites was analysed for three diagnostic allozyme markers, with a subset of 131 colonies from 49 sites in Mississippi analysed both for these allozyme markers and for one to seven RAPD markers. Pie diagrams represent the proportion of *S. invicta*, *S. richteri*, and hybrid colonies at each site. The city of Mobile, Alabama, where both parental species were introduced, is also indicated.



largely substantiating earlier reports based on biochemical markers (Vander Meer *et al.* 1985; Lofgren 1986; Diffie *et al.* 1988), also reveal a number of interesting features that warrant more detailed study, as described below.

The major distributional outlines reflect the historical patterns of colonization and genetic interaction between the two species (e.g. Vander Meer *et al.* 1985; Lofgren 1986). *Solenopsis richteri* was introduced into Mobile, Alabama ca. 1918 and came to occupy much of Alabama and Mississippi by the mid-1930s through both natural and anthropogenic dispersal. *Solenopsis invicta* also was introduced into Mobile, apparently in the mid-1930s, and this species subsequently began to replace *S. richteri* in the southern parts of their range. By 1953 the range of introduced fire ants had expanded to cover all or most of ten states in the south-eastern USA, including Mississippi, Alabama, and Georgia (Culpepper 1953), with most of this range expansion attributable to *S. invicta*. Interestingly, an isolated population of *S. richteri* was reported at this time in north-central Mississippi well ahead of the northerly-moving front of *S. invicta*. By 1974, *S. richteri* occupied only a small isolated area in northern Mississippi, whereas *S. invicta* was found throughout the southern USA (Buren *et al.* 1974).

Hybridization between the two species was first reported in 1985 (Vander Meer *et al.* 1985). Subsequent studies of museum specimens have indicated that hybridization occurred as early as 1949 near Mobile, and it is reasonable to conclude that it occurred even earlier wherever the two species came into contact in newly colonized areas (Vander Meer & Lofgren 1988). Thus, it appears that hybridization ensued early and that the zone of hybridization has moved northward as *S. invicta* has expanded

its distribution and replaced *S. richteri* in the southernmost areas (Lofgren 1986; Diffie *et al.* 1988; Vander Meer & Lofgren 1988).

Our data suggest that the ranges of the parental species may come into contact in west-central Mississippi (i.e. they are parapatric; Fig. 2). The history of this region is rather well known, and examination of earlier distributional maps (Buren *et al.* 1974; Diffie *et al.* 1988), combined with recent observations of low colony densities in this area (Shoemaker *et al.* unpubl. data), suggest that this area has been colonized only recently by fire ants. The near or complete absence here of a zone of hybridization between the pure parental populations is compatible with recent contact between the parental species, perhaps by means of *S. invicta* colonizing this area from the south and *S. richteri* from the north-east. Future studies will focus on determining whether an expanding zone of hybridization is forming in this area.

Another unexpected feature apparent from our map is a mosaic distribution of both hybrid and *S. invicta* colonies throughout much of central Alabama (Fig. 2). The history of colonization of this area is not as well documented as for Mississippi, where previous studies (and the current study) document a hybrid zone characterized by a smooth transition from pure *S. richteri* to only hybrid ants to pure *S. invicta* (Ross *et al.* 1987a; Diffie *et al.* 1988). We suggest that the mosaic pattern observed in central Alabama reflects recent anthropogenic dispersal of *S. invicta* and hybrid ants into this area. Determining which of these two transitional distributions represents an equilibrium situation has important implications for inferring the forces responsible for maintaining this zone. For example, mosaic distributions are counter to the 'tension

zone' model of hybridization, which states that hybrid zones are maintained by a balance between dispersal of parental individuals into the hybrid zone and selection against hybrids, leading to smooth clinal transitions at diagnostic loci (Key 1968; Barton & Hewitt 1985). The hybrid zone in eastern Mississippi, in which such clines have been found (Ross *et al.* 1987a), may represent a more advanced stage of hybridization than in central Alabama and thus may be a more appropriate location for detailed studies to evaluate various models of hybridization.

Finally, our map reveals low levels of introgression of heterospecific alleles on either side of the major zone of hybridization, most notably the presence of *S. richteri* alleles in *S. invicta* in southern Mississippi (Fig. 2). As *S. invicta* has moved northward over the past few decades from its original point of introduction, replacing *S. richteri* along the way, the hybrid zone also has moved northward (Buren *et al.* 1974; Lofgren 1986; Vander Meer & Lofgren 1988). We hypothesize that the movement of this zone northward has caused a pattern of differential introgression of *S. richteri* alleles into *S. invicta*, that is, occasional remnant *S. richteri* alleles not strongly selected against in an *S. invicta* genetic background have been left behind the advancing zone of large-scale hybridization. Additional detailed genetic studies of this area on a microgeographic scale will be required to test this hypothesis.

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