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Development of genetic markers distinguishing two invasive fire ant species (Hymenoptera: Formicidae) and their hybrids

DeWayne Shoemaker

Hybrid zones are regions where genetically distinct populations meet, mate, and produce some proportion of offspring of mixed ancestry. Such zones have been found in a wide range of animal and plant species. Studies of hybrid zones are of general interest to evolutionary biologists because they can provide insights into the effects of introgression on the maintenance of genetic distinctiveness, into the genetic and ecological nature of species differences, and into the genetic architecture of reproductive barriers (Harrison 1990, 1993). Although reduced hybrid fitness may render hybrids an evolutionary dead end, there also is the potential of hybridization as a source of genetic variation, functional or evolutionary novelty, and even new species (Kim & Rieseberg 1999; Rieseberg et al. 2003; Seehausen 2004; Arnold 2006; Martin et al. 2006). Studies of hybridization involving introduced or alien species and native species are of additional interest because in these cases hybridization can be detrimental to native species and result in loss of genetic distinctiveness, and possibly extinction of native species (Rhymer & Simberloff 1996).

The 2 fire ant species *Solenopsis invicta* Buren and *Solenopsis richteri* Forel (Hymenoptera: Formicidae) were inadvertently introduced into the USA early in the last century, where they have become significant ecological and agricultural pests (Lofgren 1986a,b). A large hybrid zone between these 2 species in the USA occurs over a broad area from western Mississippi to central Georgia (Vander Meer et al. 1985; Ross et al. 1987; Shoemaker et al. 1994, 1996). Hybridization between these 2 species, which is the result of secondary contact and apparently does not occur where the 2 species occur in sympatry in their native range (Ross & Shoemaker 2005), provides an excellent opportunity for evolutionary genetic studies aimed at understanding the genetic architecture of species differences, the consequences and fate of a hybrid zone formed in recent time, as well as the extrinsic and intrinsic factors affecting hybrid fitness.

Although these 2 fire ant species can be distinguished readily by using phenotypical characters, hybrids that form between them vary greatly in their appearance, making clear differentiation between the 2 species and their hybrids difficult. The goal of this study was to develop molecular-based assays that can be used to distinguish clearly between these 2 ant species and their hybrids.

A limited sequence data set representing 10 separate gene regions from *S. richteri* (generated via Sanger sequencing of a cDNA library) was aligned and compared with the draft genome of *S. invicta* (National Center for Biotechnology Information BioProject 49629) to identify potential single nucleotide polymorphisms (SNPs). Twelve SNPs were identified, 4 of which were within potential restriction enzyme recognition sites. Polymerase chain reaction (PCR) primers for these 4 regions were developed using Primer3 (http://bioinfo.ut.ee/primer3). The forward and reverse primer sequences for each marker (5′ to 3′) were: *Sol-Nuc1*: ATTAGCGCGCTGTCCAACTTAT and ATCTGAGACCAGGTTGCTGAGG; *Sol-Nuc2*: TCCGATTCCTTAGCGGTGTTAG and GCATGCCCATATTCCATCTTTC; *Sol-Nuc3*: GCTCGCTGTCATTTCTGTCGTC and ATCTTCGGCACAGAGAGCAAAC; *Sol-Nuc4*: TGGTCGTGAACACAGTTTCTCAAT and CATAGCAAATCTGCTGGCGTTA.

The samples used for the present study were derived from 15 colonies of *S. richteri* collected in Flatwood, Tennessee, 10 colonies of *S. invicta* collected in Gainesville, Florida, and 9 putative hybrid colonies collected in Grenada, Mississippi, which is an area previously shown to contain mostly hybrid fire ants (all colonies collected in 2005; Shoemaker et al. 1994, 1996). Putative hybrid colonies from Grenada, Mississippi, were confirmed as such using diagnostic allozymes as described in Shoemaker et al. (1996). Total genomic DNA was extracted from a single worker from each colony using the Puregene DNA isolation kit (Gentra Systems, Qiagen, Valencia, California) and subsequently used as template for PCR to amplify portions of the 4 candidate gene regions. PCR cocktails for each of the 4 markers contained 2′ Hot-Start Taq Mastermix (Denville Scientific, Holliston, Massachusetts), 0.2 µL of each primer (50 µM stocks), 1 µL of total genomic DNA (final concentration of approx. 25 to 100 ng/µL), and water to a final volume of 15 µL. PCR was performed using the following parameters: 2 min at 94 °C, followed by 35 cycles at 94 °C for 30 s, 50 °C (*Sol-Nuc4*) or 55 °C (*Sol-Nuc1*, *2*, and *3*) for 30 s, and 72 °C for 1 min. A small amount of resulting PCR product (2 µL) was separated on 4% agarose gels and visualized by ethidium bromide staining to confirm success of PCR. A negative control (water) was PCR-amplified and run alongside the study samples. PCR products (2.5 µL) from each individual for each marker were digested with appropriate restriction enzyme (1 µL) following the manufacturer's protocol (see Table 1 for restriction enzymes used; New England Biolabs, Ipswich, Massachusetts). Digestion products were electrophoresed in 4% agarose gels, stained in ethidium bromide, and visualized under UV light.

Inspection of results clearly revealed that 3 of these SNPs were completely diagnostic (i.e., no shared alleles) in distinguishing *S. invicta* and *S. richteri* (*Sol-Nuc1*, *Sol-Nuc2*, and *Sol-Nuc3*; Table 1). Although the 4th marker (*Sol-Nuc4*) was not fully diagnostic, it was still informative given that 1 allele (allele 2) was found only in *S. richteri*. Importantly, all hybrids were easily and consistently identified as such when these 4 markers were used in conjunction (Table 1). In every case, the surveyed hybrid individual had alleles that were unique to both *S. in-*

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Table 1. Genotypic data and details for PCR amplification of 4 single nucleotide polymorphism–restriction fragment length polymorphism markers developed to distinguish 2 fire ant species and their hybrids in the USA.

		Marker			
Species ID	Colony ID	*Sol-Nuc1*	*Sol-Nuc2*	*Sol-Nuc3*	*Sol-Nuc4*
Solenopsis invicta	US2006-381	1/1[a]	1/1	1/1	1/1
	US2006-382	1/1	1/1	1/1	1/1
	US2006-383	1/1	1/1	1/1	1/1
	US2006-384	1/1	1/1	1/1	1/1
	US2006-385	1/1	1/1	1/1	1/1
	US2006-386	1/1	1/1	1/1	1/1
	US2006-387	1/1	1/1	1/1	1/1
	US2006-388	1/1	1/1	1/1	1/1
	US2006-389	1/1	1/1	1/1	1/1
	US2006-390	1/1	1/1	1/1	1/1
Solenopsis richteri	US2005-78	2/2	2/2	2/2	1/2[b]
	US2005-79	2/2	2/2	2/2	2/2
	US2005-80	2/2	2/2	2/2	2/2
	US2005-81	2/2	2/2	2/2	2/2
	US2005-82	2/2	2/2	2/2	2/2
	US2005-83	2/2	2/2	2/2	1/2[b]
	US2005-84	2/2	2/2	2/2	2/2
	US2005-85	2/2	2/2	2/2	2/2
	US2005-86	2/2	2/2	2/2	2/2
	US2005-87	2/2	2/2	2/2	2/2
	US2005-88	2/2	2/2	2/2	2/2
	US2005-89	2/2	2/2	2/2	1/2[b]
	US2005-90	2/2	2/2	2/2	2/2
Hybrids	US2005-1	1/2	1/1	2/2	1/2
	US2005-2	2/2	1/2	1/2	2/2
	US2005-3	1/2	1/1	1/2	1/2
	US2005-4	1/1	2/2	1/1	1/1
	US2005-5	1/2	1/1	1/1	1/1
	US2005-6	1/2	1/1	2/2	1/2
	US2005-7	1/1	1/1	1/2	1/1
	US2005-8	1/1	1/1	2/2	1/1
	US2005-9	2/2	1/2	2/2	1/2
PCR amplicon size		348 bp	342 bp	305 bp	307 bp
Restriction enzyme		*Ssp I*	*Bgl II*	*Mlu I*	*Bgl II*
Sizes of digested fragments	*S. invicta* (allele 1)	33,141,174 bp	78,264 bp	305 bp	77,230 bp
	S. richteri (allele 2)	141,207 bp	342 bp	122,183 bp	307 bp

National Center for Biotechnology Information reference sequences: XM_011160725.1, XM_011171784.1, and XM_011165480.1.
[a]Alleles 1 and 2 at each locus correspond to alleles of *S. invicta* and *S. richteri*, respectively.
[b]Locus not completely diagnostic, but allele 2 is confined to *S. richteri*.

victa and *S. richteri*. In some cases, an individual was heterozygous for the alleles of each parental species (i.e., individuals from US2005-1, -3, -5, and -6 at *Sol-Nuc1*), whereas in other cases, an individual possessed alleles of one species at a locus and alleles of the alternate species at a separate locus (i.e., individuals from US2005-4 and US2005-8). One caveat is that although all hybrids were confirmed as such here, the use of only 4 markers clearly means not all hybrid nests will be detected, especially in cases where hybrid individuals represent multigenerational backcross hybrids to one of the parental species. Indeed, as with any such diagnostic markers, their utility depends partly on allele frequencies, and the more similar hybrids are to a parental species, the more difficult it is to detect introgression, even with a large number of markers (i.e., proportion of genome that is derived from each of the parental species). These SNP markers provide a simple and cost-effective (relative to allozyme markers) means of distinguishing easily the 2 introduced fire ant species and their hybrids.

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Summary

Three single nucleotide polymorphism (SNP) markers were developed that were completely diagnostic in distinguishing the 2 fire ant species *Solenopsis invicta* Buren and *Solenopsis richteri* Forel (Hymenoptera: Formicidae). Although a 4th marker was not fully diagnostic, it was still useful given that one of the variants was confined to *S. rich-*

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teri. Joint use of these markers was shown to consistently distinguish hybrids from the 2 parental species. The development of these SNP markers distinguishing the 2 introduced fire ant species and their hybrids represents a significant advance over existing methods because this method is robust, easily transferable, and cost effective.

Key Words: *Solenopsis invicta*, *Solenopsis richteri*, SNP

Sumario

Se desarrollaron marcadores de tres polimorfismo nucleótido singulares (PNS) que fueron completamente diagnósticos para distinguir 2 especies de hormigas de fuego *Solenopsis invicta* Buren y *Solenopsis richteri* Forel (Hymenoptera: Formicidae). Aunque el cuarto marcador no fue totalmente diagnóstico, toadavia fue útil dado que una de las variantes fue limitada a *S. richteri*. Se mostró que el uso conjunto de estos marcadores puede distinguir consistentemente los híbridos de las 2 especies parentales. El desarrollo de estos marcadores PNS que distinguen las 2 especies de hormigas de fuego introducidas y sus híbridos representa un avance significativo sobre los métodos existentes ya que este método es robusto, fácilmente transferible y rentable.

Palabras Clave: *Solenopsis invicta*; *Solenopsis richteri*; PNS

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