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PHYLOGEOGRAPHIC STRUCTURE OF THE FIRE ANT *SOLENOPSIS INVICTA* IN ITS NATIVE SOUTH AMERICAN RANGE: ROLES OF NATURAL BARRIERS AND HABITAT CONNECTIVITY

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Abstract.—We generated mitochondrial DNA (mtDNA) sequence data from 402 individuals of the fire ant *Solenopsis invicta* collected from 11 native populations and analyzed these data using a combination of demographic, phylogenetic, and phylogeographic methods to infer features of the evolutionary history of this species. Prior expectations regarding high levels of genetic structure and isolation by distance among populations were supported by the data, but we also discovered several unanticipated patterns. Our analyses revealed a major genetic break between *S. invicta* mtDNA haplotypes that coincides with the Mesopotamia wetlands region of South America, resulting in two higher level nested clade groupings. In addition, we identified contrasting patterns of genetic differentiation within these two major groups, which may reflect differences in connectivity of suitable habitat in different parts of the native range of *S. invicta*. Our study represents the first attempt to understand the phylogeographic history of *S. invicta* across its native range.

Key words.—Fire ant, genetic structure, mitochondrial DNA, nested clade, phylogeography, *Solenopsis invicta*, *Wolbachia*.

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Population genetic analyses examining the distribution of variation within and among populations have been used extensively to explore questions regarding microevolutionary processes, macroevolutionary patterns, and many other fundamental features of the natural history of both animal and plant populations (Barton and Clark 1990; Shoemaker and Jaenike 1997). In animals, mitochondrial DNA (mtDNA) variants are a common molecular marker employed to provide insights into these subjects (Avise 2000). Despite some potential drawbacks of using mtDNA (for a recent review, see Ballard and Whitlock 2004), a high degree of intraspecific variation, a lack of recombination, and maternal transmission make this marker generally amenable to studying patterns of molecular variation within and between populations (Avise 2000; Wiens and Penkrot 2002). While traditional genetic structure analyses may reveal whether populations are differentiated, failure to make use of all the historical information in mtDNA gene genealogies in such analyses precludes one from fully gleaning the relative roles of contemporary evolutionary forces and past events in shaping patterns of genetic structure (Templeton 1998; Althoff and Pellmyr 2002; McMillen-Jackson and Bert 2003; Verovnik et al. 2004). However, as recently shown by several studies, we can potentially gain insight into the relative roles played by recent and historical processes by combining demographic studies with phylogenetic and, in particular, phylogeographic analyses (Templeton 1998; Avise 2000; Bernatchez 2001; Althoff and Pellmyr 2002; Hoffman and Blouin 2004; Morando et al. 2004). Thus, it is becoming increasingly clear that we can obtain a more comprehensive picture of the evolutionary history of populations, or at the very least obtain corroborating evidence for observed patterns, by using a combination of analytical approaches rather than any single approach (Bernatchez 2001; Althoff and Pellmyr 2002; Hoffman and Blouin 2004; Morando et al. 2004).

In the present study, we examined the nature and distribution of mtDNA variation in the fire ant *Solenopsis invicta* throughout much of its native South American range. Specifically, we generated mtDNA sequence data from 402 individuals from 11 geographic populations and subsequently analyzed these data using a series of analyses to infer features of their evolutionary history. Such an approach allows us to make full use of the historical information contained within the gene genealogies to infer the relative roles of recent and historical factors in shaping patterns of mtDNA diversity. Similar to several recent studies (Bernatchez 2001; Althoff and Pellmyr 2002; Hoffman and Blouin 2004; Morando et al. 2004) advocating the use of a combined analytical approach to investigate intraspecific phylogeography, we inferred patterns of structure and processes of demographic change and gene flow that were neither anticipated nor evident from traditional analyses of population structure alone.

Background Biology of Solenopsis invicta

Solenopsis invicta is a particularly important species in which to address questions concerning population history and speciation. It is a member of the *Solenopsis saevissima* species-group which, after recent comprehensive taxonomic revision, consists of 13 described species native to various regions of South America (Trager 1991; Pitts 2002). *Solenopsis invicta* was unintentionally introduced into the United States in the last century, where it has flourished and become an agricultural and ecological pest as well as a danger to public health (Buren et al. 1974; Lofgren et al. 1975; Lofgren 1986a,b). Due to these detrimental effects, many studies regarding the basic biology of *S. invicta* have been published

(see Tschinkel 2005 and references therein), making this ant species an emerging model system for evolutionary and ecological studies.

In comparison to most other species within the *S. saevissima* species-group, the native range of *S. invicta* is remarkably broad, extending through central and southern Brazil, Uruguay, northern Argentina, Paraguay, Bolivia, and southern Peru (Pitts 2002). This large region encompasses a wide range of environments including grasslands, tropical forests, elevated forests, flooded forests, and wetlands (Eva et al. 2002), within which colonies of *S. invicta* are limited to open or disturbed habitats. Due to a previous lack of known species-diagnostic morphological characters, the *S. saevissima* species-group was once considered to consist of a single widespread, polytypic species (Wilson 1952). More recently, the revisionary work of Buren et al. (1974), Trager (1991), and Pitts (2002) has resulted in the description of numerous species in the group recognized on the basis of unique suites of morphological characters. Nonetheless, the occurrence of cryptic species detected by means of genetic markers (Ross and Shoemaker 2005) has led to continuing difficulties in the alpha-taxonomy of these ants. Aside from the practical problems created for fire-ant classification, these results suggest that the *S. saevissima* species-group is a relatively young group undergoing an evolutionary radiation (Ross and Shoemaker 2005).

Previous population genetic studies of nominal *S. invicta* in South America using an array of nuclear markers have found evidence for clinal geographic changes in allozyme allele frequencies over short distances (approximately 100 km) and strong levels of differentiation between pairs of populations separated by distances of a couple hundred to several hundred kilometers (Ross and Trager 1990; Ross et al. 1997; Shoemaker et al. 2003b; Ross and Shoemaker 2005). Remarkably, allozyme differentiation between two of these regional *S. invicta* populations is as great, if not greater, than the differentiation between sympatric *S. invicta* and *S. richteri* (a closely related species) from the same regions (Ross and Shoemaker 2005). Even higher levels of population differentiation have been observed for mtDNA markers, suggesting that patterns of maternal (queen-mediated) gene flow have played a significant role in creating current population structure. Based on these earlier results and knowledge of the natural history of fire ants, we predict that several factors likely influence patterns of maternal gene flow in *S. invicta*, including the relatively low dispersal ability of newly mated queens (generally less than 2 km during mating flights; Markin et al. 1971); the presence of two different social forms, colonies of which are intolerant of queens of the alternate form (Shoemaker and Ross 1996); the presence in many populations of endosymbiotic *Wolbachia* bacteria that can induce reproductive incompatibilities (Shoemaker et al. 2003a,b); and the potential for occasional long-distance natural or human-mediated dispersal (Hölldobler and Wilson 1990). No prior study has specifically measured the impact of these factors across a broad scale or inferred how the biogeography of South America and demographic history of *S. invicta* have influenced patterns of genetic variation in this insect.

Materials and Methods

Sample Collection and Determination of Social Form

Individuals of *S. invicta* were collected from 10–66 colonies in each of 11 different geographic populations spanning an area across Brazil and Argentina that includes a large portion of the known native range (Fig. 1; Mescher et al. 2003). The numbers of colonies sampled in and geographical coordinates of each collection site are summarized in Table 1. Geographical distances between pairs of populations range from 77.2 to 1966.8 km (Appendix I available online only at http://dx.doi.org/10.1554/05-067.1.s1). All collected individuals were identified as *S. invicta* by J. P. Pitts using species-informative morphological characters (Trager 1991; Pitts 2002).

Two different social forms differing mainly in the number of egg-laying queens within nests exist in *S. invicta*: monogyne (M) nests contain a single egg-laying queen and polygyne (P) nests possess multiple fertile queens. Colony social form was previously determined for all 402 sampled colonies using a diagnostic polymerase chain reaction (PCR) assay for the *b*-like alleles of the gene *Gp-9* that invariably are associated with polygyny (Mescher et al. 2003). Only four populations were found to contain polygyne colonies: Corrientes, Formosa, Campo Grande, and Arroio dos Ratos (Mescher et al. 2003). Given the restricted occurrence of polygyny and limited sample size for polygyne nests in two of the populations, we pooled individuals of both social forms and performed our analyses on these pooled populations under the assumption that gene flow between sympatric forms was more likely than gene flow between allopatric populations of either social form (Shoemaker and Ross 1996; Ross et al. 1997, 1999; Ross and Shoemaker 1997). However, to confirm that such pooling of individuals did not significantly alter our results, we also performed two additional data analyses using M colonies only and M and P colonies treated as separate populations.

Laboratory Procedures

Total genomic DNA was extracted from single individuals using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN; Ross and Shoemaker 1997). A 920-bp fragment of the mitochondrial genome that includes portions of the cytochrome oxidase subunit I (COI) and subunit II (COII) genes was amplified using the primers C1-J-2195 (COI-RLR; 5′-TTGATTTTTTGGTCATCCAGAAGT-3′; Simon et al. 1994) and DDS-COII-4 (5′-TAAGATGGTTAATGAAGAG TAG-3′; Ross and Shoemaker 1997). PCR reactions were carried out in 30-μl volumes containing 11.25 μl PCR supermix (Denville, Metuchen, NJ), 15.75 μl water, 1 μl of each 50-μM primer, and 1 μl genomic DNA in a Bio-Rad *i*Cycler under the following cycling profile: 1 min at 94°C for one cycle; 30 sec at 94°C, 1 min at 48°C, 2 min at 68°C for 35 cycles; and 5 min at 72°C for one terminal cycle. Mitochondrial DNA amplicons were purified using Agencourt (Beverly, MA) Ampure magnetic beads and used directly in standard fluorescent cycle-sequencing PCR reactions (ABI Prism Big Dye terminator chemistry; Applied Biosystems, Foster City, CA). Sequencing reactions were purified

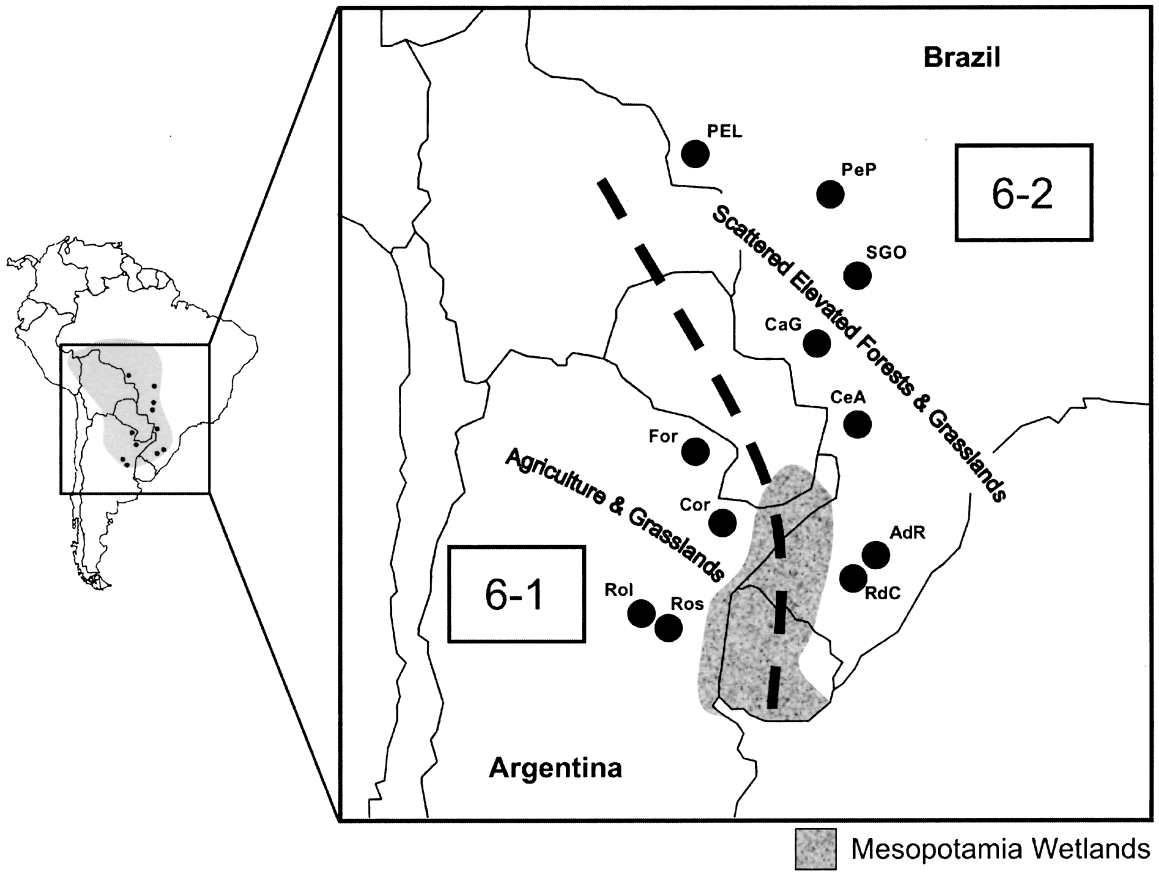


FIG. 1. Maps of South America showing native range of *Solenopsis invicta* (gray shading; from Mescher et al. 2003) and locations of population sampling sites (black dots; population codes are in Table 1). Inset depicts the coincidence of the Mesopotamia wetlands with a sharp genetic discontinuity (indicated by a dashed line) between two groups of fire-ant populations (groups 6-1 and 6-2).

using Agencourt CleanSEQ magnetic beads and run on an ABI 3700 sequencer at the University of Wisconsin Biotechnology Center DNA Sequencing Laboratory. All mtDNA amplicons were sequenced in both directions and corresponding sequence pairs were edited and combined into a single consensus sequence file using the program AutoAssembler (Applied Biosystems). All 402 sequences were then incorporated into a single master file and aligned by eye using previously published fire-ant mtDNA sequences obtained from GenBank (*S. geminata*, AY254476; *S. invicta*, AY2490093). *Solenopsis geminata* belongs to a different species-group than *S. invicta*, and so the sequence of this species was included in our dataset as an outgroup (Trager 1991; Pitts 2002).

Molecular Diversity and Tests for Neutrality

The number and frequency of all unique mtDNA haplotypes as well as summary data regarding estimates of molecular diversity were determined using ARLEQUIN ver. 2.000 (Schneider et al. 2000).

When using molecular variation to study population struc-

TABLE 1. Locations of 11 sampled populations of *Solenopsis invicta*. Codes are population abbreviations used in subsequent tables and figures. *N* represents the number of individuals (one per nest) sampled from each population.

City	Province or state	Country	Code	*N*	Latitude	Longitude
Corrientes	Corrientes	Argentina	Cor	54	27°34′09″S	58°50′23″W
Formosa	Formosa	Argentina	For	40	26°09′34″S	58°09′57″W
Roldán	Santa Fe	Argentina	Rol	14	32°49′59″S	60°51′39″W
Rosario	Santa Fe	Argentina	Ros	29	32°54′15″S	60°47′13″W
Ceu Azul	Parana	Brazil	CeA	66	25°08′30″S	53°53′56″W
Pedra Preta	Mato Grosso	Brazil	PeP	48	16°42′42″S	54°34′22″W
Pontes E Lacerda	Mato Grosso	Brazil	PEL	28	15°11′27″S	59°17′20″W
Campo Grande	Mato Grosso do Sul	Brazil	CaG	29	20°21′10″S	54°34′22″W
Rincão dos Cabrais	Rio Grande do Sul	Brazil	RdC	10	29°43′60″S	52°57′0″W
Arroio dos Ratos	Rio Grande do Sul	Brazil	AdR	33	30°08′21″S	51°30′11″W
São Gabriel do Oeste	Mato Grosso do Sul	Brazil	SGO	51	19°17′24″S	54°34′22″W

ture and infer gene flow patterns, it is assumed that the observed variation evolves neutrally in all populations. To ascertain the validity of this assumption, we tested for departures from neutrality using Tajima's (1989) *D*-test, Fu and Li's (1993) *D*-test, and Fu's (1997) *Fs*-test for each population as implemented in ARLEQUIN and DNAsp ver. 3 (Rozas and Rozas 1999). Each of these tests has its own strengths and varies in its statistical power (Simonsen et al. 1995; Wayne and Simonsen 1998; Ford 2002), but significant values may indicate that populations are not evolving in a neutral manner (i.e., are not in migration-drift or mutation-drift equilibrium). Three populations (Pedra Preta, Rincão dos Cabrais, and São Gabriel do Oeste) were found to have one haplotype (H60, H67, and H49, respectively) limited to one or two individuals that were highly divergent from all other mtDNA haplotypes from the same population. To assess what affect these divergent sequences had on the diversity estimates and neutrality tests, we repeated the above analyses with these sequences excluded.

Genetic Structure and Gene Flow Analyses

Average pairwise sequence divergence within and among populations was calculated using DNAsp ver. 3 (Rozas and Rozas 1999). Total genetic differentiation as well as differentiation between all population pairs was estimated as F_{ST}, which assumes that all haplotypes are equally divergent (equidistant) from one another, and ϕ_{ST}, which takes molecular divergence (Euclidean squared distance) between haplotypes into account, using ARLEQUIN (Excoffier et al. 1992; Schneider et al. 2000). Estimates of effective levels of gene flow were calculated using the formula $F_{ST} = 1/(2N_em + 1)$ (Neigel 1997, 2002; Ross and Shoemaker 2005; Slatkin 1987; Whitlock and McCauley 1999), which represents maternal (queen-mediated) gene flow.

Isolation by Distance

Higher genetic similarity between individuals in close geographic proximity than in distantly separated locations is known as isolation by distance (IBD; Garnier et al. 2004; Wright 1943). To determine if there was a significant positive correlation between genetic differentiation and geographic distance indicative of IBD, pairwise estimates of F_{ST} and ϕ_{ST} were plotted against the logarithms of pairwise geographic distances.

Nested Clade Phylogeographic Analyses

The programs TCS ver. 1.13 (Clément et al. 2001) and ARLEQUIN (Schneider et al. 2000) were used to generate a minimum spanning network of haplotypes, and nesting categories were assigned following Templeton et al. (1995) and Templeton and Sing (1993). Nested clades with geographic and haplotypic variation were then used for nested clade phylogeographic analyses (NCPA), which were implemented with the program GeoDis ver. 2.0 (Posada et al. 2000). Ambiguous connections (loops) pose a problem with this method, because the genetic and geographic variation within a particular clade may be altered depending on how haplotypes are connected. To break loops within our network, the methods described by Templeton and Sing (1993) and predictions derived from coalescent theory were used (reviewed in Nordborg 2001; Rosenberg and Nordborg 2002). Haplotype distribution within a clade, clade distance (D_c, the geographic distribution of haplotypes within a clade), nested clade distance (D_n, the geographic distribution of clades within the next higher clade), and contrasting measures between tips and interiors ($I\text{-}T_c$ and $I\text{-}T_n$) were all assessed statistically using 1000 random permutations of clades and haplotypes. Statistically significant large or small results for these values ($P < 0.05$) were interpreted using the inference key in appendix 2 of Templeton (2004).

Phylogenetic Analyses

Only nonredundant mtDNA haplotypes (selected with ARLEQUIN) were used for the phylogenetic analyses. To find an appropriate model of sequence evolution for our data we used the program MODELTEST (Posada and Crandall 1998), which uses likelihood-ratio tests to compare successively nested, increasingly parameter-rich, models. Genetic distance values were calculated between pairs of haplotypes using the chosen model of sequence evolution (HKY85 + I + Γ; I = 0.587; Γ = 0.788), and the resulting distance data matrix was used to infer sequence relationships using neighbor-joining (NJ), as implemented in the program PAUP* 4.0b10 (Swofford 1999). Ties were broken randomly. Bootstrap analyses were performed with 50,000 data resamplings to assess the support for particular nodes within the NJ tree.

Bayesian analyses executed in the program MrBayes 2.0 (Huelsenbeck et al. 2001) were used to generate posterior probability distribution values for clades (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003). No a priori assumptions about the tree topology were made and all Metropolis-coupled Markov chain Monte Carlo (MCMCMC) searches were provided with a uniform prior based upon the values obtained from MODELTEST. To thoroughly explore the parameter space and ensure that searches were mixing well, we ran the program four separate times and compared corresponding posterior probability distributions. Each search was run with four chains, started from a random tree, and run for 4 million generations, with every 100th tree sampled to obtain the 40,001 sample points. Burn-in length, the number of generations required for sample parameter values to reach stationarity, was set after determining by inspection when the log-likelihood values reached an asymptote. Posterior probabilities (the percentage of run samples that recovered any particular clade) were calculated using the trees visited by the Markov chains after burn-in samples were discarded.

RESULTS

Molecular Diversity and Tests for Neutrality

A total of 81 unique mtDNA haplotypes distinguished by 149 segregating sites and 168 mutations were represented among the 402 individuals (sequences) from the 11 populations of our study (Table 2). The frequency of haplotypes within each population is listed in Appendix II (available online only at http://dx.doi.org/10.1554/05-067.1.s1). Se-

TABLE 2. Estimates of diversity statistics and of statistics testing departures from neutrality for mitochondrial DNA sequences from 11 sampled populations of *Solenopsis invicta*. *N* is the number of individual sequences obtained, *L* is the average length of sequences for each population, *h* is the number of different haplotypes per population, *H* is the haplotype diversity, *S* is the number of polymorphic (segregating) sites, *n* is the number of mutations (substitutions) for each population, θ is the nucleotide polymorphism, and π represents the nucleotide diversity. Estimates for each social form within a population are indicated with M (monogyne) or P (polygyne).

Population[1]	*N*	*L*	*h*	*H*	*S*	*n*	θ	π	Tajima's *D*	Fu and Li's *D*	Fu's *Fs*
Cor	54	903	20	0.795	74	79	0.018	0.025	1.442	1.034	5.267
Cor M	32	907	11	0.647	65	69	0.018	0.021	0.715	1.080	7.851
Cor P	22	901	14	0.939	58	61	0.018	0.027	2.015*	0.978	1.983
For	40	909	18	0.923	57	58	0.015	0.015	−0.057	1.020	0.699
For M	23	909	12	0.905	33	34	0.010	0.012	0.983	0.608	1.006
For P	17	893	11	0.949	51	52	0.017	0.017	0.041	0.568	1.093
Rol	14	897	5	0.593	50	50	0.018	0.014	−0.842	1.264	7.043
Ros	29	905	6	0.591	65	67	0.018	0.020	0.406	−0.383	15.537
CeA	66	910	11	0.722	15	15	0.003	0.002	−1.167	−1.216	−2.436
PeP	48	910	3	0.082	63	64	0.016	0.003	−2.803***	−6.051**	6.900
(H60)[2]	47	910	2	0.043	12	12	0.003	0.001	−2.428**	−4.785**	1.951
PEL	28	910	6	0.331	20	20	0.006	0.003	−1.705	0.020	1.190
CaG	29	910	5	0.717	45	44	0.012	0.011	−0.440	1.797**	10.859
CaG M	27	910	5	0.692	44	45	0.013	0.012	−0.270	1.779**	10.990
CaG P	2	910	1	0.000	0	0	0.000	0.000	0.000	0.000	N/A
RdC	10	910	5	0.844	33	35	0.013	0.012	−2.381	−0.860	4.010
(H67)[2]	9	910	4	0.806	16	16	0.006	0.008	1.374	1.076	3.902
AdR	33	910	7	0.705	4	4	0.001	0.001	0.844	0.938	−1.738
AdR M	26	910	6	0.717	4	4	0.001	0.001	0.442	0.968	−1.253
AdR P	7	910	3	0.762	2	2	0.001	0.001	0.687	1.178	−0.056
SGO	51	910	2	0.077	31	31	0.008	0.003	−2.177*	1.845**	8.326
(H49)[2]	49	910	1	0.000	0	0	0.000	0.000	0	0.000	N/A
Total	402	910	81	0.947	149	168	0.025	0.033	0.942	−1.323	−0.095

* $P < 0.005$; ** $P < 0.02$; *** $P < 0.001$.
[1] Codes are population abbreviations found in Table 1.
[2] Estimates with highly divergent outlier sequences removed (removed haplotypes in parentheses).

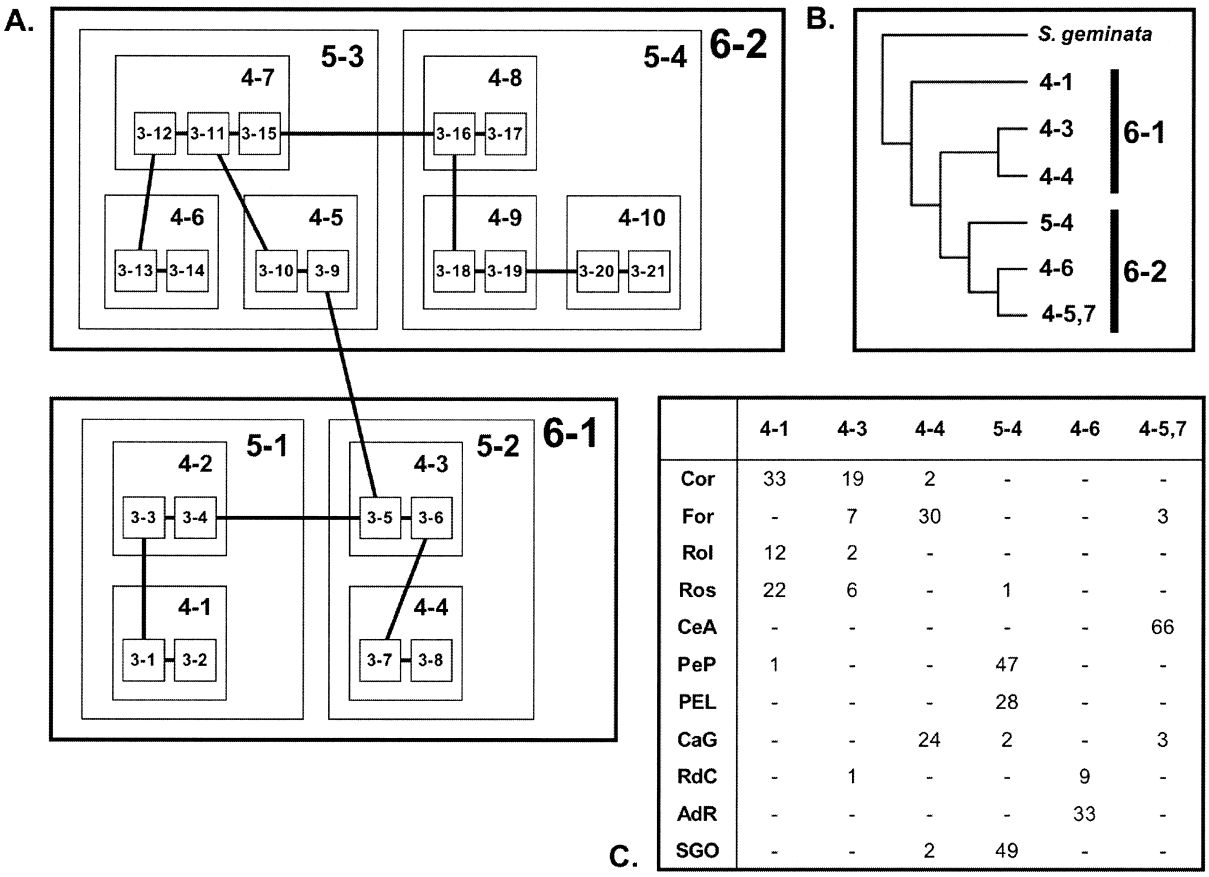
quences representing each unique mtDNA haplotype have been deposited in GenBank (see Appendix II online for accession numbers). Five of the 81 haplotypes (6.2%; H1, H5, H7, H48, and H49) were found in more than one population, whereas the remaining haplotypes (93.8%) were exclusive to single populations (Appendix II, available online). The number of unique mtDNA haplotypes within populations ranged from two to 20 (Table 2). Haplotype diversity and nucleotide diversity estimates for the full dataset (all 402 sequences) were 0.947 and 0.033, respectively. Haplotype diversity within populations ranged from 0.077 to 0.923, while nucleotide diversity ranged from 0.001 to 0.025. Both the number of unique mtDNA haplotypes and estimates of nucleotide diversity were greatest in populations southwest of the Mesopotamia wetlands (Corrientes, Formosa, Roldán, and Rosario), with 44 of the 81 haplotypes (54%) occurring in just these four populations.

The removal of haplotypes H49, H60, and H67, all of which are rare and highly divergent from the remaining haplotypes found in Pedra Preta, Rincão dos Cabrais, and São Gabriel do Oeste, respectively, dramatically reduced the molecular diversity estimates for these three populations, and in fact reduced such diversity to zero in the São Gabriel do Oeste population (Table 2).

Results of the neutrality tests were all nonsignificant and consistent with neutral evolution with the exception of four populations: Corrientes, Campo Grande, Pedra Preta, and São Gabriel do Oeste (Table 2). Tajima's *D*-test was significant for the polygyne colonies from Corrientes, Pedra Preta, and São Gabriel do Oeste. However, Tajima's *D* was not significant for the combined monogyne and polygyne haplotypes in Corrientes. Fu and Li's *D* was significant for Pedra Preta, Campo Grande (combined sample as well as the monogyne form only), and São Gabriel do Oeste. Fu's *Fs*-test was not significant for any population (Fu 1997). None of the test statistics for São Gabriel do Oeste remained significant when the divergent sequence H49 was removed from the analyses (Table 2). Overall, these results suggest that, with the possible exception of Pedra Preta, our study populations are in mutation-drift equilibrium.

Genetic Structure and Gene Flow Analyses

Combining monogyne and polygyne nests for analyses did not qualitatively change the patterns in or significance of our results. Therefore, we report only the values for our pooled dataset and for the subset of monogyne samples in populations where both social forms were sampled. Analyses using both the equidistant and Euclidean metrics revealed highly significant ($P < 0.001$) genetic differentiation among *S. invicta* populations ($F_{ST} = 0.441$ with all samples included and 0.499 with polygyne samples excluded; $\phi_{ST} = 0.736$ with all samples included and 0.783 with polygyne samples excluded). Based on these estimates, from about one-half to three-fourths of total mtDNA variation resides among populations, with the remainder occurring within populations. Estimates of pairwise genetic differentiation between populations using the equidistant metric were all significant; estimates using the Euclidean metric were all significant with the exception of comparisons involving three populations located in the



	4-1	4-3	4-4	5-4	4-6	4-5,7
Cor	33	19	2	-	-	-
For	-	7	30	-	-	3
RoI	12	2	-	-	-	-
Ros	22	6	-	1	-	-
CeA	-	-	-	-	-	66
PeP	1	-	-	47	-	-
PEL	-	-	-	28	-	-
CaG	-	-	24	2	-	3
RdC	-	1	-	-	9	-
AdR	-	-	-	-	33	-
SGO	-	-	2	49	-	-

FIG. 2. (A) Nested clade levels 4–6 for a haplotype network of unique mitochondrial DNA sequences with nested design created according to Templeton et al. (1995). Nested clade levels 1–3 can be found online in Supplementary Data. (B) Simplified phylogenetic tree of mitochondrial DNA sequences from *Solenopsis invicta* representing the six major clades detected using neighbor-joining, Bayesian, and nested clade analyses. Bootstrap/posterior probability values for all clades are greater than 88%. The six major clades are indicated to the right of the tree (see text). (C) Number of haplotypes from each sampled population within each of the six major clades.

southwestern portion of the species range: Corrientes/Roldán, Corrientes/Rosario, and Roldán/Rosario (Appendix III available online only at http://dx.doi.org/10.1554/05-067.1.s1). The ϕ_{ST}-values are consistently greater than the corresponding F_{ST}-values, indicating that there is less genetic divergence among haplotypes within a population than among haplotypes from different populations, a pattern consistent with strong phylogeographic structure (Goropashnaya et al. 2004).

Euclidean metric estimates of effective gene flow between populations (N_em) were all well below the threshold value of 0.5, denoting a balance between gene flow and drift for a neutral maternally inherited marker (Slatkin 1987), except for the three population pairs listed above as well as Campo Grande/Formosa and Corrientes/Formosa (Appendix III, available online). Equidistant metric estimates of N_em were more variable and implied more instances of gene flow levels greater than 0.5 than the Euclidean-based estimates.

Isolation by Distance

Euclidean metric estimates of genetic differentiation (ϕ_{ST}) show a significant positive correlation with geographic distance for both the M and pooled M + P datasets ($R^2 = 0.1638$ and 0.1723, respectively; $P < 0.002$ in both cases). There was no significant association between genetic differentiation and pairwise geographic distance using the equidistant estimates (F_{ST}).

Nested Clade Phylogeographic Analyses

Nesting of mtDNA haplotypes according to Templeton et al. (1995) resulted in six nested clade levels, with level 7 encompassing the entire haplotype network (for nested clade levels 4–6, see Fig. 2A; for nested clade levels 1–3, see Supplementary Material available online only at http://dx.doi.org/10.1554/05-067.1.s2). Statistical parsimony did not connect all mitochondrial haplotypes within the 13 mutational steps permitted under a 95% confidence limit set by TCS, creating multiple independent haplotype networks. The minimum spanning network of haplotypes created by ARLEQUIN, however, did result in a linkage of all haplotypes. Aside from the nine cases in which TCS did not link portions of the network, the two methods produced identical networks that portrayed virtually identical haplotype relationships as inferred from NJ and Bayesian analyses (see below and Fig. 2B). Thus, all methods we employed provided concordant pictures of mtDNA sequence evolution in *S. invicta*.

Six major groupings of haplotypes were identified using NCPA (clades 4-1; 4-3; 4-4; 4-5,7; 4-6; and 5-4). These six clades were connected by relatively long branches, in every

TABLE 3. Significant demographic inferences for 16 nested clades based upon the results from nested clade phylogeographic analysis performed with GeoDis ver. 2.0 and the inference key in Templeton (2004).

Clade	Inference chain	Inferred pattern
2-3	1-2-3-5-15-NO	Long-distance colonization (LDC)
2-13	1-2-11-17-4-NO	Restricted gene flow with isolation by distance (RGF/IBD)
3-1	1-2-3-5-6-13-YES	LDC coupled with subsequent fragmentation
3-7	1-19-20-2-3-5-6-7-YES	RGF/dispersal but with some long-distance dispersal
3-10	1-2-3-5-6-13-14-YES	Sampling design inadequate to discriminate between contiguous range expansion (CRE), LDC, or past fragmentation (PF)
3-13	1-2-11-17-4-NO	RGF/IBD
4-1	1-19-20-2-11-12-13-YES	LDC coupled with subsequent fragmentation
4-4	1-2-3-5-6-13-YES	LDC coupled with subsequent fragmentation
4-6	1-2-11-12-13-YES	LDC coupled with subsequent fragmentation
4-9	1-19-20-2-11-17-4-NO	RGF/IBD
5-2	1-2-11-12-13-YES	LDC coupled with subsequent fragmentation
5-3	1-2-3-5-6-13-14-YES	Sampling design inadequate to discriminate between (CRE), LDC, or PF
5-4	1-19-20-2-11-12-13-YES	LDC coupled with subsequent fragmentation
6-1	1-2-3-4-NO	RGF/IBD
6-2	1-2-11-12-NO	Contiguous range expansion
Total	1-2-11-17-4-NO	RGF/IBD

case differing from all others by a minimum of 15 and a maximum of 41 mutational steps. Importantly, none of the ambiguous connections (loops) in the network required complete resolution because alternative connections did not change the nesting of clades. Multiple runs of GeoDis were performed to determine if alternate connections that changed the tip/interior node status of haplotypes would have an effect on the significance of different clades. No such alterations were identified.

No significant demographic inferences were obtained for one-step clades, but 16 higher-level clades yielded significant inferences (Table 3). The lack of such inferences for lower-level clades suggests that the predominant evolutionary forces shaping mtDNA variation in native *S. invicta* act at the scales of entire populations or regional groups of populations rather than at smaller scales.

Using the inference key of Templeton (2004), a general trend found at every level is restricted gene flow coupled with IBD (RGF/IBD; five of 16 significant cases; Table 3). A prominent example that involves the total cladogram is the break between the major clades 6-1 and 6-2, a discontinuity that coincides geographically with the Mesopotamia wetlands of northeastern Argentina and southernmost Brazil (Fig. 1). Additionally, RGF/IBD is inferred for the clades 2-13, 3-13, 4-9, 6-1. Finally, restricted gene flow is inferred for clade 3-7, which is composed of haplotypes from Formosa, Campo Grande, and São Gabriel do Oeste, but the restricted gene flow here is inferred to be coupled with some long-distance dispersal rather than IBD.

Long-distance colonization coupled with subsequent fragmentation (divergence) from the source population is inferred for seven of the 10 remaining clades with significant demographic inferences (Table 3). A primary example of inferred long-distance colonization is between the southwestern region (Corrientes, Roldán, Rosario) and Pedra Preta in the north (clade 4-1). Subsequent fragmentation is inferred for all of these clades because lower-level nested clades are connected to each other by a larger than average number of mutational steps (Templeton 2004). The demographic inference for clade 2-3, which is composed of haplotypes from Corrientes, Formosa, Roldán, and Rosario, also is long-distance colonization and stems from the fact that branch lengths among haplotypes are relatively short (Templeton 2004).

Clade 6-2 is the only clade for which contiguous range expansion is inferred, suggesting a unique demographic history for *S. invicta* in the northeastern part of its range. Finally, for two of the clades with significant D_c (clade distance) or D_n (nested clade distance) values, clades 5-3 and 3-10, the inference key was unable to suggest a demographic scenario because the sampling design was inadequate to discriminate between contiguous range expansion, long-distance colonization, and past fragmentation.

Phylogenetic Analyses

NJ and Bayesian analyses recovered virtually identical trees, and posterior probability values for each node differed by only 2% or less across the four runs of the Bayesian analysis. Our Bayesian support values generally are greater than the corresponding bootstrap values, as has been reported in other studies (Simmons et al. 2004), but differences between the two sets are not substantial enough to question the concordance of the results from the two methods. While there often is low support for relationships among haplotypes at the tips of clades, there is consistently high support for six major clades ($>$ 88% in all cases), which correspond exactly to the six clades identified using NCPA (Fig. 2B).

None of the six major clades consists of haplotypes from only a single population. For example, clades 4-1 and 4-3 each consist of multiple haplotypes from Corrientes, Roldán, and Rosario, but also contain single haplotypes from Pedra Preta (4-1 only) and Rincão dos Cabrais (4-3 only; See Fig. 2C). In both of these cases, the uncharacteristic haplotypes were found in only a single individual. This same pattern is observed for all populations and clades except for Ceu Azul, the haplotypes from which are restricted entirely to clade 4-5,7, and for clade 4-6, which is composed entirely of haplotypes from Arroio dos Ratos and Rincão dos Cabrais.

Discussion

We used a sequential phylogeographic approach (Althoff and Pellmyr 2002; Bernatchez 2001; Hoffman and Blouin 2004; Morando et al. 2004) to examine the nature and distribution of mtDNA sequence variation that occurs in the fire ant *S. invicta* throughout much of its native South American range. Our analyses confirmed several anticipated patterns and also revealed several unanticipated patterns that were discernable only from use of multiple methods of analysis. Specifically, four noteworthy findings include: pronounced genetic differentiation among populations separated by hundreds to thousands of kilometers, a major phylogeographic break between the southwestern and northeastern portions of the range that coincides with the Mesopotamia wetlands region, several inferred long-distance colonization events, and contrasting patterns of genetic differentiation within each of the two portions of the range separated by the Mesopotamia wetlands. Below we discuss each of these four findings in light of the natural history of fire ants, known or presumed factors affecting maternal gene flow, previous fire-ant population genetic studies, and available ecological data for the portion of the range of *S. invicta* that we sampled.

Restricted Gene Flow and Isolation by Distance

One consistent pattern revealed by our analyses is a combination of restricted gene flow and IBD. Our overall estimate of genetic differentiation among *S. invicta* populations is extremely high ($\phi_{ST} = 0.736$), and the pairwise estimates reveal that strong and significant differentiation can occur between populations separated by as little as 80 km (see Appendices I, III available online only). Additional evidence for strongly reduced gene flow is the finding that almost no mtDNA haplotypes are shared among populations: of 81 unique mtDNA haplotypes discovered in this study, only five were found to occur in individuals from more than one geographic population. Lastly, one of the most common inferences from NCPA was restricted gene flow with IBD, with this pattern being inferred at almost every clade level (Table 3).

Our finding of patterns of genetic structure consistent with IBD and restricted gene flow at even modest scales corroborates and extends earlier population genetic studies of *S. invicta* based on nuclear and mtDNA markers (Ross et al. 1997; Ross and Shoemaker 2005; Ross and Trager 1990; Shoemaker and Ross 1996). Several factors may act to limit maternally mediated gene flow in *S. invicta* including the relatively low dispersal ability of newly mated monogyne queens (Markin et al. 1971), with their polygyne counterparts showing even lower vagility (DeHeer et al. 1999), the restricted ability of queens of each social form to be accepted as reproductives in nests of the alternate form (Shoemaker and Ross 1996), and the presence at varying population frequencies of different *Wolbachia* strains (Shoemaker et al. 2003a), which can lead to reproductive incompatibilities between individuals from different populations. These factors presumably may act alone or in concert to reduce maternal gene flow.

Major Genetic Break Coincident with the Mesopotamia Wetlands

One must be cautious when inferring IBD, even in situations where such a pattern may be predicted, because sharp discontinuities or other phylogeographic patterns may be overlooked when testing for IBD (Garnier et al. 2004). Indeed, support for such sharp regional discontinuities in native *S. invicta* comes from NCPA, which identified a major genetic break between *S. invicta* mtDNA haplotypes. This break demarcates two higher-level groups (clades 6-1 and 6-2), each of which represents haplotypes mostly in individuals from either the southwestern or northeastern portions, respectively, of the species range (Fig. 1). The location of this major phylogeographic division coincides with the Mesopotamia wetlands region, which is located along the border between Argentina and Brazil and includes the Paraná River, second in size only to the Amazon among South American rivers (see Figure 1: Eva et al. 2002). The Paraná River joins the Uruguay River to form the Río de la Plata estuary at the southern extreme of the Mesopotamia wetlands. With its extensive river system, this entire region historically has been subject to repeated, widespread flooding as a result of both large rainfalls and changes in sea level during periods of glacial melting. As such, the region probably constitutes unsuitable habitat for the long-term persistence of fire ants, thus serving as a geographic barrier to gene flow that extends at least 2500 km between two large portions of the range of *S. invicta*. While Ross et al. (1997) hypothesized that the Paraná River may serve as a major barrier to gene flow that has influenced the structure and evolution of native fire-ant populations, until now no explicit phylogeographic or historical information has been provided to support this conjecture.

While we did not specifically address the effects of differences in colony social form on patterns of gene flow, earlier studies have revealed pronounced mtDNA differentiation between sympatric populations of the alternate forms, presumably due to the inability of queens to be accepted as new reproductives by workers in colonies of the alternate form (Keller and Ross 1993; Ross 1992; Ross and Keller 1998). More relevant to the broad-scale phylogeographic concerns of the present study, the polygyne social form has been shown to be limited largely to the southern portion of the range of *S. invicta* (Mescher et al. 2003), coincident with our group 6-1, a pattern paralleled by the distribution of *Wolbachia* infection frequencies (Shoemaker et al. 2003a). These concordant distributions, while unexplained, lend support to the idea that *S. invicta* is composed of at least two evolutionarily independent entities divided by the Mesopotamia wetlands.

Long-Distance Colonization

Although it seems unlikely that fire-ant queens are able to disperse often across the vast floodplains of the Mesopotamia wetlands, we did find evidence for historical long-distance colonization or migration between the northeastern and southwestern regions. Thus, this presumed extrinsic barrier to gene flow is not complete. Indeed, NCPA inferred three long-distance colonization events (clades 4-1, 4-4, and 4-6), all of which bridged the Mesopotamia wetlands and provided some degree of gene flow between groups 6-1 and 6-2 (Table

3). In addition to these events, NCPA also identified long-distance dispersal in conjunction with restricted gene flow for clade 3-7, which is composed of haplotypes from both Formosa in the southwestern region and Campo Grande and São Gabriel do Oeste in the northeastern region. Long-distance dispersal may be more likely between these three populations because they are located near the northern reaches of the wetlands, where the terrain is not likely to create as effective a barrier as it does closer to the mouth of the river, where the majority of flooding occurs.

Long-distance migration of queens may be attributable to strong winds during nuptial flights, to rafting of entire colonies on the water surface during flooding (Hölldobler and Wilson 1990), or, more recently, to inadvertent human transport of queens or queenright colony fragments. However, one argument against frequent recent human-mediated fire-ant migration in South America is the finding that in every inferred case of long-distance colonization the presumed migrants harbor mtDNA haplotypes that differ by one or a few base pair substitutions from those in the inferred source population, suggesting that the inferred long-distance colonization occurred some time ago. Thus, while we initially feared that long-distance colonization via human transport might be common enough to overwhelm the effects of biogeographic barriers and physical distance on gene flow, our analyses suggest that in fact such movement may not be sufficiently frequent or regular to prevent the continued genetic divergence of geographic populations. This conclusion contrasts with the apparently frequent anthropogenic transport of *S. invicta* in the United States since its introduction there (Lofgren 1986b), which presumably facilitated its spread and may have helped homogenize populations from different parts of the new range.

Contrasting Patterns of Genetic Differentiation

Another unexpected result from the combined analyses of our data was the contrasting patterns of genetic differentiation within the two major haplotype groups, 6-1 and 6-2. Pairwise estimates of genetic differentiation between all population pairs within group 6-2, composed mainly of haplotypes from populations in the northeastern region, are all significant, regardless of the geographic distance between the populations. In fact, significant genetic differentiation even occurs between Arroio dos Ratos and Rincão dos Cabrais, which are only 77 km apart. In contrast, levels of differentiation among populations within group 6-1 generally are much lower, and in some cases nonsignificant, despite rather large distances separating many of the populations. For example, although Corrientes is more than 533 km from both Rosario and Roldán, only modest or weak significant differentiation was evident among any of these three populations.

One possible explanation for these contrasting patterns of genetic differentiation is that the distribution of suitable habitats differs between the two regions. Patterns of vegetation, which reflect both the quality of a given habitat and the patchiness of preferred habitats, differ dramatically between the two regions (Eva et al. 2002; Fig. 1). Specifically, the southwestern region, containing haplotypes within group 6-1, is composed almost entirely of uninterrupted grasslands and land developed for agriculture. Such large, open areas are ideal environments for *S. invicta* colonies, which require exposed, sunlit conditions to develop, and apparently pose minimal geographic barriers to movement of these ants. Given such large, uninterrupted suitable habitats and presumably increased opportunities for dispersal, it perhaps is not surprising that gene flow levels appear to be very high within this region even across rather large distances. In contrast, *S. invicta* populations northeast of the Mesopotamia wetlands occupy grasslands regularly interrupted by scattered and elevated forests, resulting in a more patchily distributed network of suitable fire-ant habitat. Similar habitat patchiness has been shown to severely limit gene flow in the polygyne ant species *Formica exsecta* (Liautard and Keller 2001), and it is reasonable to conclude that environmental features play a significant role in shaping patterns of gene flow within native *S. invicta* as well.

The overall patterns of genetic variation we observe suggest that the southwestern region is characterized by long-term fire ant persistence and uninterrupted gene flow. If this is the region where *S. invicta* originated, then northeastern populations derived from colonization across the Mesopotamia wetlands possibly expanded into a more patchily distributed environment where restricted gene flow would be expected. NCPA infers contiguous range expansion for the populations north and east of the wetlands (group 6-2), which lends support to the idea that *S. invicta* has expanded northward into more patchy environments. Monogyne queens have a far greater capacity for long-distance dispersal and colonization than their polygyne counterparts due to their larger fat reserves (DeHeer et al. 1999), so it is not surprising that the majority of colonies found in the northeast are monogyne (Mescher et al. 2003), even if the source populations for colonizing queens contained nests of both social forms.

A potential alternative explanation for the contrasting patterns of differentiation within each of the two major clades is that they result from the influence of *Wolbachia* on mtDNA variation rather than reflecting differences in the distribution of suitable habitats. Indeed, while low levels of gene flow between populations may allow their continual divergence via drift, even very small amounts of gene flow should quickly erode such divergence if *Wolbachia* are present (Shoemaker et al. 2003b). Consistent with this prediction, *Wolbachia* infections in *S. invicta* are limited largely to the southern portion of the species range, an area where modest genetic differentiation exists among populations. However, a recent study revealed that transmission of *Wolbachia* and the mtDNA genome are largely uncoupled in *S. invicta* (Ahrens and Shoemaker 2005), which means that the effects of *Wolbachia* on mtDNA evolution within *S. invicta* are less substantial than previously anticipated. Furthermore, the fact that the frequency spectra of mtDNA variation within *Wolbachia*-infected populations are largely consistent with neutral expectations (Table 2) also supports the view that transmission of the two genomes is uncoupled historically or, at the very least, that there has not been a recent mtDNA sweep as a result of the spread of *Wolbachia*.

Conclusions

Our data suggest roles for both historical and recent forces in shaping the distribution of mtDNA sequence variation in

S. invicta in its native range. Both demographic analyses and NCPA revealed a consistent pattern of restricted gene flow with IBD among geographic populations. Furthermore, the use of NCPA, in conjunction with data on the geography of South America, provided insights into the evolutionary history of *S. invicta* not evident from traditional population genetic structure analyses by revealing several phylogeographic discontinuities within *S. invicta*, including a sharp genetic break between populations from the northeastern and southwestern portions of its range. Our analyses also suggest that, while the limited dispersal ability of fire ants may be a factor determining the degree of genetic differentiation between *S. invicta* populations, as is the presence of variable *Wolbachia* infections and alternative forms of colony social organization, the structure of the physical and biotic environment clearly creates barriers to dispersal, thus playing an important role in determining the nature and degree of population genetic structure at varying scales. Finally, our study represents a major step toward understanding the complex causes of barriers to gene flow and, ultimately, reproductive isolation in this important group of ants.

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Literature Cited

Ahrens, M., and D. D. Shoemaker. 2005. Evolutionary history of *Wolbachia* infections in the fire ant *Solenopsis invicta*. BMC Evol. Biol. 5:35.

Althoff, D. M., and O. Pellmyr. 2002. Examining genetic structure in a bogus yucca moth: a sequential approach to phylogeography. Evolution 56:1632–1643.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard Univ. Press, Cambridge, MA.

Ballard, J. W. O., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Mol. Ecol. 13:729–744.

Barton, N. H., and A. G. Clark. 1990. Population structure and process in evolution. Pp. 115–173 *in* K. Wohrmann and S. K. Jain, eds. Population biology: ecological and evolutionary viewpoints. Springer-Verlag, Berlin.

Bernatchez, L. 2001. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. Evolution 55: 351–379.

Buren, W. F., G. E. Allen, W. H. Whitcomb, F. E. Lennartz, and R. N. Williams. 1974. Zoogeography of the imported fire ants. J. NY Entomol. Soc. 82:113–124.

Clément, J.-L., A.-G. Bagnères, P. Uva, L. Wilfert, A. Quintana, J. Reinhard, and S. Dronnet. 2001. Biosystematics of *Reticulitermes* termites in Europe: morphological, chemical and molecular data. Insectes Soc. 48:202–215.

DeHeer, C. J., M. A. D. Goodisman, and K. G. Ross. 1999. Queen dispersal strategies in the multiple-queen form of the fire ant *Solenopsis invicta*. Am. Nat. 153:660–675.

Eva, H. D., E. E. deMiranda, C. M. DiBella, V. Gond, O. Huber, A. S. Belward, S. Fritz, M. Sgrenzaroli, G. DeGrandi, S. Jones, F. Bartholomé, A. Achard, M. Dorado, A. Guimardães, C. Coutinho, P. Elvidge, and P. Vogt. 2002. A vegetation map of South America. The European Commission, Ispra, Italy.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Ford, M. J. 2002. Applications of selective neutrality tests to molecular ecology. Mol. Ecol. 11:1245–1262.

Fu, Y.-X. 1997. Statistical test of neutrality of mutations against population growth, hitchhiking, and background selection. Genetics 147:915–925.

Fu, Y.-X., and W.-H. Li. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709.

Garnier, S., P. Alibert, P. Audiot, B. Prieur, and J. Y. Rasplus. 2004. Isolation by distance and sharp discontinuities in gene frequencies: implications for the phylogeography of an alpine insect species, *Carabus solieri*. Mol. Ecol. 13:1883–1897.

Goropashnaya, A. V., V. B. Fedorov, B. Seifert, and P. Pamilo. 2004. Limited phylogeographical structure across Eurasia in two red wood ant species *Formica pratensis* and *F. lugubris* (Hymenoptera, Formicidae). Mol. Ecol. 13:1849–1858.

Hoffman, E. A., and M. S. Blouin. 2004. Evolutionary history of the northern leopard frog: reconstruction of phylogeny, phylogeography, and historical changes in population demography from mitochondrial DNA. Evolution 58:145–159.

Huelsenbeck, J. P., F. Ronquist, R. Nielsen, and J. P. Bollback. 2001. Evolution: Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294:2310–2314.

Hölldobler, B., and E. O. Wilson. 1990. The ants. The Belknap Press of Harvard Univ. Press, Cambridge, MA.

Keller, L., and K. G. Ross. 1993. Phenotypic basis of reproductive success in a social insect: genetic and social determinants. Science 260:1107–1110.

Liautard, C., and L. Keller. 2001. Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica exsecta*. Evolution 55:2484–2492.

Lofgren, C. S. 1986a. The economic importance and control of imported fire ants in the United States. Pp. 227–256 *in* S. B. Vinson, ed. Economic impact and control of social insects. Praeger, New York.

———. 1986b. History of imported fire ants in the United States. Pp. 36–49 *in* C. S. Lofgren and R. K. Vander Meer, eds. Fire ants and leaf cutting ants: biology and management. Westview Press, Boulder, CO.

Lofgren, C. S., W. A. Banks, and B. M. Glancey. 1975. Biology and control of imported fire ants. Annu. Rev. Entomol. 20:1–30.

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* (Hymenoptera: Formicidae). J. GA. Entomol. Soc. 6:145–156.

McMillen-Jackson, A. L., and T. M. Bert. 2003. Disparate patterns of population genetic structure and population history in two sympatric penaeid shrimp species (*Farfantepenaeus aztecus* and *Litopenaeus setiferus*) in the eastern United States. Mol. Ecol. 12:2895–2905.

Mescher, M. C., K. G. Ross, D. D. Shoemaker, L. Keller, and M. J. B. Krieger. 2003. Distribution of the two social forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) in the native South American range. Ann. Entomol. Soc. Am. 96: 810–817.

Morando, M., L. J. Avila, J. Baker, and J. W. Sites. 2004. Phylogeny and phylogeography of the *Liolaemus darwinii* complex (Squamata: Liolaemidae): evidence for introgression and incomplete lineage sorting. Evolution 58:842–861.

Neigel, J. E. 1997. A comparison of alternative strategies for estimating gene flow from genetic markers. Annu. Rev. Ecol. Syst. 28:105–128.

———. 2002. Is F_{ST} obsolete? Conserv. Genet. 3:167–173.

Nordborg, M. 2001. Coalescent theory. Pp. 1–37 *in* M. Balding, P. M. Bishop, and C. Cannings, eds. Handbook of statistical genetics. Wiley, Chichester, U.K.

Pitts, J. P. 2002. A cladistic analysis of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae). Ph.D. diss., University of Georgia, Athens, GA.

Posada, D., and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 19:817–818.
Posada, D., K. A. Crandall, and A. R. Templeton. 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol. Ecol. 9:487–488.
Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.
Rosenberg, N. A., and M. Nordborg. 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. Nat. Rev. Genet. 3:380–390.
Ross, K. G. 1992. Strong selection on a gene that influences reproductive competition in a social insect. Nature 355:347–349.
Ross, K. G., and L. Keller. 1998. Genetic control of social organization in an ant. Proc. Natl. Acad. Sci. USA 95:14232–14237.
Ross, K. G., and D. D. Shoemaker. 1997. Nuclear and mitochondrial genetic structure in two social forms of the fire ant *Solenopsis invicta*: insights into transitions to an alternate social organization. Heredity 78:590–602.
———. 2005. Species delimitation in native South American fire ants. Mol. Ecol. *In press*.
Ross, K. G., and J. C. Trager. 1990. Systematics and population genetics of fire ants (*Solenopsis saevissima* complex) from Argentina. Evolution 44:2113–2134.
Ross, K. G., M. J. B. Krieger, D. D. Shoemaker, E. L. Vargo, and L. Keller. 1997. Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. Genetics 147:643–655.
Ross, K. G., D. D. Shoemaker, M. J. Krieger, C. J. DeHeer, and L. Keller. 1999. Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant *Solenopsis invicta*. Mol. Biol. Evol. 16:525–543.
Rozas, J., and R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.
Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.000: a software for population genetic data analysis. Genetics Biometry Laboratory, Univ. of Geneva, Switzerland.
Shoemaker, D. D., and J. Jaenike. 1997. Habitat continuity and the genetic structure of *Drosophila* populations. Evolution 51: 1326–1332.
Shoemaker, D. D., and K. G. Ross. 1996. Effects of social organization on gene flow in the fire ant *Solenopsis invicta*. Nature 383:613–616.
Shoemaker, D. D., M. Ahrens, L. Sheill, M. Mescher, L. Keller, and K. G. Ross. 2003a. Distribution and prevalence of *Wolbachia* infections in native populations of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Environ. Entomol. 32:1329–1336.
Shoemaker, D. D., G. Keller, and K. G. Ross. 2003b. Effects of *Wolbachia* on mtDNA variation in two fire ant species. Mol. Ecol. 12:1757–1771.
Simmons, M. P., K. M. Pickett, and M. Miya. 2004. How meaningful are Bayesian support values? Mol. Biol. Evol. 21: 188–199.
Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 81: 651–701.
Simonsen, K. L., G. Churchill, and C. F. Aquadro. 1995. Properties of statistical tests of neutrality for DNA polymorphism data. Genetics 141:413–429.
Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.
Swofford, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (* and other methods). Ver. 4.0. Sinauer Associates, Sunderland, MA.
Tajima, F. 1989. The effect of change in population size on DNA polymorphism. Genetics 123:597–601.
Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7:381–397.
———. 2004. Statistical phylogeography: methods of evaluating and minimizing inference errors. Mol. Ecol. 13:789–809.
Templeton, A. R., and C. F. Sing. 1993. A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. Genetics 134:659–669.
Templeton, A. R., E. Routman, and C. A. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140: 767–782.
Trager, J. C. 1991. A revision of the fire ants, *Solenopsis geminata* group (Hymenoptera: Formicidae: Myrmicinae). J. NY Entomol. Soc. 99:141–198.
Tschinkel, W. R. 2005. The fire ants. Harvard Univ. Press, Cambridge, MA.
Verovnik, R., B. Sket, and P. Trontelj. 2004. Phylogeography of subterranean and surface populations of water lice *Asellus aquaticus* (Crustacea: Isopoda). Mol. Ecol. 13:1519–1532.
Wayne, M. L., and K. L. Simonsen. 1998. Statistical tests of neutrality in the age of weak selection. Trends Ecol. Evol. 13: 236–240.
Whitlock, M. C., and D. E. McCauley. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. Heredity 82: 117–125.
Wiens, J. J., and T. A. Penkrot. 2002. Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). Syst. Biol. 51:69–91.
Wilson, E. O. 1952. O complexo *Solenopsis saevissima* na America do Sul (Hymenoptera: Formicidae). Mem. Inst. Oswaldo Cruz 50:49–59.
Wright, S. 1943. Isolation by distance. Genetics 28:114–138.

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