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Putative native source of the invasive fire ant *Solenopsis invicta* in the USA

Eric J. Caldera · Kenneth G. Ross · Christopher J. DeHeer · D. DeWayne Shoemaker

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Abstract The ecological and evolutionary dynamics of newly introduced invasive species can best be understood by identifying the source population(s) from which they originated, as many species vary behaviorally, morphologically, and genetically across their native landscapes. We attempt to identify the source(s) of the red imported fire ant (*Solenopsis invicta*) in the southern USA utilizing data from three classes of genetic markers (allozymes, microsatellites, and mitochondrial DNA sequences) and employing Bayesian clustering simulations, assignment and exclusion tests, and phylogenetic and population genetic analyses. We conclude that the Mesopotamia flood plain near Formosa, Argentina represents the most probable source region for introduced *S. invicta* among the 10 localities sampled across the native South American range. This result confirms previous suspicions that the source population resides in northern Argentina, while adding further doubts to earlier claims that the Pantanal region of Brazil is the source area. Several lines of evidence suggest that *S. invicta* in the southern USA is derived from a single location rather than being the product of multiple invasions from widely separated source localities. Although finer-scale sampling of northern Argentina and Paraguay combined with the use of additional genetic markers will be necessary to provide a highly precise source population assignment, our current results are of immediate use in directing future sampling and focusing ongoing biological control efforts.

Keywords Allozymes · Fire ant · Genetic structure · Invasive species · Microsatellites · mtDNA · Native source population · *Solenopsis invicta*

E. J. Caldera
Department of Zoology, University of Wisconsin, Madison, WI 53706, USA

E. J. Caldera
Department of Bacteriology, University of Wisconsin, Madison, WI 53706, USA

K. G. Ross
Department of Entomology, University of Georgia, Athens, GA 30602-2603, USA

C. J. DeHeer
LI-COR Biotechnology, 4647 Superior Street, P.O. Box 4000, Lincoln, NE 68504, USA

D. D. Shoemaker (✉)
USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, 1600/1700 SW 23rd Drive, Gainesville, FL 32608, USA
e-mail: dewayne.shoemaker@ars.usda.gov

Introduction

Invasive species can pose major threats to agricultural and natural environments as well as to the public

health (Sax et al. 2005; Wilcove et al. 1998). Considering just insects in agricultural systems, the negative impact of invasives is evident from the fact that a vast number of major pest species in such settings in the USA are of exotic origin (Carruthers 2003). Although the basis for the success of different exotic pests varies, one common contributing element appears to be ecological release from the natural assemblages of competitors, parasites, and predators that occur in their native environments and normally act to suppress their populations (Mitchell and Power 2003; Torchin et al. 2003). Thus, a reasonable approach to managing populations of undesirable exotics is to identify the natural enemies attacking them in their native ranges and to deliberately introduce one or more of these biological control agents into the introduced range.

While biological control has proved successful in suppressing pest populations in a number of cases, one potential difficulty is a failure to recognize biologically important variation among native pest populations that correlates with variation in natural enemy assemblages (Goolsby et al. 2006; Sakai et al. 2001; Waage and Mills 1992). As an example, if strong genetic differentiation exists among native populations of an introduced species, then it is likely either that the species composition of the natural enemy assemblage varies locally over the native range of the exotic or that widespread individual enemy species are locally adapted to it. In such cases, the efficacy and success of natural enemies in controlling their host will likely depend on an interaction between natural enemy and host genotypes (Dybdahl and Storfer 2003; Foitzik et al. 2003; Goolsby et al. 2006; Kaltz and Shykoff 1998; Kraaijeveld and Godfray 1999; Lively and Dybdahl 2000; Lively et al. 2004; Thrall et al. 2002). If so, efforts to pinpoint the source population of an invasive pest are of paramount importance in identifying appropriate agents of its biological control (Waage and Mills 1992).

In addition to directing efforts toward appropriate locations for identifying potential control agents, determination of the geographical source of an invasive pest may facilitate reconstruction of the invasion history, help reveal important regional genetic variation relevant to its natural history, and shed light on important post-invasion changes in its biology that may affect its pest attributes (Downie 2002; Giraud et al. 2002; Gwiazdowski et al. 2006; Lee 2002; Provan et al. 2005; Ross et al. 1996; Tsutsui et al. 2003). While identification of the source population of an invasive species can be challenging, the development of new molecular tools and statistical methods for analyzing the resulting data have greatly improved the prospects for meaningful results, as illustrated by several recent studies (Gwiazdowski et al. 2006; Harter et al. 2004; Havill et al. 2006; May et al. 2006; Miura et al. 2006).

The red imported fire ant, *Solenopsis invicta*, is an invasive pest of significant economic, agricultural, and medical importance in the USA and elsewhere (Jouvenaz 1990; Lofgren 1986a; Orr 1996; Patterson 1994). This insect was inadvertently introduced into the USA from South America some 75 years ago. Since that time, it has spread throughout the southern part of the country (Callcott and Collins 1996; Lofgren 1986b) and, more recently, to several western states, the Carribean, Australia, and Taiwan (Buckley 1999; Chen et al. 2006; Davis et al. 2001; Huang et al. 2004; MacKay and Fagerlund 1997; McCubbin and Weiner 2002). *Solenopsis invicta* is of economic importance in these areas of introduction because: (1) it is an aggressive stinging insect causing mass envenomation incidents and hypersensitivity reactions in humans, (2) it occurs primarily in human-modified habitats, (3) it constructs large mounds that are unsightly and capable of damaging farm machinery, (4) it feeds on several important cultivated plants and tends homopterans that are also plant pests, and (5) it may negatively affect populations of native ants and other ground-dwelling animals (Allen et al. 1998; Carroll and Hoffman 2000; Gotelli and Arnett 2000; Lofgren 1986a; Lofgren et al. 1975; Morrison 2002; Porter and Savignano 1990; Tschinkel 2006; Vinson 1994; Wojcik et al. 2001). These pest traits of *S. invicta* presumably are exacerbated by the relative lack of natural enemies in invasive populations that normally act to suppress fire ant populations, with the effect that population densities in the USA are orders of magnitude greater than in South America (Morrison 2000; Porter et al. 1992, 1997b).

Concerns about the negative economic and ecological impacts of *S. invicta* have led to the development of many different control methods that target individual colonies (e.g., contact insecticides) or are intended to suppress the colonies inhabiting larger areas (e.g., baits containing poisons, growth regulators, or

reproductive inhibitors) (Kemp et al. 2000; Williams 1994; Williams and Porter 1994). Because these methods generally have failed to halt the continued spread and enormous population buildups following new introductions, alternative approaches to population management, including those based on biological control by natural enemies from the native range, are being developed (Jouvenaz 1990; Lofgren 1986a; Morrison et al. 2000; Orr 1996; Patterson 1994; Porter 1998, 2000; Porter and Briano 2000; Porter et al. 2004; Williams and deShazo 2004). Clearly, however, sustained success in such novel management approaches requires detailed knowledge of the biology of this ant in its native habitat, including such crucial ecological information as the identity of key competitors and natural enemies and the nature of their interactions with *S. invicta*. Given that this species occupies a vast native range characterized by profound regional genetic differentiation (Ross et al. 1997, 2007; Ross and Shoemaker 2005), the most scientifically and practically relevant information of this type will come from the native population(s) that served as the source of the USA colonists.

The current study uses genetic data generated from three classes of genetic markers to attempt to identify the native source of *S. invicta* introduced into the USA. Such attempts would have been difficult previously, given the somewhat problematic α-taxonomy of South American fire ants, the lack of a sufficient number of informative molecular markers, and the limited availability of samples from the native range. However, substantial progress in resolving these shortcomings has been made recently (Pitts et al. 2005; Ross et al. 2007; Ross and Shoemaker 2005). An attempt to identify the origin of *S. invicta* is particularly appropriate at this juncture in light of recent studies detailing the nature and extent of geographic population genetic differentiation within the native range (Ross et al. 2007; Ross and Shoemaker 2005). While this recent work provides necessary baseline data for source identification, the observed strong regional differentiation also suggests that natural enemies of *S. invicta* may be locally adapted to their host and that *S. invicta* may differ regionally in some important aspects of its natural history. Thus, identification of the source of the USA colonists is important not only for focusing biological control collections but also to anchor studies of the natural history of *S. invicta* in its native range, an increasingly important endeavor given the emergence of the species as a prominent model for ecological and evolutionary studies (Gotzek and Ross 2007; Tschinkel 2006).

Materials and methods

All of the samples used here were subjected to extensive population genetic analyses in several earlier studies (Ross et al. 2007; Ross and Shoemaker 2005; Shoemaker et al. 2006a, b). These previous studies described patterns of genetic variation within native and introduced populations of *S. invicta*, important baseline data for attempting to identify the native source of the USA colonists. Below we summarize briefly the sampling and genetic methods employed in generating these data; additional details can be found in Shoemaker et al. (2006b) and Ross et al. (2007).

Samples

Two social forms of *S. invicta* occur in both the native and introduced ranges. The monogyne (*M*) social form is characterized by the presence of one reproductive queen per colony, while the polygyne (*P*) form is characterized by multiple such queens per colony. The two forms differ not only in colony queen number but also in many other important features of their reproductive and dispersal biology that are expected to have important effects on the distribution of genetic variation at various spatial scales (Ross and Keller 1995; Tschinkel 2006). Samples of introduced *S. invicta* were collected from 258 colonies at two exemplar localities in the southern USA (Fig. 1), with both social forms well sampled at each locality. The Mississippi locality was chosen as an exemplar because of its close proximity to the port of Mobile, Alabama, the suspected initial point of entry of *S. invicta* into the USA (Lofgren 1986a), and because Shoemaker et al. (2006b) demonstrated that the patterns of diversity within this population appear to closely resemble those of a hypothetical original colonizing population. We included ants from west Louisiana because they appear to be somewhat divergent from other ants sampled throughout the southern USA (Shoemaker et al. 2006b), possibly as the result of a secondary introduction. All sampled colonies at each locality were located within 40 km of one another.

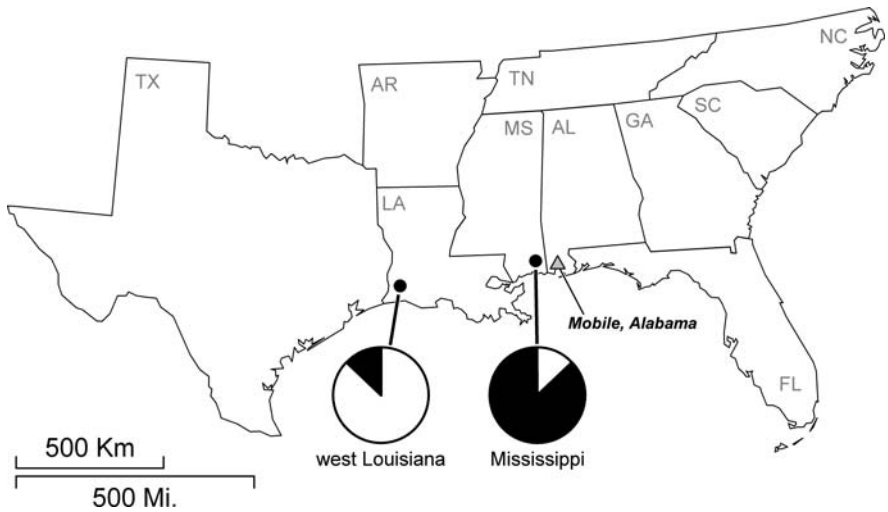

Fig. 1 Sampling localities for *S. invicta* within the introduced range in the USA. Pie charts represent the proportions of individuals (colonies) within each geographic locality that were placed in each of two genetic clusters inferred by the program STRUCTURE

Samples of native *S. invicta* were collected from 567 colonies at 10 localities in Brazil and Argentina that span a large portion of the native range (Fig. 2; see also Ahrens et al. 2005; Mescher et al. 2003; Ross et al. 2007). All sampled colonies at a locality were located within 20 km of one another, while distances between pairs of localities ranged from 90 to 1,967 km. Samples of each social form were collected in sufficient numbers from the Corrientes and Formosa localities to warrant separate analyses. Following Ross et al. (2007), two distinct populations were distinguished within the Arrorio dos Ratos locality, designated as Arroio X and Y. Three of the Brazilian localities, Pedra Preta, São Gabriel do Oeste, and Campo Grande, lie at the eastern edge of the Pantanal, a large flood plain hypothesized by earlier authors to be the source area for the USA colonists (Allen and Buren 1974; Buren 1972; Buren et al. 1974).

All sampled colonies were identified as *S. invicta* by J. P. Pitts using species-informative morphological characters (Pitts 2002; Trager 1991). The social form of each colony was identified using the methods described in Shoemaker et al. (2006b). Geographic coordinates for the sampling localities and numbers of samples from each are summarized in Appendix I.

Genetic markers

We genotyped one female alate (winged virgin queen) or dealate (wingless reproductive queen) per colony at 12–14 nuclear loci. Nuclear markers included seven allozyme loci (*Aat-2*, *Acoh-1*, *Acoh-5*, *Est-2*, *G3pdh-1*, *Gpi*, *Pgm-1*) and seven microsatellite loci (*Sol-6*, *Sol-11*, *Sol-18*, *Sol-20*, *Sol-42*, *Sol-49*, *Sol-55*). Methods for allozyme electrophoresis and staining are found in Shoemaker et al. (1992) and Ross et al. (1997). Because the allozyme loci *Est-2* and *Gpi* are monomorphic in *S. invicta* in the USA (Shoemaker et al. 1996), they were not scored in the Mississippi and west Louisiana ants. Primers and procedures for microsatellite amplification and visualization are described in Ross et al. (2007).

We sequenced a 920-bp fragment of the mitochondrial DNA (mtDNA) genome for 644 of the individuals described above using the primers COI-RLR (Simon et al. 1994) and DDS-COII-4 (Ross and Shoemaker 1997; see Appendix II for sample sizes for each population); All sequences for ants from the USA were newly generated for this study (GenBank accession numbers EU352605–EU352610). Primer sequences, PCR reaction conditions, and sequencing methods were identical to those described in Ahrens et al. (2005). All sequences were aligned by eye using sequence data deposited in GenBank (accession number AY2490093), and each was assigned a specific haplotype identification code according to Shoemaker et al. (2006a). For STRUCTURE analyses incorporating mtDNA data from the native range (see below), haplotypes were binned into one of seven well supported clades described in Ross et al. (2007) and Shoemaker et al. (2006a). All unique mtDNA sequences have been deposited in GenBank

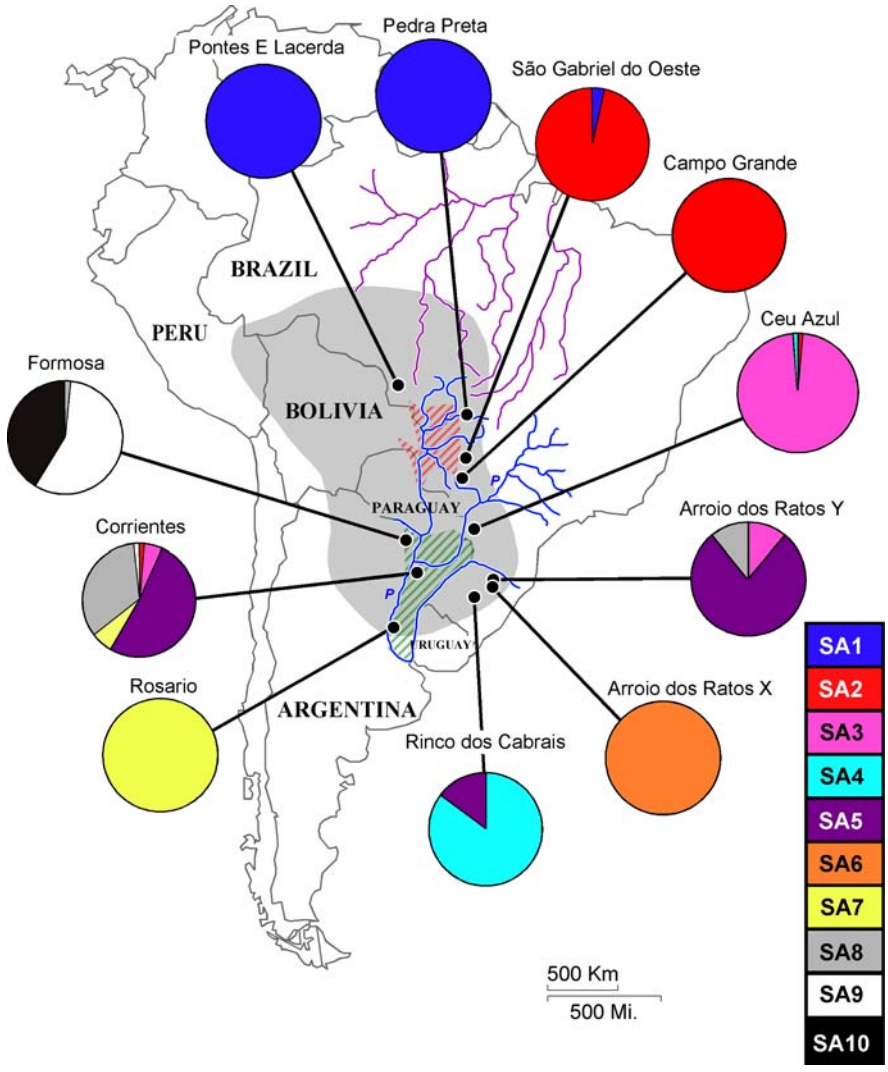

Fig. 2 Sampling localities for *S. invicta* within the native range of the species (area shaded gray). Pie charts represent the proportions of individuals (colonies) within each geographic locality that were placed in one of ten genetic clusters inferred by the program STRUCTURE (designated clusters SA1–SA10). The Pantanal and Mesopotamia floodplain regions are depicted with red and green stippling, respectively, while relevant portions of waterways in the Amazon and La Plata River Basins are depicted with purple and blue lines, respectively. The Paraná River is indicated by the letter "*P*"

(see Table 1 of Shoemaker et al. 2006a for GenBank accession numbers).

Assignment tests

We used Bayesian assignment tests implemented in the program GENECLASS2 (Piry et al. 2004) as one approach to determine the likely source(s) of invasive *S. invicta*. Bayesian assignment tests were selected over distance-based tests because the former appear to perform better (Cornuet et al. 1999), and assignment tests utilizing the Rannala and Mountain (1997) methods were selected over approaches employing a uniform prior because the former produce less ambiguous results (Baudouin et al. 2004). Initially, assignment tests for the introduced ants were conducted at both the individual and population levels using the native sampling localities (geographic populations) as reference populations.

Because power in assigning individuals or groups to a reference population can be gained by grouping individuals on the basis of genetic similarity rather than geographic proximity (Baudouin et al. 2004), we also performed the above analyses after grouping ants into distinctive genetic clusters rather than collection localities. To accomplish this, we used the Bayesian program STRUCTURE (Pritchard et al. 2000) to sort individuals from the native or introduced ranges into *K* genetic clusters. We did not include the mtDNA data for these analyses because GENECLASS2 is not

equipped to handle data derived from both haploid and diploid markers. All simulations performed in STRUCTURE featured 300,000 runs following a burn-in period consisting of 100,000 runs. We used the admixture model assuming correlated allele frequencies and did not include collection site or social form as priors. Analyses were performed separately for ants from the USA ($K = 1$–4) and from South America ($K = 1$–19). For both analyses, ten separate runs were performed for each value of K. We ensured accurate estimates of the simulation values by checking that model parameters equilibrated before the end of the burn-in phase and that posterior probabilities were consistent across all ten runs for each data and parameter set. After determining the appropriate value of K for each dataset using the ΔK method of Evanno et al. (2005), each individual was placed in the cluster in which it had predominant membership (determined from the run with the highest posterior probability for a particular cluster). The resulting new clusters of native ants then were used as reference populations for assignment tests performed on the introduced ants in GENECLASS2. Again, these assignment tests utilizing the STRUCTURE-defined genetic clusters were conducted at both the individual and population levels.

In a somewhat different approach, we used STRUCTURE to determine whether *S. invicta* from Mississippi and west Louisiana grouped predominantly with any distinct genetic cluster comprising ants from the native range. We ran these simulations using the entire set of individuals from both ranges ($K = 1$–17). Runs were performed with the parameters described above, using only the nuclear DNA data as well as the combined nuclear and mtDNA data.

Once a native source was tentatively identified, we ran STRUCTURE simulations again on a more restricted dataset comprising only this native population and a single geographic population from the USA (Mississippi or west Louisiana). These analyses were repeated with the introduced ants distinguished by membership in the genetic clusters determined from STRUCTURE rather than by locality.

Exclusion tests

One potential drawback of Bayesian assignment methods is that they assume the actual source population is represented among the reference populations, with the result that individuals of unknown origin will always be assigned with high probability to one or another reference population. In contrast, exclusion tests allow the possibility of excluding all reference populations as potential sources. GENECLASS2 offers three different Monte Carlo sampling algorithms for performing exclusion analyses (Cornuet et al. 1999; Paetkau et al. 2004; Rannala and Mountain 1997). We judged the algorithm of Cornuet et al. (1999) to be the most appropriate for our dataset, given that it assumes that source and introduced populations diverged in the absence of migration. All exclusion analyses were conducted with 100,000 simulated individuals and an alpha level of 0.01.

Phylogenetic analyses of mtDNA sequences

To make full use of the information available from the mtDNA, we performed phylogenetic analyses on 92 sequences representing all unique haplotypes from the native and introduced ranges. MtDNA sequences from two other fire ant species, *Solenopsis geminata* and *S. electra* (GenBank accession numbers AY254476 and AY249092, respectively), were used as outgroups for these analyses. We used neighbor-joining (NJ) and Bayesian methods implemented in the programs PAUP 4.08b (Swofford 1999) and MrBayes 3 (Ronquist and Huelsenbeck 2003), respectively, to reconstruct the mtDNA trees. The program MODELTEST (Posada and Crandall 1998) was used to determine the appropriate model of sequence evolution and proportion of invariant sites. The NJ tree was constructed using $\mathrm{TrN} + \mathrm{I} + \Gamma$ distances (determined by MODELTEST to have the lowest likelihood ratio; $\mathrm{I} = 0.6970$ and $\Gamma = 1.8861$), with the additional constraint that ties were broken randomly. All branches of zero length were collapsed during searches. Bootstrap support values for each node within the tree were calculated by performing 10,000 data resamplings. All MCMC searches in the Bayesian analyses employed a uniform prior based on output from MODELTEST, and no priors regarding tree topology were assumed. To ensure that parameter space was thoroughly explored, four separate runs with four heated chains each were performed; each chain featured four million generations (with sampling every 100th generation) following a burn-in period of 100,000 generations. Posterior probabilities were calculated using the

trees visited by the Markov chains after burn-in samples were discarded.

Additional analyses

We constructed a consensus genetic distance tree depicting the relationships of geographic populations at their nuclear genomes. The programs SEQBOOT, GENDIST, and NEIGHBOR within the PHYLIP package (Felsenstein 2004) were used to create replicate data sets, calculate Nei's chord distance (Nei et al. 1983), and construct NJ trees, respectively. Node stability was assessed by performing 1,000 bootstrap resamplings. The final consensus tree was produced in the CONSENSE program of PHYLIP by employing the majority rule criterion.

We used the program ARLEQUIN 2.000 (Schneider et al. 2000) to estimate F_{ST} values for all nuclear loci and Φ_{ST} values for the mtDNA between all pairs of native and introduced geographic populations, as well as to estimate allele and haplotype frequencies within geographic populations.

Finally, we employed STRUCTURE to generate F_K values for each geographic population in both the native and introduced ranges based on the nuclear data; this statistic can be interpreted as equivalent to F_{ST} between a sampled population and a hypothetical population assumed to be ancestral to all the study populations (Falush et al. 2003; Pritchard et al. 2000). We incorporated geographic location and social form as priors for these simulations.

The majority of data format conversions necessary for the different software programs used in this study were conducted with the program CONVERT (Glaubitz 2004). Graphical displays of STRUCTURE output were created using the program DISTRUCT (Rosenberg 2004).

Results

General descriptions of the types and distributions of nuclear genetic and mtDNA variation in native and introduced *S. invicta* populations are provided elsewhere (Ross et al. 2007; Shoemaker et al. 2006a, b). Allele and haplotype frequencies for the populations included in this study are provided in Appendix II (electronic versions available upon request).

Assignment tests

We first used GENECLASS2 to assign geographic populations from the USA to native geographic populations on the basis of the nuclear marker data. At the population level, both the Mississippi and the west Louisiana populations were assigned to the native Formosa, Argentina population with probabilities greater than 0.999. Population-level assignment probabilities to the remaining native localities were all below 0.0001.

We repeated the above analyses after grouping individuals from the native and introduced ranges into genetic clusters. STRUCTURE simulations revealed that ants from the introduced populations comprise two distinct genetic clusters ($K = 2$) based on calculation of ΔK (Evanno et al. 2005). These clusters correspond roughly to the two sampling localities (Fig. 1); over 87% of individuals with predominant genetic membership in the first cluster were from west Louisiana, while a similar proportion of individuals with predominant membership in the second cluster were from Mississippi. Thus, we subsequently refer to these two clusters as the "west Louisiana cluster" and the "Mississippi cluster." Very similar results were obtained when the two social forms at each locality were considered separately. For the South American ants, STRUCTURE simulations yielded ΔK values strongly supporting the existence of ten distinct genetic clusters ($K = 10$). We subsequently refer to these as clusters SA1 through SA10 (Fig. 2; see also Ross et al. 2007). As in the introduced range, cluster representation was very similar for the two social forms within Corrientes and Formosa.

After placing each individual in the cluster in which it had predominant membership, population-level assignment tests for the introduced ants were repeated in GENECLASS2. Both the Mississippi and west Louisiana clusters of introduced *S. invicta* were assigned to native cluster SA9 with probabilities greater than 0.999. This cluster is composed almost entirely of individuals from the Formosa population (one individual from the neighboring Corrientes, Argentina population was also placed in this cluster). Thus, GENECLASS2 population analyses of the nuclear data based on both geographic locality and genetic clustering implicate Formosa, Argentina as the source of invasive *S. invicta* in the USA, congruence that is not surprising given that the native genetic

clusters are strongly geographically structured (Ross et al. 2007). Notably, while the introduced ants are differentiated into two clusters corresponding roughly to the two sampling localities, their assignment to a single locality and cluster in the native range suggests that all *S. invicta* in the USA may be derived from colonists that originated in the same area.

For the individual-level GENECLASS2 analyses using nuclear genetic clusters as references, 231 (90%) of the individuals from Mississippi and west Louisiana were assigned with high probability (>0.99) to native cluster SA9, the cluster associated almost exclusively with Formosa. The remaining 27 individual assignments were deemed ambiguous because of probabilities <0.99. Nonetheless, the highest assignment probabilities for 21 of these were to cluster SA9. Moreover, among the remaining six ambiguous cases, five assignments were to SA10, a cluster found only in Formosa. The final ambiguous individual was assigned to SA5, a cluster commonly represented in the neighboring Corrientes population as well as the southern Brazil populations of Arroio Y and Rinco dos Cabrais (Fig. 2).

Results of individual-level assignment tests using GENECLASS2 with geographic reference populations were qualitatively similar to the above results using genetic clusters, but a much larger proportion of assignments were deemed ambiguous. Ants from Mississippi and west Louisiana invariably were assigned to the Formosa population, but only 52 (20%) of these assignments were made with probabilities >0.99. This underscores the importance of learning about the boundaries of natural genetic units within the native (and introduced) range in order to extract the most useful information for evaluating potential native sources.

STRUCTURE simulations run using all ants sampled from both ranges yielded evidence for just $K = 2$ clusters, regardless of whether only the nuclear data or both the nuclear and mtDNA data were considered. (This low estimate of K compared to that for just the native ants reflects a bias of the Evanno et al. (2005) method toward detection of the highest level of genetic structure.) Remarkably, native ants from almost all localities have membership predominantly in just one of the two clusters, while the introduced ants have membership predominantly in the other (Fig. 3). The conspicuous exception to this partitioning of the native and introduced gene pools is the native Formosa population. Ants here appear highly admixed, with average membership coefficients split nearly equally between the two clusters (0.58 and 0.44 with the nuclear markers only; 0.59 and 0.41 with the mtDNA included as well). Thus, this analysis also reveals a stronger genetic link of *S. invicta* in the USA to the Formosa ants than to any other sampled native population.

Given the multiple lines of evidence of strong genetic affinity between ants from the USA and Formosa, we ran additional STRUCTURE simulations

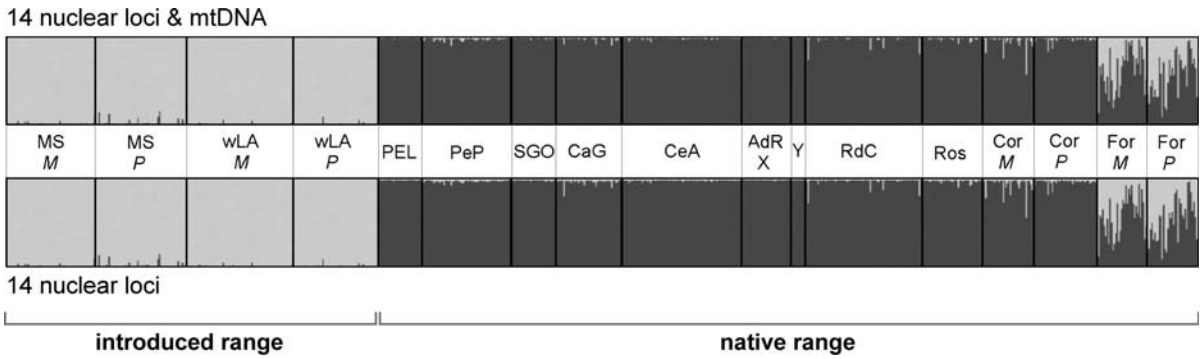

Fig. 3 Individual membership coefficients for introduced (USA) and native *S. invicta* determined from STRUCTURE simulations. The top panel shows results for 14 nuclear markers and the mtDNA combined, whereas the bottom panel shows results for the nuclear markers only. Within each population demarcated by a rectangle, individuals are represented by vertical lines divided into parts proportional to their proposed ancestry in each STRUCTURE-defined genetic cluster. MS, Mississippi; wLA, west Louisiana; PEL, Pontes E Lacerda; PeP, Pedra Preta; SGO, São Gabriel do Oeste; CaG, Campo Grande; CeA, Ceu Azul; AdR X, Arroio dos Ratos X; Y, Arroio dos Ratos Y; RdC, Rinco dos Cabrais; Ros, Rosario; Cor, Corrientes; For, Formosa. *M*, monogyne social form; *P*, polygyne social form

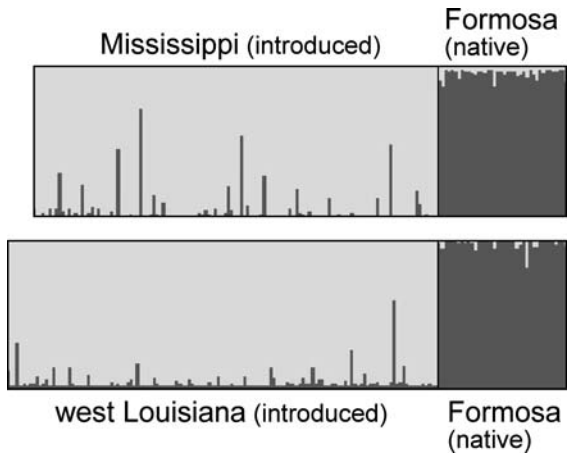

Fig. 4 Individual membership coefficients for introduced (Mississippi and west Louisiana, USA) and native (Formosa, Argentina) *S. invicta* determined from STRUCTURE simulations using 14 nuclear markers (analyses conducted separately for each introduced population). Individuals are represented by vertical lines divided into parts proportional to their proposed ancestry in each STRUCTURE-defined genetic cluster

using all 14 nuclear markers for ants from just these areas. Each of the analyses conducted separately on ants from Mississippi and west Louisiana yielded evidence for two clusters ($K = 2$), with the Formosa ants and introduced ants having membership predominantly in separate clusters (Fig. 4). Thus, despite the relative genetic similarity between the Formosa and USA ants when all samples are considered simultaneously, significant genetic differentiation between the putative source and introduced populations is nonetheless detectable. Essentially the same results were obtained when STRUCTURE-defined clusters were substituted for geographically defined populations in the introduced range or when the mtDNA data were incorporated (data not shown).

Exclusion tests

Exclusion tests potentially allow the rejection of all native populations as sources of the USA ants if the actual source was not sampled. Nonetheless, the exclusion tests we performed strongly support our findings from the assignment tests and STRUCTURE simulations that the likely source population for *S. invicta* in the USA occurs near Formosa, Argentina. In the individual-level analyses, only 38 (15%) of the ants from Mississippi and west Louisiana could be excluded as originating from the characteristically Formosan cluster SA9 (at $P < 0.01$; Fig. 5). Among these individuals, all but one were excluded from every other potential source cluster as well. Only three other clusters were not excluded entirely as sources for some introduced ants (Fig. 5). These are SA10, a cluster confined to Formosa, SA8, a cluster best represented at the neighboring Corrientes locality, and SA5, a cluster well represented at Corrientes and two southern Brazilian localities (Fig. 2).

It is noteworthy that genetic clusters composed solely or largely of ants from Brazilian localities (SA1, SA2, SA3, SA4, and SA6) were rejected as potential sources for any individual from the USA (Fig. 5). Identical results were obtained when we used geographic populations as the native references; all Brazilian localities invariably were rejected as potential sources.

Phylogenetic analyses of mtDNA sequences

The NJ and Bayesian phylogenetic analyses of the mtDNA sequences produced identical trees comprising seven well-supported clades, each of which has a distinctive geographic distribution (Fig. 6; see also Shoemaker et al. 2006a). The six haplotypes found in ants from Mississippi and west Louisiana fall within three clades distributed chiefly in central and north-central Argentina (designated clades 2, 3, and 4; see Shoemaker et al. 2006a). Four of the six introduced haplotypes are identical to variants from Formosa. A fifth (H5) is identical to a haplotype occurring in Corrientes and a third Argentine locality, Rosario. The sole mtDNA sequence unique to the introduced ants (USA4) differs from a recovered Formosa haplotype by a single point substitution.

Additional results

The NJ tree depicting the nuclear genetic relationships of the sampled populations is presented in Fig. 7, along with estimates of F_K, a measure of the nuclear genetic divergence of each sampled population from a hypothetical population ancestral to all of them. The introduced populations are most closely allied genetically to the Formosa ants among the

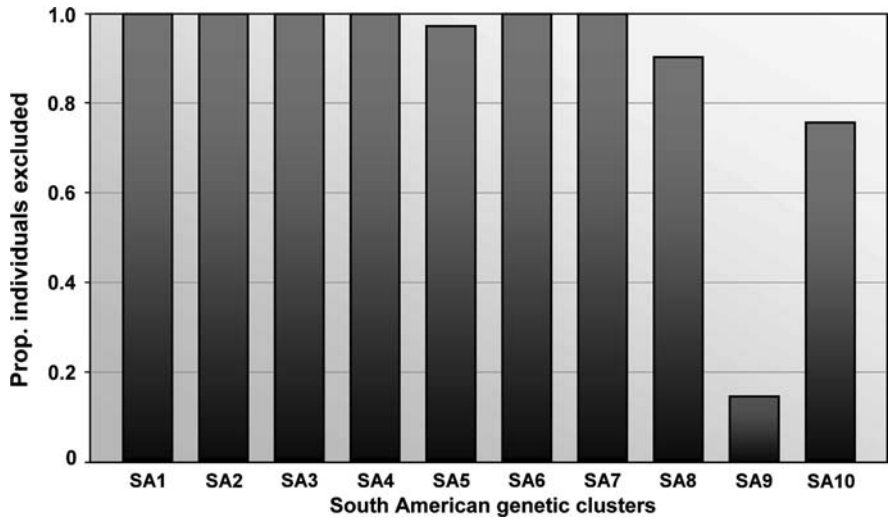

Fig. 5 Results of exclusion tests for individual *S. invicta* from the introduced (USA) range based on 14 nuclear markers. The histogram shows the proportions of individuals from both Mississippi and west Louisiana excluded as originating from each STRUCTURE-defined genetic cluster in the native range

native populations studied. Values of F_K equal to or exceeding 0.2 are confined to the Brazilian and introduced populations, whereas values less than 0.1 characterize only Corrientes and Formosa. This result reflects the conclusions of Ross et al. (2007) that the more peripheral Brazilian populations are relatively recently derived from ancestral *S. invicta* populations that resided in northern Argentina. More importantly with respect to the present study, it also implies that the introduced populations are among the most divergent relative to this hypothetical ancestral form. Such a pattern is expected given the pronounced changes in the extent and type of genetic variation predicted for bottlenecked populations (Chakraborty and Nei 1977), changes that have been demonstrated in *S. invicta* in the USA for allozyme and sex-determination loci (Ross et al. 1993).

Inspection of the nuclear and mtDNA variants in our samples from Formosa and the USA confirms that the variation in the introduced ants generally represents a very restricted subset of the variation found in Formosa, and that most variants in the USA can also be found in Formosa (Fig. 8). Specifically, 43% of the Formosa nuclear alleles and 22% of the Formosa mtDNA haplotypes were detected in the introduced ants, while only four of the 59 total variants from the introduced range (6.8%) were not detected in Formosa (two microsatellite alleles and two mtDNA haplotypes). Importantly, an atypical interrupted-repeat microsatellite allele recovered from both USA localities (*Sol-55*^{152}) was found only in Formosa among the native populations. Because mutations giving rise to such variants occur relatively infrequently (e.g., Jarne and Lagoda 1996), their common occurrence in Formosa and the introduced range is most likely due to a relatively recent genealogical connection between ants in the two areas.

Estimates of F_{ST} (nuclear markers) and Φ_{ST} (mtDNA sequences) between paired native and introduced populations are presented in Fig. 9. The lowest F_{ST} values invariably involve the Formosa ants. Estimates of Φ_{ST} yield similar patterns, with one exception; the Mississippi polygyne form is more similar overall in its mtDNA composition to ants from the Argentine populations of Corrientes and Rosario than to ants from Formosa. This result reflects the fact that one common haplotype in the Mississippi polygyne ants occurs only in Corrientes and Rosario in the native range (see Fig. 6).

Discussion

The primary objective of this study was to use genetic data generated from three classes of genetic markers (allozymes, microsatellites, and mtDNA sequences) to attempt to identify the native source population(s) from which the fire ant *Solenopsis invicta* in the USA originated. To accomplish this goal, we performed assignment and exclusion tests, as well as population genetic and phylogenetic analyses, utilizing ants collected from diverse native and introduced populations. One result important in paving the way for source identification is that, consistent with earlier genetic

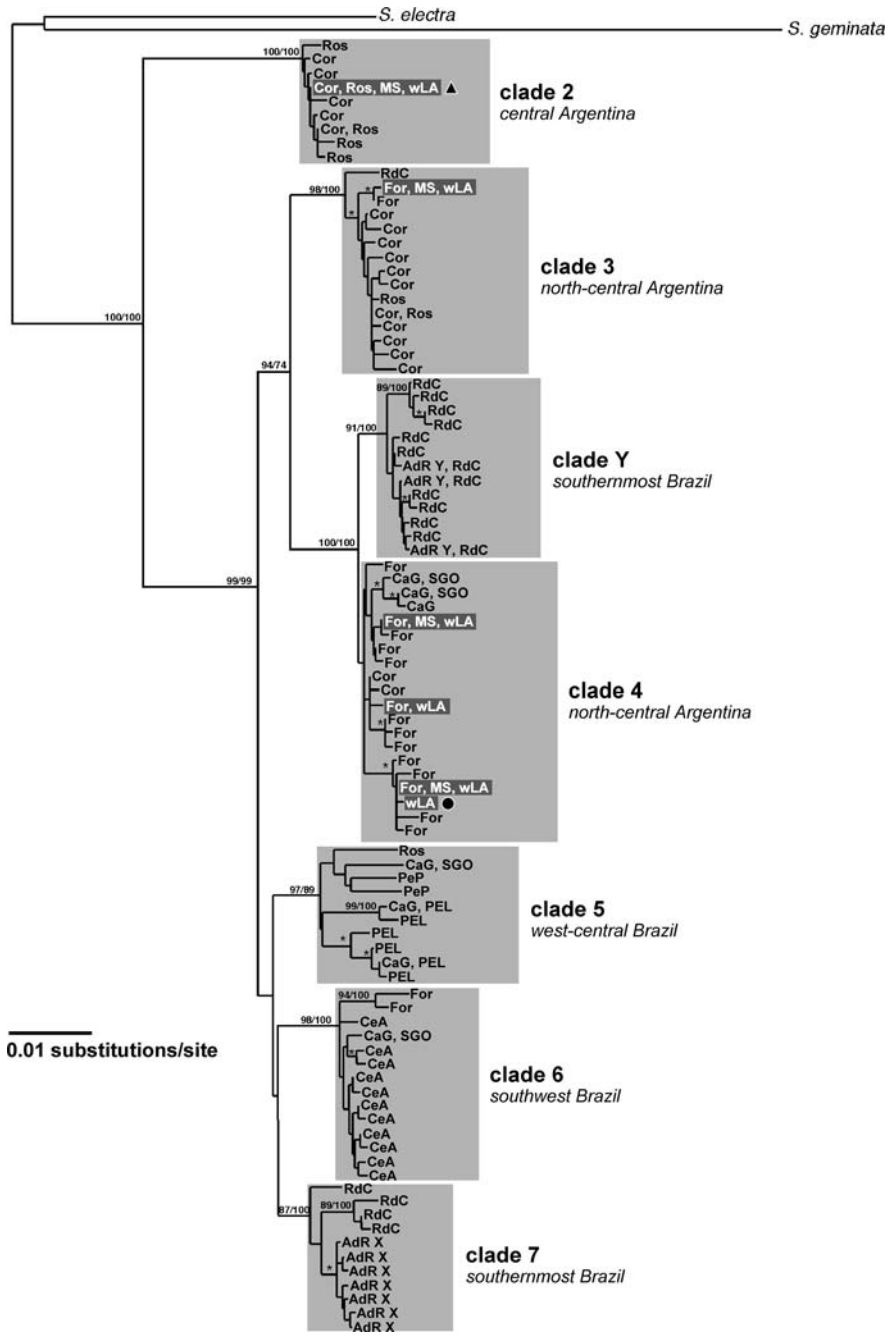

Fig. 6 Tree depicting phylogenetic relationships of unique *S. invicta* mtDNA sequence haplotypes obtained from both NJ and Bayesian analyses. Codes at terminals indicate geographic localities where each haplotype was found (see Fig. 3 legend). Terminals labeled with white lettering indicate haplotypes found in the USA; haplotype H5 is denoted by a black triangle and haplotype USA4 is denoted by a black circle. Seven major haplotype clades are indicated along with their regional affiliations (see Ross et al. 2007; Shoemaker et al. 2006a). Numbers on branches represent NJ bootstrap support values followed by Bayesian posterior probability values (only values greater than 70% are shown); asterisks indicate additional nodes with bootstrap and posterior probability values greater than 70%

studies, significant genetic differentiation was detected among sampled populations within both the native and introduced ranges (Ross et al. 2007; Shoemaker et al. 2006b). The especially marked differentiation among native populations is noteworthy because it constitutes a nearly ideal circumstance for identifying the native source of invasive *S. invicta* by allowing information contained in the unique regional genetic makeups to be exploited. Moreover, such pronounced structure highlights the practical and scientific importance of identifying the source population(s), as it implies that the natural enemies of this pest ant are likely to be locally adapted to their genetically distinct hosts from different areas (see also below). Recognition of the

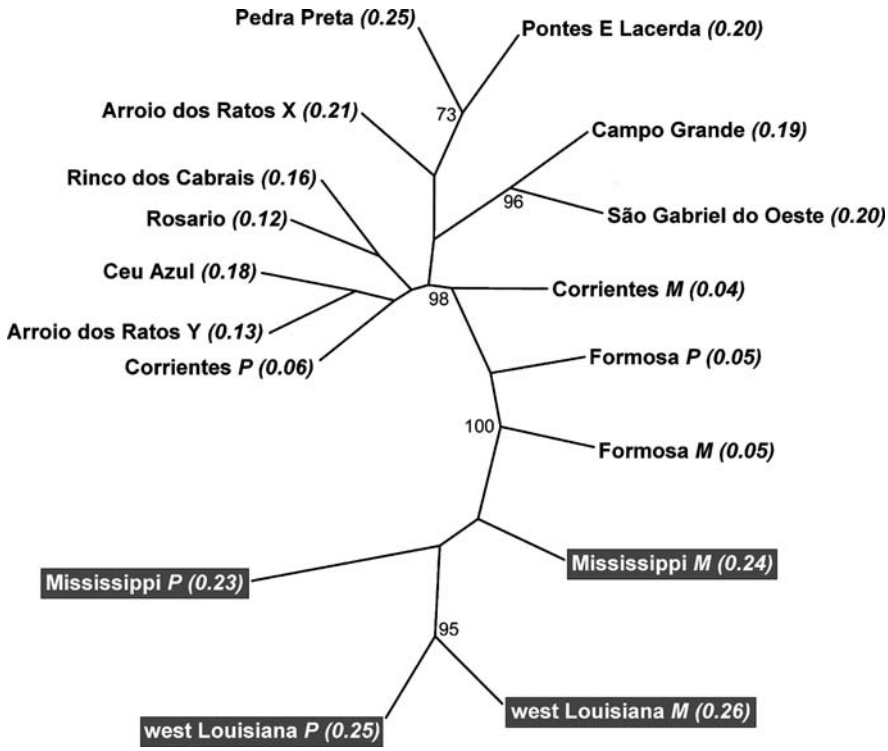

Fig. 7 Neighbor-joining (NJ) consensus tree depicting nuclear genetic relationships of native (black lettering) and introduced (white lettering) populations of *S. invicta* based on Nei's chord distance. Numbers at nodes are percentages of bootstrap replicates (out of 1,000) in which population clusters distal to that node were recovered (only values greater than 70% are shown). Numbers in parentheses correspond to population F_K values generated by STRUCTURE simulations using the nuclear data. *M*, monogyne social form; *P*, polygyne social form

significant differentiation between the two introduced study populations, earlier taken to signify possible multiple invasions of *S. invicta* from the native range, is important in that separate analyses involving each population are thus warranted.

A major result consistently obtained by our analyses is that the likely source population for all invasive *S. invicta* in the USA occurs at or near Formosa, Argentina. Furthermore, while some of our analyses did not provide unequivocal evidence implicating this specific source, virtually every analysis ruled out all sampled Brazilian populations as a potential source. These findings are consistent with speculation that northeastern Argentina is more likely to contain the native source population(s) (Mescher et al. 2003; Ross and Trager 1990) than is the earlier suggested Pantanal Region of southwestern Brazil (Allen and Buren 1974; Buren 1972; Buren et al. 1974). While further work will be needed to conclusively pinpoint the source of the USA colonists, our data clearly are of value in geographically focusing such efforts.

Early collections of *S. invicta* in the USA provide a rather detailed picture of the history of the spread of this ant in the USA, but the question of how the original colonists arrived remains unanswered (Tschinkel 2006). One early hypothesis that the ant was introduced from Brazil (Allen and Buren 1974; Buren 1972; Buren et al. 1974) envisioned a scenario in which colonies from the Pantanal either drifted naturally on floodwaters or were carried by commerce to rivers in one of two major drainage systems, the Amazon River Basin to the north or the La Plata River Basin to the south (see Fig. 2). Ultimately, commercial ships would have carried the ants from ports on either of these waterways to the port of Mobile, Alabama, where they first appeared in the USA. Another scenario proposed by Buren (1972) envisioned natural or anthropogenic movement of colonies southward down the Paraná River (in the La Plata Basin), with eventual transport to the USA from ports near Buenos Aires. Any scenario invoking waterways in the La Plata Basin remains feasible in light of the results of the present study. However, our data further suggest that colonies likely were transported to such a waterway from the flood plains of the Mesopotamia region of northeastern Argentina rather than the flood plains of the Brazilian Pantanal. Mesopotamia is subject to recurrent flooding (as is

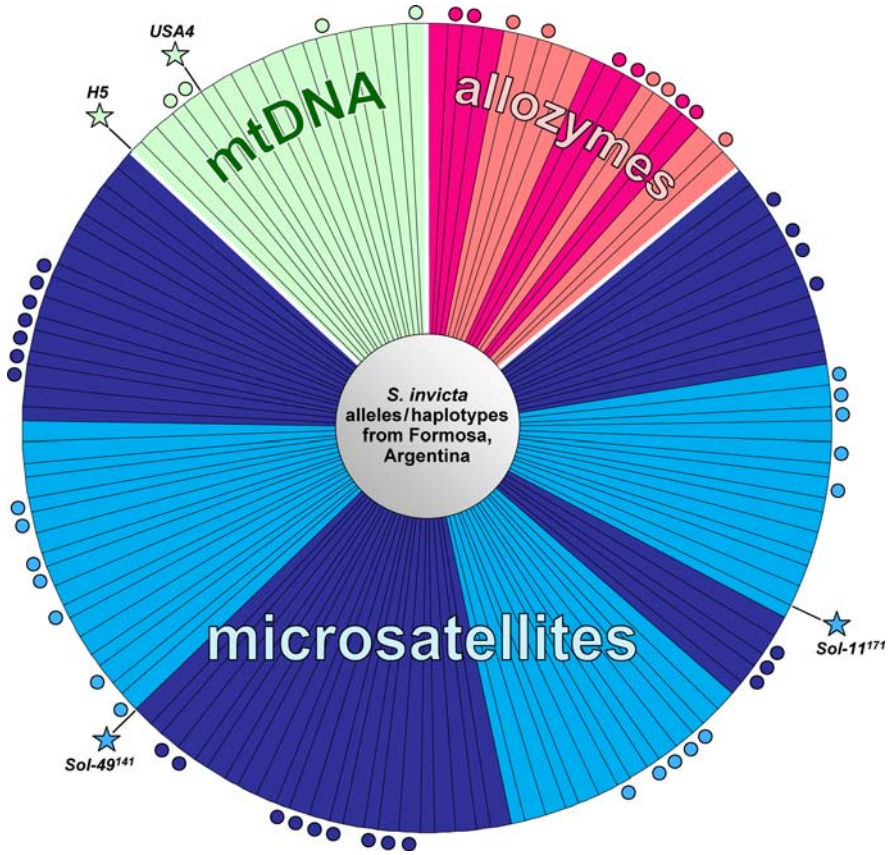

Fig. 8 Genetic variation in native Formosa and introduced USA populations of *S. invicta*. Variants detected in Formosa are shown as pie slices, with the nuclear loci and alleles ordered as in Appendix II (the allozyme locus *Gpi* is excluded because it is monomorphic for the same allele in Formosa and the USA). Variants detected in the introduced range are shown outside the pie perimeter, with stars indicating the two microsatellite alleles and two mtDNA haplotypes found in the USA but not Formosa

the Pantanal), an important characteristic posited for the source region by Buren (1972).

Our results are highly relevant to programs intended to control invasive populations of *S. invicta* using natural enemies. Strong genetic differentiation among native populations of *S. invicta* suggests that its various natural enemies, such as parasitic phorid flies, are likely to be locally adapted to their geographically unique ant hosts. The success of such enemies in attacking *S. invicta* thus may depend on genotype matching between the enemies and the ants (Kaltz and Shykoff 1998; Laine 2005; Lively and Dybdahl 2000; Lively et al. 2004; Thrall et al. 2002), and the search for sustainable biological control agents is likely to be more effective if they are collected from the specific local variants of *S. invicta* that were introduced into the USA. Several lines of evidence suggest the possibility of such genotype matching. First, current success in rearing and propagating South American phorid flies on fire ants in the USA is highly variable across trials (Morrison and Porter 2005; Porter and Alonso 1999; Porter et al. 1995, 1997a). Second, two biotypes of the phorid fly *Pseudacteon curvatus* have been shown to differ markedly in their host preferences, with each strongly preferring to attack the fire ant host species on which it was collected (Vazquez et al. 2004). Moreover, the *P. curvatus* biotype most successful in parasitizing *S. invicta* in the USA comes from Formosa, Argentina (Vazquez et al. 2004), the area inferred from our analyses to be the most probable source of these introduced ants. While climate matching between the native and introduced ranges for parasites such as phorids undoubtedly is also an important consideration for choosing potential control agents (Folgarait et al. 2005), this evidence argues that genetic matching of hosts and parasites may be equally or more important. Unfortunately, several studies of the impact of the phorid *P. tricuspis* on introduced *S. invicta* have utilized flies collected from Brazil (e.g., Mehdiabadi and Gilbert 2002; Mehdiabadi et al. 2004; Pereira and Porter 2006), making it more

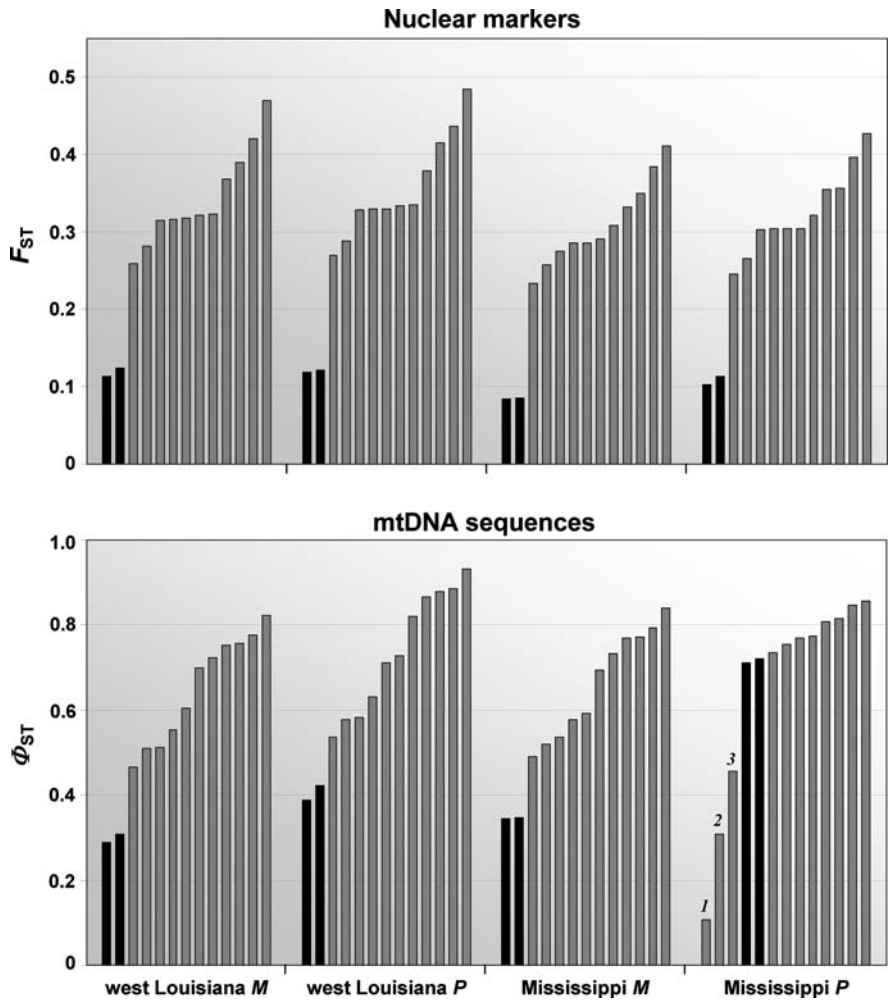

Fig. 9 Estimates of F_{ST} (nuclear markers) and Φ_{ST} (mtDNA sequences) between paired native and introduced populations of *S. invicta*. Values for comparisons between the introduced ants and the native Formosa, Argentina populations are shown in black. Numbers in the Φ_{ST} graph for the Mississippi *P* population indicate comparisons to the Corrientes *M* (1), Rosario (2), and Corrientes *P* (3) populations. *M*, monogyne social form; *P*, polygyne social form

difficult to judge the potential effectiveness of this species in the biological control of fire ants.

Our results are of special significance because *S. invicta* recently has been inadvertently introduced into a number of regions around the world, including the Carribean, Australia, China, and Taiwan (Buckley 1999; Chen et al. 2006; Davis et al. 2001; Huang et al. 2004; MacKay and Fagerlund 1997; McCubbin and Weiner 2002). The extensive dataset we have generated provides necessary baseline information for researchers studying *S. invicta* in any area to readily make initial diagnoses concerning the regional source of the invaders, and then to implement subsequent fine-scale sampling or collection of natural enemies in the implicated region. We expect that such efforts will become increasingly necessary as *S. invicta* continues to be spread globally through commerce.

A recent detailed study of the population genetic structure of introduced *S. invicta* in the USA (Shoemaker et al. 2006b) concluded that at least two introductions may have occurred, one near the presumed original site of entry (Mobile, Alabama) more than 70 years ago, and a second near Port Arthur, Texas at a more recent point. It might be expected that any such secondary introduction involved ants from a different location in the native range that served as the primary source of introduction, yet our data consistently implicate Formosa, Argentina as the native population most closely allied genetically to ants derived from both introductions (as represented in the Mississippi and west Louisiana samples). In addition to

the various statistical analyses based on the overall information content of diverse nuclear and mtDNA markers pointing to this conclusion, the unique possession of an atypical interrupted-repeat microsatellite allele in both the Formosa and USA populations constitutes strong corroborating evidence. Furthermore, some added support comes from recent sequence analyses of the candidate social behavior gene *Gp-9* in introduced and native *S. invicta* (Gotzek et al. 2007; Krieger and Ross 2002); only one allele from the USA was found as well in South America, in ants from Formosa and a second Argentine locality between Formosa and Rosario.

Although these various results implicate Formosa as the source region of *S. invicta* in the USA, inspection of our mtDNA haplotype phylogeny suggests the possibility of a more nuanced picture. Most haplotypes from the USA belong to clades 3 and 4, and they either are found exclusively in Formosa or are nearly identical to haplotypes found only there (Fig. 6). However, a single haplotype in clade 2 (H5) found in both introduced populations was not recovered from Formosa ants, but does occur in ants collected from the nearby locality of Corrientes and the more southerly Argentine locality of Rosario. Indeed, all of the clade 2 haplotypes are known only from these latter two localities. While it is possible that clade 2 haplotypes such as H5 occur in Formosa but simply were missed due to sampling error, a perhaps more likely possibility is that the actual source locality lies in an unsampled part of the Mesopotamia region close to both Corrientes and Formosa. The absence of two microsatellite alleles in Formosa that are found in the USA may also support this scenario, although these alleles were not detected anywhere in Argentina (see Appendix II). Despite this uncertainty, one consistent result across our analyses is that all of the sampled Brazilian populations can be excluded as potential source populations. More extensive collections from northeastern Argentina and Paraguay, combined with the use of additional genetic markers currently under development, must now be employed to refine our hypotheses concerning the origin of invasive *S. invicta* in the USA.

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Appendices

Appendix I Locations of sampled populations of *S. invicta*

City	Province or State	Country	Code	*N*	Latitude	Longitude
Hurley	Mississippi	USA	MS	125	30°65′17″ N	88°49′15″ W
De Quincy	Louisiana	USA	wLA	135	37°06′25″ N	93°47′35″ W
Corrientes	Corrientes	Argentina	Cor	79	27°34′09″ S	58°50′23″ W
Formosa	Formosa	Argentina	For	70	26°09′34″ S	58°09′57″ W
Rosario	Santa Fe	Argentina	Ros	42	32°54′15″ S	60°47′13″ W
Ceu Azul	Paraná	Brazil	CeA	83	25°08′30″ S	53°53′56″ W
Pedra Preta	Mato Grosso	Brazil	PeP	62	16°42′42″ S	54°34′22″ W
Pontes E Lacerda	Mato Grosso	Brazil	PEL	30	15°11′27″ S	59°17′20″ W
Campo Grande	Mato Grosso do Sul	Brazil	CaG	43	20°21′10″ S	54°34′22″ W
Rinco dos Cabrais	Rio Grande do Sul	Brazil	RdC	81	29°43′60″ S	52°57′00″ W
Arroio dos Ratos	Rio Grande do Sul	Brazil	AdR	44	30°08′21″ S	51°30′11″ W
Sáo Gabriel do Oeste	Mato Grosso do Sul	Brazil	SGO	31	19°17′24″ S	54°34′22″ W

Codes are population abbreviations used in figures and Appendix II. *N* represents the number of individuals (one per nest) sampled from each population

Appendix II Nuclear allele and mtDNA haplotype frequencies within 17 sampled sites in the USA and South America

	MS *M*	MS *P*	wLA *M*	wLA *P*	PEL	PeP	SGO	CaG	CeA	AdR X	AdR Y	RdC	Ros	Cor *M*	Cor *P*	For *M*	For *P*
Allozyme loci																	
Pgm-1	**60**	**62**	**73**	**59**	**30**	**62**	**31**	**43**	**83**	**27**	**8**	**80**	**42**	**36**	**43**	**35**	**35**
85	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0.023	0	0.014
90	0	0	0	0	0	0.008	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0
96	0.142	0.145	0.034	0.042	0.983	0.621	0.145	0.07	0	0	0.188	0.063	0.119	0.069	0.151	0.171	0.114
98	0	0	0	0	0	0	0	0	0.03	0.37	0	0	0	0	0	0	0
100	0.858	0.855	0.966	0.958	0.017	0.371	0.839	0.93	0.97	0.63	0.813	0.919	0.667	0.903	0.767	0.829	0.857
104	0	0	0	0	0	0	0.016	0	0	0	0	0	0	0	0	0	0.014
107	0	0	0	0	0	0	0	0	0	0	0	0.019	0.214	0.014	0.047	0	0
Acoh-1	**58**	**61**	**70**	**59**	**30**	**62**	**31**	**43**	**82**	**35**	**4**	**81**	**41**	**36**	**43**	**35**	**35**
82	0.086	0.033	0.214	0.093	0	0	0	0	0	0	0	0	0	0	0	0.057	0.071
87	0	0	0	0	0.033	0	0	0	0	0	0	0	0	0	0.012	0	0
92	0	0	0	0	0	0	0	0	0.092	0	0	0	0	0	0	0.029	0
100	0.914	0.967	0.786	0.907	0.967	1	1	1	0.909	0.986	1	1	1	0.986	0.988	0.886	0.929
114	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0	0.014	0
130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0
139	0	0	0	0	0	0	0	0	0	0.014	0	0	0	0	0	0	0
Acoh-5	**62**	**60**	**72**	**59**	**30**	**62**	**31**	**42**	**83**	**35**	**4**	**81**	**42**	**36**	**43**	**35**	**35**
84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0.043
88	0	0	0	0	0	0	0.307	0.202	0.018	0.014	0	0.025	0	0	0	0	0
93	0.234	0.292	0.292	0.195	0.833	0.307	0.355	0.5	0.837	0.986	1	0.877	0.869	0.806	0.861	0.3	0.214
100	0.766	0.708	0.708	0.805	0.167	0.694	0.339	0.298	0.145	0	0	0.099	0.131	0.194	0.14	0.686	0.743
G3pdh-1	**61**	**61**	**73**	**59**	**30**	**60**	**31**	**43**	**81**	**34**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
40	0.41	0.353	0.438	0.424	0	0	0	0	0	0	0	0	0	0	0	0.129	0.143
100	0.59	0.648	0.562	0.576	1	1	1	1	1	1	1	1	1	1	1	0.871	0.857
Aat-2	**62**	**60**	**74**	**59**	**30**	**62**	**31**	**43**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
100	0.927	0.958	0.966	0.949	0	0	0	0	0	0	0	0.068	0.012	0.083	0	0.629	0.443
144	0.073	0.042	0.034	0.051	1	1	1	1	1	0.957	1	0.932	0.988	0.903	1	0.371	0.557
172	0	0	0	0	0	0	0	0	0	0.043	0	0	0	0.014	0	0	0
Gpi	–	–	–	–	**30**	**62**	**31**	**43**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
79	–	–	–	–	0	0	0	0	0	0	0.111	0.025	0	0	0	0	0
88	–	–	–	–	0	0	0	0.023	0	0	0	0	0	0	0	0	0
100	–	–	–	–	1	0.968	1	0.977	1	1	0.889	0.975	1	1	1	1	1
104	–	–	–	–	0	0.032	0	0	0	0	0	0	0	0	0	0	0
Est-2	–	–	–	–	**30**	**62**	**31**	**43**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
58	–	–	–	–	0	0	0	0	0	0	0	0	0	0	0.023	0	0
65	–	–	–	–	0	0	0	0	0	0	0	0	0	0	0.012	0.029	0
68	–	–	–	–	0	0	0	0	0	0	0	0.012	0.012	0	0.012	0	0
73	–	–	–	–	0	0	0	0	0	0	0	0	0	0	0.012	0	0
76	–	–	–	–	0	0	0	0	0	0	0	0	0.048	0	0.012	0	0
81	–	–	–	–	0	0	0	0	0.036	0	0	0.006	0	0	0	0	0
88	–	–	–	–	0	0	0	0	0	0.014	0	0.395	0	0	0	0	0
100	–	–	–	–	1	1	0.903	0.988	0.265	0.014	0	0.303	0.917	0.681	0.326	0.957	1
108	–	–	–	–	0	0	0	0	0	0.971	0	0.185	0.024	0	0	0	0
111	–	–	–	–	0	0	0.097	0.012	0.699	0	1	0.099	0	0.319	0.605	0.014	0

Appendix II continued

	MS *M*	MS *P*	wLA *M*	wLA *P*	PEL	PeP	SGO	CaG	CeA	AdR X	AdR Y	RdC	Ros	Cor *M*	Cor *P*	For *M*	For *P*
Microsatellite loci																	
Sol-6	**62**	**63**	**74**	**58**	**30**	**62**	**31**	**45**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
87	0	0	0	0	0	0	0	0	0.265	0	0.056	0.426	0.048	0.139	0.128	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014
97	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0	0	0
107	0	0	0	0	0	0	0.016	0	0	0	0	0	0	0	0	0.014	0
109	0.129	0.111	0.027	0.017	0	0	0	0	0.06	0	0	0	0	0	0	0.014	0.043
111	0	0	0	0	0.017	0.202	0	0	0	0.3	0.167	0.099	0.262	0.014	0.058	0.014	0.014
113	0.734	0.675	0.628	0.759	0.05	0.339	0.048	0.022	0.199	0.057	0.389	0.253	0.214	0.319	0.267	0.414	0.614
115	0.121	0.183	0.345	0.224	0.017	0.298	0.21	0.344	0.392	0.614	0	0.074	0.012	0.194	0.174	0.229	0.157
117	0	0	0	0	0.067	0.161	0	0.122	0.078	0.029	0.278	0.117	0.083	0.153	0.186	0.129	0.086
119	0.016	0.032	0	0	0.083	0	0	0.167	0	0	0	0	0.31	0.042	0.047	0.071	0.029
121	0	0	0	0	0.55	0	0.694	0.322	0.006	0	0.111	0	0.06	0.083	0.058	0	0
123	0	0	0	0	0.133	0	0	0	0	0	0	0	0	0	0.07	0.071	0.029
125	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0.012	0	0
127	0	0	0	0	0	0	0	0	0	0	0	0.031	0	0.028	0	0.014	0.014
129	0	0	0	0	0	0	0.032	0	0	0	0	0	0	0	0	0.014	0
131	0	0	0	0	0.033	0	0	0	0	0	0	0	0.012	0.014	0	0	0
133	0	0	0	0	0	0	0	0.022	0	0	0	0	0	0	0	0	0
135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0
Sol-11	**62**	**63**	**73**	**59**	**30**	**62**	**31**	**45**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
143	0.21	0.183	0.027	0.068	0	0	0	0	0.006	0.071	0	0	0.191	0.014	0.023	0.1	0.029
145	0.04	0.103	0	0	0	0	0.032	0	0	0	0	0.006	0	0	0	0.071	0.086
147	0.169	0.151	0	0.144	0.533	0	0	0.078	0	0.614	0	0	0.036	0.042	0.035	0.114	0.129
149	0	0	0	0	0	0	0	0	0	0.114	0.111	0.469	0.048	0	0.012	0.114	0.1
151	0.427	0.405	0.623	0.661	0.2	0	0.613	0.767	0.669	0.143	0.444	0.198	0.25	0.486	0.581	0.314	0.457
153	0	0	0	0	0.033	0.016	0.016	0.022	0.121	0.057	0	0.222	0.333	0.208	0.174	0.143	0.014
155	0.153	0.159	0.343	0.127	0.017	0.339	0	0	0	0	0	0.025	0.036	0.056	0	0	0.057
157	0	0	0	0	0	0.073	0	0	0	0	0.056	0	0.06	0.042	0.035	0.071	0.071
159	0	0	0	0	0.067	0.008	0	0	0.048	0	0	0.049	0.024	0.069	0.023	0.014	0
161	0	0	0	0	0.033	0.008	0.032	0.1	0.072	0	0.111	0.019	0.024	0.014	0.023	0	0
163	0	0	0	0	0	0	0	0	0.042	0	0	0	0	0.028	0.012	0	0.014
165	0	0	0	0	0	0	0.032	0.022	0	0	0.111	0	0	0.028	0.035	0.014	0
167	0	0	0	0	0.117	0	0.274	0.011	0.042	0	0.167	0.012	0	0.014	0.023	0	0.029
169	0	0	0	0	0	0.057	0	0	0	0	0	0	0	0	0.023	0.043	0
171	0	0	0.007	0	0	0.186	0	0	0	0	0	0	0	0	0	0	0
173	0	0	0	0	0	0.307	0	0	0	0	0	0	0	0	0	0	0
177	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014
189	0	0	0	0	0	0.008	0	0	0	0	0	0	0	0	0	0	0
Sol-18	**62**	**63**	**74**	**59**	**30**	**62**	**31**	**45**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0
121	0	0	0	0	0.05	0.008	0.032	0	0.024	0	0	0	0	0	0	0	0
123	0	0	0	0	0.5	0.774	0.065	0	0	0.714	0.167	0.08	0.107	0.208	0.116	0.043	0.029
125	0.766	0.786	0.919	0.924	0.033	0.218	0.274	0.433	0.843	0.029	0.722	0.883	0.857	0.694	0.744	0.9	0.9
127	0.186	0.151	0.081	0.076	0.15	0	0.629	0.567	0.133	0.257	0.111	0.006	0	0.083	0.093	0.014	0.043
129	0.048	0.064	0	0	0.25	0	0	0	0	0	0	0.031	0	0	0.012	0.043	0
131	0	0	0	0	0.017	0	0	0	0	0	0	0	0.036	0.014	0.012	0	0.029
135	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0

Appendix II continued

	MS *M*	MS *P*	wLA *M*	wLA *P*	PEL	PeP	SGO	CaG	CeA	AdR X	AdR Y	RdC	Ros	Cor *M*	Cor *P*	For *M*	For *P*
Sol-20	**62**	**63**	**74**	**59**	**30**	**62**	**31**	**45**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
114	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0	0	0	0
116	0	0	0	0	0	0	0	0	0	0	0	0.006	0	0	0	0	0
120	0	0	0	0	0	0.016	0	0	0.036	0	0	0.006	0.071	0.014	0.035	0	0.014
122	0	0	0	0	0	0	0	0	0.06	0	0	0.006	0.06	0.069	0.198	0.043	0.029
124	0.25	0.183	0.115	0.212	0.083	0	0.081	0.167	0.753	0.214	0.722	0.29	0.357	0.347	0.384	0.129	0.157
126	0.589	0.5	0.345	0.322	0.367	0.282	0.258	0.3	0.06	0.086	0.111	0.068	0.191	0.139	0.128	0.357	0.429
128	0.161	0.318	0.264	0.085	0.4	0.694	0.194	0.222	0.078	0.1	0	0.099	0.214	0.111	0	0.214	0.129
129	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0	0	0
130	0	0	0	0.009	0.033	0.008	0.468	0.311	0	0	0	0.019	0	0.139	0.058	0.1	0.071
131	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0	0	0
132	0	0	0	0	0	0	0	0	0	0	0	0.056	0	0.014	0.035	0.1	0.071
133	0	0	0	0	0	0	0	0	0	0.429	0	0	0	0	0	0	0
134	0	0	0	0	0	0	0	0	0	0	0	0.037	0	0.028	0.012	0	0
135	0	0	0	0	0	0	0	0	0	0.086	0	0	0	0	0	0	0
136	0	0	0.014	0	0	0	0	0	0	0	0.056	0.025	0.06	0	0.047	0.014	0
138	0	0	0	0	0.1	0	0	0	0	0.057	0	0.241	0.048	0.014	0.023	0	0.014
140	0	0	0	0	0.017	0	0	0	0	0.029	0.111	0.124	0	0.028	0.047	0.014	0.014
142	0	0	0	0	0	0	0	0	0.012	0	0	0.012	0	0	0.012	0.014	0.043
144	0	0	0.264	0.348	0	0	0	0	0	0	0	0	0	0.014	0	0	0
146	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0.014
148	0	0	0	0	0	0	0	0	0	0	0	0	0	0.056	0	0	0.014
150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0
152	0	0	0	0.025	0	0	0	0	0	0	0	0	0	0	0.012	0	0
Sol-42	**62**	**63**	**74**	**59**	**30**	**62**	**31**	**45**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
91	0	0	0	0	0	0	0	0	0	0	0.111	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.023	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0	0	0.028	0	0	0
103	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0.035	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0.012	0	0
107	0	0	0	0	0.067	0	0.016	0	0	0.071	0	0.025	0.012	0.208	0.244	0.029	0.014
109	0	0	0	0	0	0	0	0	0.006	0	0.056	0.117	0.143	0.139	0.093	0.014	0.014
111	0	0	0	0	0	0	0	0.133	0.115	0.114	0.056	0.025	0.214	0.097	0.035	0	0.029
113	0	0	0	0	0	0	0.032	0.1	0.036	0.029	0	0.124	0	0.042	0.035	0.114	0.029
115	0	0	0	0	0	0	0.274	0.278	0	0.029	0.056	0.006	0.012	0.042	0.116	0.029	0.043
117	0.323	0.294	0.297	0.466	0.133	0	0.113	0.044	0.205	0.143	0	0.019	0	0.083	0.105	0.129	0.114
119	0.113	0.103	0.169	0.22	0	0.016	0.307	0.089	0.06	0.129	0.111	0.099	0.095	0.028	0.058	0.114	0.171
121	0.161	0.079	0.088	0.059	0.017	0.008	0.048	0.044	0.042	0.243	0	0.191	0.095	0.069	0.058	0.086	0.157
123	0	0	0	0	0.033	0.04	0.21	0.089	0.277	0.1	0.056	0.13	0.048	0.014	0.047	0.114	0.1
125	0.016	0.056	0	0.009	0.05	0	0	0.133	0.012	0.029	0.222	0.099	0.036	0.014	0.047	0.043	0.014
127	0	0.008	0	0	0.033	0.04	0	0.067	0.054	0.029	0.111	0.062	0.024	0.056	0.047	0.071	0.057
129	0	0.024	0	0	0	0.161	0	0	0.012	0	0.056	0.093	0.071	0.028	0	0.029	0
131	0.226	0.325	0.284	0.161	0.017	0.516	0	0	0.042	0.043	0	0.006	0.012	0.042	0.012	0.071	0.043
133	0	0	0	0	0.1	0.129	0	0	0.024	0	0	0	0	0.028	0	0.014	0.014
135	0	0	0	0	0.017	0	0	0	0.084	0.043	0	0.006	0.071	0.014	0.012	0	0.014
137	0	0	0	0	0	0.089	0	0	0	0	0.167	0	0.06	0.014	0.012	0.014	0.029
139	0	0	0	0	0.183	0	0	0	0	0	0	0	0.083	0	0	0.029	0.014

Appendix II continued

	MS *M*	MS *P*	wLA *M*	wLA *P*	PEL	PeP	SGO	CaG	CeA	AdR X	AdR Y	RdC	Ros	Cor *M*	Cor *P*	For *M*	For *P*
141	0	0	0	0	0.2	0	0	0.022	0	0	0	0	0	0.028	0	0.014	0.057
143	0.153	0.095	0.155	0.085	0	0	0	0	0	0	0	0	0.024	0	0	0.014	0.014
145	0.008	0.016	0.007	0	0	0	0	0	0	0	0	0	0	0.014	0.012	0.029	0.043
147	0	0	0	0	0.033	0	0	0	0	0	0	0	0	0	0	0	0
149	0	0	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0.014	0.014
151	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.029	0.014
159	0	0	0	0	0.017	0	0	0	0.03	0	0	0	0	0	0	0	0
Sol-49	**62**	**63**	**73**	**59**	**30**	**62**	**31**	**45**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
141	0.129	0.127	0	0	0.883	0.532	0.048	0	0	0	0	0	0	0	0	0	0
142	0	0	0.096	0.085	0	0	0	0	0	0	0	0	0	0.028	0.023	0.086	0.057
146	0	0	0	0	0	0	0	0	0	0.1	0.111	0	0	0	0.012	0	0.014
148	0.073	0.064	0.151	0.22	0	0	0	0	0	0	0	0	0	0	0	0.029	0.043
150	0	0	0	0	0	0	0.113	0.111	0	0	0.111	0.049	0.238	0.069	0.047	0.029	0.029
152	0	0	0	0	0	0	0.032	0	0	0.057	0	0.043	0.012	0	0.047	0.114	0.043
154	0	0	0	0	0	0	0.452	0.289	0.169	0	0	0.006	0.012	0.097	0.047	0.071	0.057
156	0	0.008	0	0	0	0.073	0.032	0	0.133	0.3	0	0.049	0	0.042	0.081	0.043	0.057
158	0	0	0	0	0	0.008	0	0	0.06	0.229	0.111	0.148	0.024	0.111	0.105	0.029	0.071
160	0.444	0.516	0.404	0.373	0	0.024	0.016	0.222	0.241	0.014	0	0.142	0.036	0.167	0.093	0.071	0.214
162	0.145	0.151	0.021	0.042	0.033	0.024	0.048	0.078	0.024	0.086	0.167	0.049	0.191	0.125	0.186	0.057	0.129
164	0	0	0	0	0.05	0.323	0.048	0.189	0.018	0.029	0.222	0.117	0.06	0.139	0.105	0.114	0.086
166	0.21	0.135	0.322	0.28	0.017	0.016	0	0.022	0.072	0.029	0	0.08	0.131	0.042	0.128	0.143	0.086
168	0	0	0.007	0	0.017	0	0	0	0.09	0.014	0.278	0.031	0.024	0.069	0.07	0.057	0.029
170	0	0	0	0	0	0	0	0.011	0.084	0.1	0	0.167	0.262	0.056	0.023	0.086	0.029
172	0	0	0	0	0	0	0.016	0	0.102	0	0	0.062	0.012	0.028	0.023	0.014	0
174	0	0	0	0	0	0	0.145	0.056	0.006	0.014	0	0.031	0	0.014	0	0.043	0.029
176	0	0	0	0	0	0	0	0.022	0	0	0	0.025	0	0	0.012	0.014	0.029
178	0	0	0	0	0	0	0.016	0	0	0	0	0	0	0.014	0	0	0
180	0	0	0	0	0	0	0	0	0	0.029	0	0	0	0	0	0	0
182	0	0	0	0	0	0	0.016	0	0	0	0	0	0	0	0	0	0
184	0	0	0	0	0	0	0.016	0	0	0	0	0	0	0	0	0	0
Sol-55	**62**	**63**	**72**	**59**	**30**	**62**	**31**	**45**	**83**	**35**	**9**	**81**	**42**	**36**	**43**	**35**	**35**
145	0	0	0	0	0	0	0	0	0.09	0	0	0	0	0	0.058	0	0.014
147	0	0	0	0	0	0	0	0.056	0.03	0	0.167	0.006	0.214	0.069	0.093	0.014	0.257
149	0.419	0.77	0.597	0.636	0	0.008	0	0.044	0	0.086	0	0.006	0	0.097	0.267	0.114	0.1
151	0.04	0.008	0.063	0.042	0.067	0	0.113	0.011	0.127	0	0	0.068	0.048	0.222	0.058	0.1	0.143
152	0.105	0.024	0.097	0.076	0	0	0	0	0	0	0	0	0	0	0	0.029	0
153	0.04	0.056	0.007	0	0.05	0.557	0.145	0.178	0.398	0.557	0.611	0.21	0.155	0.222	0.174	0.257	0.171
155	0.137	0.04	0.049	0.085	0.233	0.218	0.645	0.511	0.115	0.114	0.111	0.154	0.345	0.069	0.105	0.071	0.071
157	0.032	0.024	0.007	0.025	0.017	0	0	0.178	0.163	0	0	0	0.083	0.056	0.012	0.071	0.014
159	0.226	0.079	0.181	0.136	0.017	0	0	0.011	0.03	0.014	0	0.161	0.012	0.056	0.081	0.114	0.129
161	0	0	0	0	0.033	0.073	0	0	0.006	0.1	0	0.235	0.107	0.111	0.128	0.071	0.043
163	0	0	0	0	0.433	0.024	0.097	0.011	0.042	0.086	0	0.006	0.012	0.056	0.012	0.043	0.029
165	0	0	0	0	0.133	0.024	0	0	0	0	0.056	0.117	0.012	0	0.012	0.043	0.014
167	0	0	0	0	0	0	0	0	0	0.014	0	0.031	0	0	0	0.029	0.014
169	0	0	0	0	0.017	0	0	0	0	0	0	0	0	0.028	0	0.014	0
171	0	0	0	0	0	0	0	0	0	0	0	0.006	0	0	0	0	0
173	0	0	0	0	0	0	0	0	0	0.014	0	0	0	0.014	0	0.014	0

Appendix II continued

	MS *M*	MS *P*	wLA *M*	wLA *P*	PEL	PeP	SGO	CaG	CeA	AdR X	AdR Y	RdC	Ros	Cor *M*	Cor *P*	For *M*	For *P*
175	0	0	0	0	0	0	0	0	0	0.014	0	0	0	0	0	0.014	0
177	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0	0	0
181	0	0	0	0	0	0	0	0	0	0	0.056	0	0	0	0	0	0
183	0	0	0	0	0	0.048	0	0	0	0	0	0	0	0	0	0	0
187	0	0	0	0	0	0.008	0	0	0	0	0	0	0	0	0	0	0
189	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0
mtDNA clade	**61**	**63**	**72**	**31**	**28**	**50**	**18**	**31**	**64**	**33**	**9**	**55**	**41**	**31**	**21**	**20**	**16**
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0.05	0.841	0.014	0.032	0	0	0	0.032	0	0	0	0	0.78	0.71	0.381	0	0
3	0.148	0	0.222	0	0	0	0	0	0	0	0	0.018	0.195	0.226	0.619	0.3	0.063
4	0.803	0.159	0.764	0.968	0	0	0.125	0.742	0	0	0	0	0	0.065	0	0.7	0.813
5	0	0	0	0	0.179	0.02	0.833	0.097	0	0	0	0	0.024	0	0	0	0
6	0	0	0	0	0.821	0.98	0.056	0.129	1	0	0	0	0	0	0	0	0.125
7	0	0	0	0	0	0	0	0	0	1	0	0.145	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	1	0.836	0	0	0	0	0

Sample sizes [number of individuals (=nests)] are indicated separately for the nuclear loci and mtDNA in bold

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