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Research article

Population genetic structure of the red imported fire ant, *Solenopsis invicta*, in Taiwan

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Abstract. We generated and analyzed microsatellite genotypic data and mtDNA sequence data from the fire ant *Solenopsis invicta* collected from two separate infested areas (Taoyuan and Chiayi) in Taiwan to infer the population and colony structure of these recently established populations. These genetic analyses revealed the following patterns: 1) Relatedness among worker nestmates was significantly greater than zero for both social forms from both populations; 2) No significant isolation by distance was found among nests within each social form from either population; 3) Significant mtDNA but no nuclear differentiation occurs between sympatric social forms in Taoyuan; 4) Molecular signatures of genetic bottlenecks associated with recent introductions are evident in both populations; and 5) The two sampled populations, Taoyuan and Chiayi, are highly genetically differentiated at both the nuclear or mtDNA genomes and most likely derive from two separate introductions into Taiwan. While results from these analyses generally were consistent with predictions based on the known biology of these ants and similar studies of *S. invicta* in the U.S.A. and South America, some patterns likely reflect the recent introduction and human-mediated inadvertent transport of ants in Taiwan. This is the first study to investigate the population and colony structure of fire ants in Taiwan and results from our study represent an important contribution to the ongoing efforts aimed at eradicating this invasive pest in Taiwan.

Keywords: Solenopsis invicta, control strategy, fire ants, invasive species, population structure.

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Introduction

Invasive species pose major threats to agricultural and natural environments and to the public health (Porter and Savignano, 1990; Williams and Porter, 1994; Lee, 2002; Sax et al., 2005). The negative impact of invasive species on agricultural systems is evident from the fact that a vast number of major pest insects in the U.S.A. are of exotic origin (Carruthers, 2003). Because many invasive species occupy human-disturbed habitats and are often found in areas of high commercial activity, the importance of invasive species is an issue of growing concern given increasing human activities, such as habitat destruction and international trade and transportation (Drake et al., 1989; Sax et al., 2005). Despite these negative impacts and increasing concerns, studies of invasive species offer unique opportunities to the study of evolutionary genetic processes as well as the genetic and evolutionary consequences of invasions (Ross et al., 1993; Tsutsui and Case, 2001; Giraud et al., 2002; Lee, 2002; Jaquiéry et al., 2005). For instance, because the history of many invasions are well documented and have occurred in recent times, inferences drawn from patterns of variation often are not confounded by historical processes. Further, the introduction of species into novel environments provides ideal scenarios for studying adaptation to novel environments, effects of bottlenecks on genetic diversity, and genetic changes occurring during invasion and spread (Sax et al., 2005). Also, comparative studies across species or within

species that have been introduced to more than one location can increase our understanding of what characteristics of these species are important for invasion success (Lee, 2002; May et al., 2006).

Many social insects, particularly ants, are well known as being important invasive pest species. Indeed, the invasive potential and abilities of ants to colonize almost every available habitat is such that they often are referred to as 'tramp species' (Hölldobler and Wilson, 1990; Vander Meer et al., 1990). One of the most notorious invasive ant species is the red imported fire ant (RIFA) *Solenopsis invicta* Buren (Lofgren, 1986; Jouvenaz, 1990; Patterson, 1994; Orr, 1996). This exotic pest was inadvertently introduced into the U.S.A. from South America about 75 years ago. Since that time, it has spread throughout the southern states in the U.S.A. *S. invicta* is of profound economic importance in the U.S.A. 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 negatively affects populations of native ants and other ground-dwelling animals (Lofgren et al., 1975; Lofgren, 1986; Porter and Savignano, 1990; Allen et al., 1994, 1998, 2000, 2001; Vinson, 1994, 1997; Giuliano et al., 1996; Carroll and Hoffman, 2000; Gotelli and Arnett, 2000; Kaspari, 2000; Kemp et al., 2000; Kopachena et al., 2000; Eubanks, 2001; Forys et al., 2001; Wojcik et al., 2001; Morrison, 2002). These pest attributes of *S. invicta* presumably are attributable largely to the fact that the introduced populations have almost none of the competitors and natural enemies that normally act to suppress their populations, with the effect that population densities in the U.S.A. are orders of magnitude greater than in the native South American range (Porter et al., 1992, 1997; Morrison, 2000).

More recently, *S. invicta* has been inadvertently introduced to other regions of the world including several western states (e.g., New Mexico, California), the Carribean, Australia, mainland China, and Taiwan (MacKay and Fagerlund, 1997; Buckley, 1999; Davis et al., 2001; Nattrass and Vanderwoude, 2001; McCubbin and Weiner, 2002; Huang et al., 2004; Chen et al., 2006). In all of these areas, *S. invicta* is considered a significant pest such that its negative agricultural, ecological, and public health impacts can only be expected to intensify in the near future.

While an extensive body of literature exists on studies of *S. invicta* from native South American and introduced populations in the U.S.A., few studies have been conducted on *S. invicta* in these more recently invaded areas (Henshaw et al., 2005; Chen et al., 2006). Such studies are of interest not only for development of effective integrated management strategies for control and eradication of fire ants in these areas, but also provide the opportunity for comparative studies of the genetic changes associated with or occurring during separate invasions (Ross et al., 1993; Tsutsui and Case, 2001; Giraud et al., 2002; Jaquiéry et al., 2005). To that end, we present here the results of a study exploring patterns of nuclear (microsatellite) and of mtDNA variation in the fire ant *S. invicta* from two separate infested areas in Taiwan (Taoyuan and Chiayi counties; Fig. 1). While the occurrence of *S. invicta* in Taiwan was first reported in 2003, analyses of the size and distribution of colonies suggest that this species likely arrived some 3–5 years earlier (Huang et al., 2004; Chen et al., 2006).

Previous studies also have shown that both known social forms of *S. invicta* are present in both of the infested areas in Taiwan (Huang et al., 2004; Chen et al., 2006). Two distinct types of colony social organization occur within this species (Glancey et al., 1973). Colonies of the monogyne (**M**) form possess a single egg-laying queen, whereas colonies of the polygyne (**P**) form possess several to hundreds of these queens. Association studies have shown that social form in this and other fire ant species depends on the presence within a colony of specific coding region variants of the single gene *Gp-9* (reviewed in Gotzek and Ross, 2007). Two classes of variants, designated as *B*-like and *b*-like alleles occur in *S. invicta.* Monogyne colonies contain only the *B*-like allele whereas polygyne colonies invariably contain the *b*-like allele as well as the *B*-like allele (Ross, 1997; Ross and Keller, 1998; Krieger and Ross, 2002). The two social forms of *S. invicta* differ not only in colony queen number and *Gp-9* genotypes but also in important features of their reproductive and dispersal behaviors, which are expected to have a number of important effects on the distribution of genetic variation at various spatial scales, as well as management strategies employed for eradication. Thus, knowledge of distribution of the two social forms is critical to constructing an effective predictive model of their spread and expansion and assessing the potential of successful eradication of *S. invicta* (Drees and Vinson, 1990). Finally, previous studies have shown that the unique social and breeding biology of each social form can act as strong constraints on particular routes of interform gene flow (Shoemaker and Ross, 1996). Specifically, the selective elimination of queens of the alternate social form by workers (Keller and Ross, 1998; Vander Meer and Porter, 2001; Krieger and Ross, 2002; Ross and Keller, 2002), and the high proportion of sterile diploid males in **P** nests in introduced areas (Ross and Fletcher, 1985a, 1986; Krieger et al., 1999) severely restrict female-mediated gene flow and limit male-mediated interform gene flow mostly to instances involving **M** males mating with **P** females (Ross and Shoemaker, 1993, 1997; Shoemaker and Ross, 1996; Shoemaker et al., 2006). Therefore, the presence of both social forms in a novel environment presents the opportunity to test the generality of these patterns observed in earlier studies in the introduced range in the U.S.A.

This study has several objectives. First, we wished to learn whether the current distribution of fire ants in these two areas is the result of a single or separate introductions into Taiwan. The finding of multiple introductions may indicate that the island of Taiwan is at high risk of experiencing additional introductions. Second, we aimed to infer whether one social form was possibly derived from local populations of the alternate social form as well as whether substantial gene flow occurs between sympatric populations of each social form. Lastly, we utilized our genetic data to assess whether a genetic signature characteristic of a recent population bottleneck is evident in either of two areas infested by *S. invicta.* While data examining the alternative colony genetic structure and associated genetic differences between native and introduced fire ant populations have been proposed for the period of several decades after their first introduction to the U.S.A. (Ross and Fletcher, 1985b; Ross et al., 1996; Ross et al., 1999), the more recent introduction of fire ants into Taiwan provides a novel opportunity to study the genetic changes characteristics of invasiveness of this species over an extremely short time period, particularly in the initial 5–10 years after the original introduction.

Materials and methods

Source of ant samples

Ants representing both social forms were collected from two locations, Taoyuan county and Chiayi county, in Taiwan (these two locations are separated by ca. 250 km). Initially, the social form of *S. invicta* was determined by visual inspection of the average size of the workers, the height of the mounds, and the number of queens (Greenberg et al., 1985). Based on the preliminary determinations of social form in the field, adult workers were collected from nests of each putative social form separated by distances of 30–5000 (polygyne) and 600–2000 m (monogyne) (Fig. 1). In total, we sampled 30 colonies from Taoyuan county and 25 colonies from the Chiayi county. Collected workers were stored in 95% alcohol and frozen at -20 °C.

DNA extractions and determination of social form

Two separate sets of total genomic DNA were extracted from individuals sampled from each nest. For the first set, 10–15 workers were pooled and extracted in bulk using the Viogene DNA extraction kit (Viogene, Inc., Taipei, Taiwan). These bulk-extracted samples were used in *Gp-9* assays for social form determination (Valles and Porter, 2003). For the second set, total genomic DNA was extracted from 10 individual workers from each sampled nest. These samples were used as source DNA for microsatellite genotyping and mtDNA sequencing (single individual per nest; see below). Inspection of the distribution of multilocus genotypes from 10 individuals per sampled nest provides a powerful and direct approach for determining the social form of each nest.

Microsatellite analyses

We genotyped 10 workers from each colony, representing 550 individuals in total, at six dinucleotide-repeat microsatellite loci (Table 1) previously developed for *S. invicta* (Table 1; Krieger and Keller, 1997). Amplification of the single locus *Sol-20* was performed in 10-µl reactions with 30–100 ng DNA, 1 µl of 10X Super-Therm Gold PCR buffer, 1 µl of 2.5 mM dNTPs, 0.3 µl of a 10 µM FAM-labeled forward primer, 0.3 µl of a 10 µM unlabeled reverse primer, and 0.15 U of Super-Therm Gold Hot-start *Taq* DNA polymerase (Applied Biosystems). The remaining five loci were amplified in two separate multiplex PCR reactions. In the first reaction, *Sol-11*, *Sol-42,* and *Sol-49* were amplified simultaneously in a single tube. Reaction cocktails and conditions for *Sol-11*, *Sol-42,* and *Sol-49* were identical to those described earlier (Ross et al., 2003), except forward primers were labeled with FAM. In the second multiplex reaction, *Sol-6* and *Sol-55* were co-amplified in a single 20-µl reaction with 30–100 ng DNA, 2.4 µl of 10X Super-Therm Gold PCR buffer, 1.6 µl of 2.5 mM dNTPs, 0.5 µl of a 10 µM FAM-labeled *Sol-6* forward primer, 0.5 µl of a 10 µM unlabeled *Sol-6* reverse primer, 0.6 µl of a 10 µM FAM-labeled *Sol-55* forward primer, 0.6 µl of a 10 µM unlabeled *Sol-55* reverse primer, and 1U of Super-Therm Gold Hot-start *Taq* DNA polymerase. PCR amplifications were carried out on an ABI 9700 thermal cycler (Applied Biosystems) using the following profile: initial denaturation at 95 °C for 10 min; 30 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min; a final extension of 72 °C for 30 min. All PCR products were visualized on an ABI 3100 genetic analyzer using laser detection, and scoring of genotypes was performed using the software GENESCAN 3.1.2 (Applied Biosystems).

MtDNA sequencing

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 from a single individual per nest using the primers C1-J-2195 (Simon et al., 1994) and DDS-COII-4 (Ross and Shoemaker, 1997). PCR reactions were carried out using identical reaction cocktails and thermal cycling profiles described in Ahrens et al. (2005). MtDNA amplicons were purified and used directly in standard fluorescent cycle-sequencing PCR reactions (ABI Prism Big Dye terminator chemistry, Applied Biosystems). Sequencing reactions were subsequently purified and run on an ABI 3730 sequencer. All mtDNA amplicons were sequenced in both directions and corresponding sequence pairs were edited and combined into a single consensus sequence file. All sequences were then aligned by eye using previously published *S. invicta* mtDNA sequence obtained from GenBank (AY950762).

Genetic analyses

The number of alleles, allele frequencies, observed heterozygosity, and expected heterozygosity at each microsatellite locus for both populations were calculated using the program MSA 3.00 (Dieringer and Schlötterer, 2002). Genotype proportions in the two populations were tested for conformity to Hardy-Weinberg expectations (HWE) using exact tests implemented in the program GENEPOP 3.3 (Raymond and Rousset, 1995b). The program DAMBE (Xia and Xie, 2001) was used to determine haplotype counts from the mtDNA sequence data. Genetic data from only one individual per nest was used for the above analyses.

Relatedness of nestmate workers in each form from both populations was estimated from genotype distributions at the six microsatellite loci using the program RELATEDNESS 5.0.8 (Goodnight and Queller, 1999). Triploid individuals were discarded when performing the relatedness calculations (see below). The reference population for relatedness estimation within social forms was defined as either all monogyne or polygyne nests from a given population. For all relatedness calculations, nests were weighted equally and standard errors and their derivative 95% confidence limits were obtained by jackknifing over nests.

Population genetic differentiation was examined in several ways. Pairwise F_{ST} values based on microsatellite and mtDNA sequence data were calculated between sympatric populations of each social form, between the two separate geographic populations, and between nest pairs within in each area using the program FSTAT (Goudet, 2001). Significance of the genetic differentiation measured between pairs of

populations was determined by means of exact tests implemented in GENEPOP 3.3 (Raymond and Rousset, 1995a). Genetic distance values (Nei's D) also were calculated to further assess divergence among populations (Nei, 1972).

F_{ST} values were also estimated simultaneously at two levels (social form, geographic population) using the hierarchical analysis of molecular variance procedure in ARLEQUIN (Excoffier et al., 1992). Statistical significance of the differentiation at each level was established by means of 20,000 data permutations.

We tested for correlations between pairwise F_{ST} values [$F_{ST}/(1-F_{ST})$] and the natural logarithm of pairwise geographic distances among nests from each social form in the two areas, except for monogynous individuals from Chiayi, at all six microsatellite loci using the Mantel test in GENEPOP 3.3 (Raymond and Rousset, 1995b) to determine if there was a significant positive correlation between genetic differentiation and geographic distance indicative of isolation by distance.

We also used an individual-based Bayesian method to statistically recognize distinct genetic clusters of individuals and to infer levels of population admixture from individual multilocus genotypic data (Pritchard et al., 2000; Mank and Avise, 2004). The method assumes a model with K populations, each with characteristic allele frequencies that are estimated while individuals are probabilistically assigned to each population. Prior information such as the geographic location of samples or colony social form also can be incorporated into the model to assist in clustering. The program STRUCTURE (Pritchard et al., 2000; URL: http://pritch.bsd.uchicago.edu) was used to explore the parameter space of each model using Markov chain Monte Carlo (MCMC) algorithms, with competing models evaluated on the basis of the posterior probabilities given the data, model, and prior information. Separate runs of STRUCTURE were performed on samples from each single geographic population only, with different numbers of assumed populations ranging from one to five ($K=1-5$) and on samples from both populations combined into a single dataset ($K=1-10$). Separate runs were performed using the nuclear data only and the nuclear and mtDNA data combined. All simulations used 100,000 MCMC iterations in the burnin phase and 300,000 iterations in the data collection phase, with four independent runs conducted on each set of data and parameter values. No prior information regarding social form or population of origin were included and all analyses were run assuming correlated allele frequencies. We ensured accurate estimates of the simulation values by checking that model parameters equilibrated before the end of the burnin phase and that the posterior probabilities were consistent across the four runs for each data and parameter set. The estimated membership coefficients (degree of admixture) of individuals, and their averages for each of the populations, were obtained from the simulations yielding the preferred estimates of numbers of distinct clusters, as determined by the method of Evanno et al. (2005). Again, for all of the analyses of genetic differentiation, we utilized the multilocus genetic data from only a single individual per sampled nest.

One signature of a recent population bottleneck is reduction in allelic diversity but not observed heterozygosity, simply because such population bottlenecks tend to reduce allelic number substantially more than genetic diversity at polymorphic loci (Luikart et al., 1998). We used the program BOTTLENECK (Cornuet and Luikart, 1996; Piry et al., 1999) to identify whether a mode-shift distortion of allele frequency distributions characteristic of recent bottlenecks was evident for either of the two recently established fire ant populations in Taiwan, using the infinite allele model (IAM) and the two phase model (TPM). Significance was assessed by the sign, standardized differences, and Wilcoxon tests. Additionally, we also compared N_A and H_E for the populations from South America (Ross et al., 2007), the U.S.A. (Shoemaker et al., 2006) and Taiwan and examined the bottleneck effect in Taiwanese populations if results showed N_A similar between U.S.A. and Taiwan but considerably lower than those found in South America.

The proportions of triploid individuals from **P** and **M** colonies of the two infested areas in Taiwan were estimated from the proportion of individuals harboring three different alleles at one or more microsatellite locus. We repeated PCR amplification and genotyping of the microsatellite loci for all individuals in which three alleles at any locus was detected to confirm they indeed were triploid. Triploid individuals were not used for any of the population genetic analyses above. Instead, we randomly selected one diploid individual from the same nest for all population genetic analyses.

Results

Distribution of the monogyne and polygyne social forms

All nests putatively identified as monogyne in the field were identified as such by examining genotype distributions for the 10 nestmate females surveyed at the six microsatellites and by detecting only the $Gp\text{-}9^B$ allele among these workers. Also, all putative polygyne nests were confirmed as such by inspection of genotype distributions of 10 nestmate offspring females for departure from those expected for offspring in monogyne nests (single female mated to a single male) surveyed at six microsatellite loci and/or by detecting individuals bearing the $Gp\text{-}9^b$ allele. In every case the two complementary approaches to determining social form were consistent in classifying nests as either monogyne or polygyne. The preliminary analyses show that the distribution of the two social forms is quite different in the two infested areas (Fig. 1). In Taoyuan, townships centrally located in the county (Fig. 1b) harbor a higher proportion of **P** colonies (**P:** 60 %), whereas, surrounding townships (Fig. 1b) are infested more commonly by ants of the **M** social form (**M:** 76.5 %). In contrast, the large infested area in Chiayi consists mostly of **P** nests (92 % of colonies collected; Fig. 1c).

Genetic diversity

From one to five alleles were found at each microsatellite locus (23 and 26 alleles in the Taoyuan and Chiayi populations, respectively; Table 1). A total of seven private alleles (i.e., unique to a single population) across all six microsatellite loci were found. Tests for conformity of genotype proportions to Hardy-Weinberg expectations revealed only one significant deviation involving *Sol-11* in the Taoyuan polygyne population (Table 1). Score tests reveal that this significant departure resulted from a deficiency of heterozygotes.

Analyses of the mtDNA sequence data revealed only three unique variants occur in Taiwan (designated haplotypes H5, H22, and H36; see Shoemaker and Ross, 1996), all of which are commonly observed in fire ants from the U.S.A. Individuals from the Taoyuan population harbored either the H36 or H5 mtDNA haplotype whereas all individuals from the Chiayi population bear mtDNA haplotype H22 (Fig. 1).

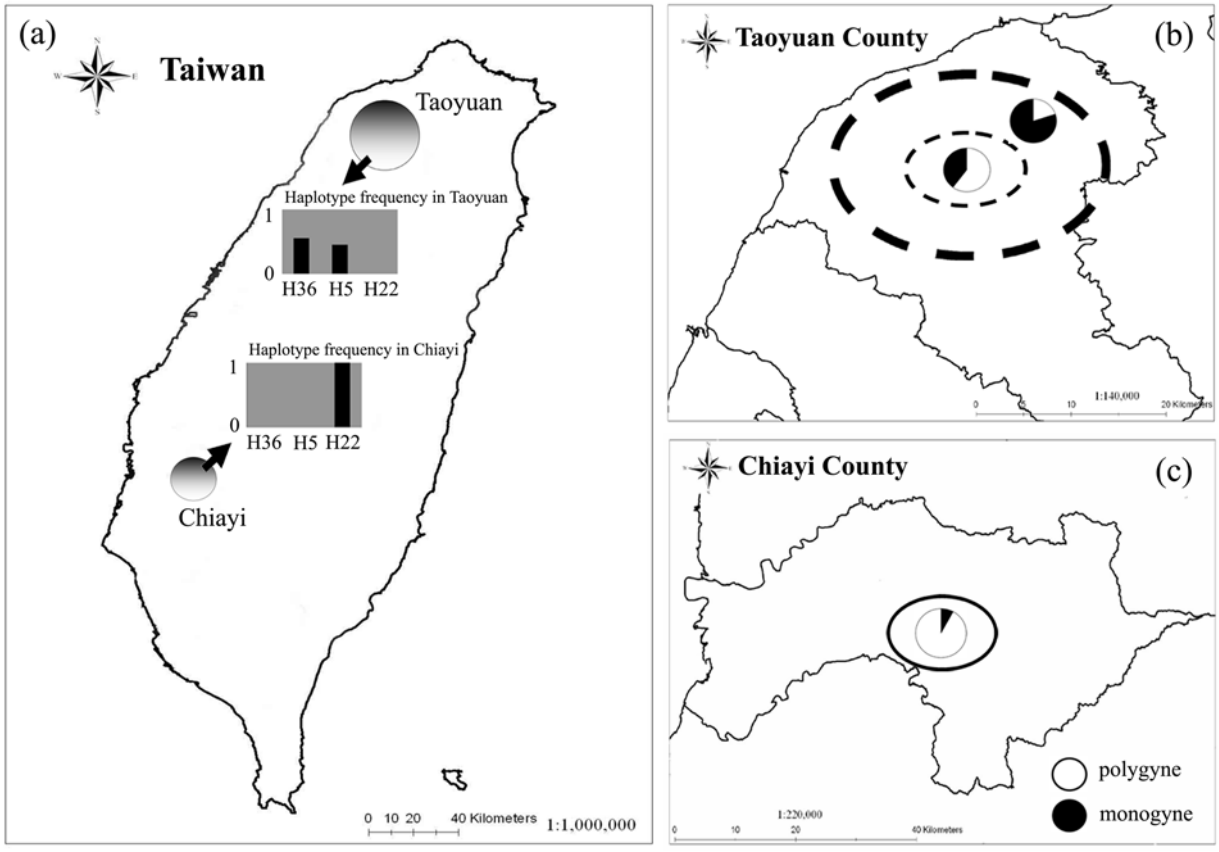

Figure 1. (a) Map of Taiwan showing the two areas (Taoyuan and Chiayi counties) infested by *Solenopsis invicta* (indicated by shading). Histograms show the proportion of nests harboring each mtDNA haplotype. Pie diagrams in the enlarged map of Taoyuan county (b) show the proportion of the monogyne and polygyne nests found in the central townships (smaller dotted-line circle) and in the surrounding townships (areas between larger and smaller dotted-line circles). A pie diagram in the enlarged map of Chiayi county (c) indicates the proportion of two social forms in the sampling area (solid-line circle). Social form of each nest was determined by using a *Gp-9* allele-specific assay and by examining the distribution of genotype arrays at six microsatellite loci within nests (see text).

Table 1. Genetic diversity measures within each social form from the two study populations of *S. invicta* in Taiwan. *n* denotes total number of individuals (nests) for a given social form in each area; N_A denotes observed number of alleles found at each locus from each population; H_E = expected heterozygosity; H_O = observed heterozygosity.

Locus	Taoyuan						Chiayi					
	Monogyne ($n=11$)			**Polygyne** ($n=19$)			**Monogyne** ($n=2$)			**Polygyne** ($n=23$)		
	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O
Sol-6	1	0.000	0.000	2	0.193	0.105	3	0.833	0.800	4	0.599	0.434
Sol-11	4	0.731	0.545	4	0.755	0.526	2	0.500	0.700	4	0.657	0.652
Sol-20	5	0.814	0.818	5	0.734	0.631	3	0.833	0.800	4	0.679	0.782
Sol-42	4	0.610	0.363	4	0.613	0.473	2	0.500	0.450	5	0.656	0.608
Sol-49	4	0.697	0.634	5	0.768	0.842	3	0.833	0.850	5	0.735	0.782
Sol-55	3	0.634	0.454	3	0.624	0.789	2	0.667	0.850	4	0.486	0.608
Mean	3.5	0.581	0.469	3.8	0.614	0.561	2.5	0.603	0.742	4.3	0.635	0.645

Within-nest relatedness

The estimates of relatedness among workers within monogyne nests from each area were consistent with each nest headed by single queen mated to a single male (Taoyuan: $R = 0.7187 \pm 0.036$; Chiayi: $R = 0.7197 \pm 0.038$; Fig. 2). Relatedness among workers within polygyne nests was significantly greater than zero but significantly lower ($P < 0.001$) than the values for monogyne nests (Taoyuan: $R = 0.1122 \pm 0.006$; Chiayi: $R = 0.1444 \pm 0.007$; Fig. 2).

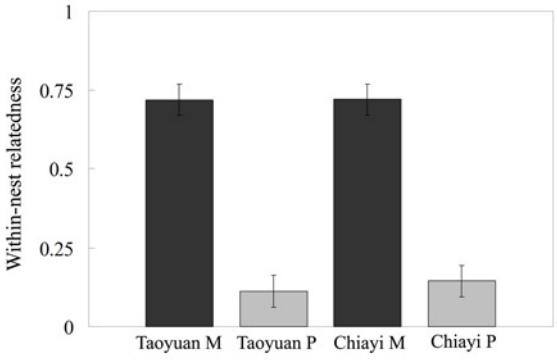

Figure 2. Average relatedness (±95% confidence interval) values among workers within nests of the polygyne and monogyne social forms from Taoyuan and Chiayi counties, Taiwan.

Population genetic differentiation

Exact tests based on six microsatellite loci revealed significant differentiation between Taoyuan and Chiayi populations ($P < 0.02$). No significant differentiation was observed between sympatric populations of the two social forms in the two areas ($P > 0.13$). Exact tests for differentiation using mtDNA sequence data revealed significant differentiation occurs between the two geographic populations ($P < 0.0001$) as well as between the two social forms in Taoyuan ($P < 0.04$) but not between the two social forms in Chiayi (only one mtDNA variant occurs here).

Nuclear and mtDNA variation was partitioned by estimating F_{ST} simultaneously at both levels using the analysis of molecular variance approach (Excoffier et al., 1992). Results of these analyses for the nuclear loci showed that only the between-population variance component is significant ($P < 0.000001$; Fig. 3a), providing additional support to the above analyses suggesting these two populations are genetically distinct. For analyses involving mtDNA data, both the between-population and between-social form variance components are significant (both $P < 0.05$, Fig. 3b).

Bayesian simulations using STRUCTURE revealed that individuals from the Taoyuan population represent a single distinct genetic cluster, suggesting no differentiation between sympatric social forms exists, whether using nuclear data only or nuclear and mtDNA combined. Identical results were obtained for individuals from the Chiayi population. In contrast, simulations incorporating all individuals from the two areas gave posterior probabilities that strongly support the existence of two distinct genetic clusters using dataset from the nuclear loci or combination of the two classes of markers ($K = 2$; Fig. 4). The two clusters hypothesized by the Bayesian method correspond roughly to geographic populations based on average membership coefficients > 0.5 for one of the two posterior clusters in each of these regions: The first cluster (indicated by lighter gray) is comprised largely of individuals from the Taoyuan population, whereas the second cluster (indicated by the darker gray) is comprised mostly of the individuals from Chiayi. Identical clustering results were obtained when simulations were run incorporating both the microsatellite and mtDNA data (Fig. 4).

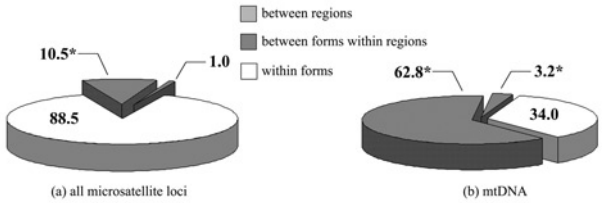

Figure 3. Partitioning of genetic variance in *S. invicta* at six microsatellite loci (a) and mtDNA (b) estimated using analysis of molecular variance. This hierarchical approach partitioned the total variance between infested regions (Taoyuan and Chiayi counties), between the two social forms within each infested region, and within the social forms in each region. The percentage of the total variance partitioned at each level is shown. Asterisks indicate statistically significant differentiation at a given level ($\alpha = 0.05$).

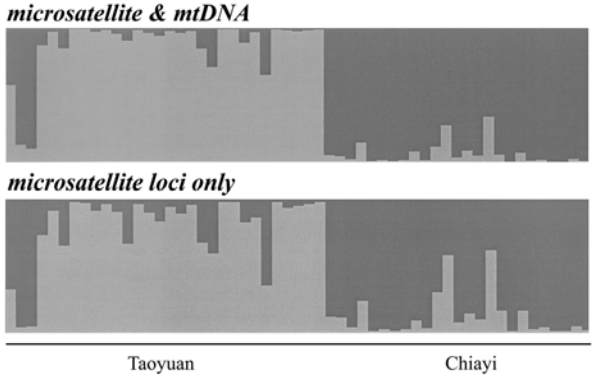

Figure 4. Assignment of individuals representing *Solenopsis invicta* from both study populations to two genetic clusters inferred from STRUCTURE simulations (average membership coefficient, Q). Each vertical bar represents the individual membership coefficients divided into parts proportional to the individual's proposed ancestry in each cluster.

Table 2. Genetic diversity measures within the two study populations of *S. invicta* in Taiwan. N_A denotes observed number of alleles found at each locus from each population; H_E = expected heterozygosity; H_O = observed heterozygosity. Numbers in parentheses represent the number of private alleles occurring in only one of the two Taiwanese populations. Genetic diversity measures for two population from the U.S.A. and South America also are shown (see text).

Source of collection	Population	Sample size	Mean no. of alleles	N_A	H_E	H_O
Taiwan						
	Taoyuan county	30	3.8	23 (2)	0.609	0.527
	Chiayi county	25	4.8	26 (5)	0.636	0.660
U.S.A.						
	Mississippi	30	5.7	34	0.651	0.559
	western Louisiana	30	5.8	35	0.628	0.607
South America						
	Formosa, Argentina	30	15.8	195	0.835	0.842
	Corrientes, Argentina	30	15.8	95	0.836	0.826

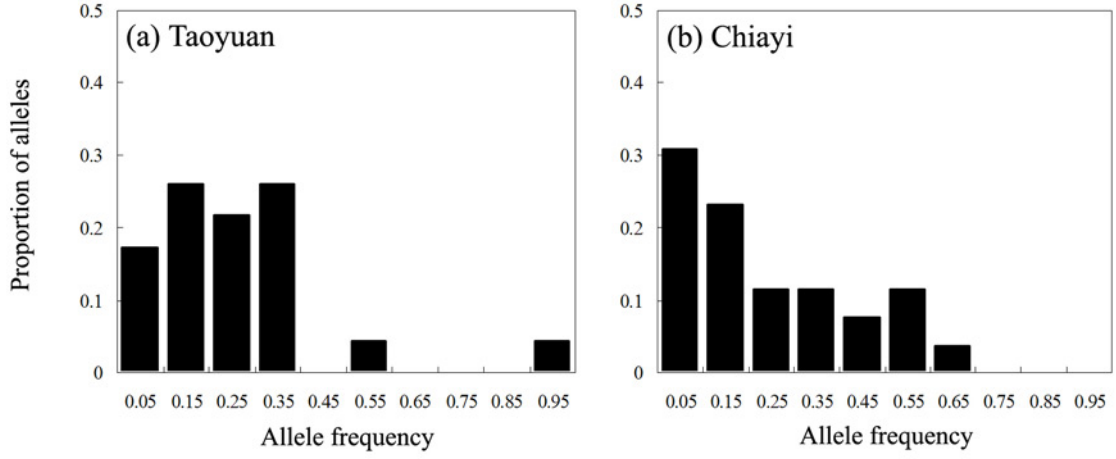

Figure 5. Distribution of allele frequencies at six microsatellite loci for ants (single individual per nest) from the Taoyuan (a) and Chiayi populations (b).

Isolation by distance and tests for genetic bottlenecks

No correlation exists between pairwise F_{ST} or $F_{ST}/(1-F_{ST})$ values and geographic distance for individuals of the polygyne social form in either population (Taoyuan: $P > 0.22$; Chiayi: $P > 0.48$). Further, no significant isolation by distance for the monogyne nests from Taoyuan population was found ($P > 0.65$). We did not perform the Mantel tests for the Chiayi monogyne population since only two nests were collected.

All tests revealed a significant excess of heterozygosity in Taoyuan under both models, except the sign test (Table 3). A significant excess of heterozygosity was found in Chiayi for the infinite allele model but not the two phase model (Table 3). Combined with a mode shift of allele frequency distribution (Table 3, Fig. 5a), the Taoyuan population appears to have undergone a recent bottleneck. In contrast, the most abundant alleles in the Chiayi population belong to the low frequency class (< 0.1; Fig. 5b), resulting in a normal L-shape distribution. We also compared N_A (number of observed alleles, Table 2) and H_E (expected heterozygosity, Table 2) among the South American, U.S.A. and Taiwanese populations to determine whether a genetic signature characteristic of a bottleneck was evident for the latter populations. In order to minimize the effect of population size, we randomly selected 30 individuals from the U.S.A. and South American dataset (Shoemaker et al., 2006; Ross et al., 2007). Results showed that the N_A and H_E in Taiwan and the U.S.A. populations were consistently lower than in South American fire ant populations. Two populations in Taiwan had slightly lower N_A than in U.S.A., but had similar H_E values.

Additional findings

Analyses of individual genotypes revealed that 10.8 % of the 190 workers sampled from polygyne nests in Taoyuan and 11 % of 230 individuals from polygyne nests in Chiayi

harbored three distinct alleles, whereas all of the individuals sampled from monogyne nests harbored at most two distinct alleles. These results indicate that a substantial number of polygyne fire ants are triploid, a finding consistent with an earlier study of fire ants from the U.S.A. (Krieger et al., 1999).

Discussion

We studied the colony genetic and population structure of the invasive fire ant *S. invicta* in Taiwan by collecting and analyzing microsatellite genotypic data and mtDNA sequence data of ants collected from two separate infested areas. Our genetic analyses revealed several patterns, which are discussed in more detail below and can be summarized as follows: 1) Estimates of within nest relatedness were significantly greater than zero for both social forms from both populations; 2) No significant isolation by distance was found among nests within each social form from either population; 3) Significant mtDNA but no nuclear differentiation occurs between sympatric social forms in Taoyuan (no differentiation between forms at either genome is evident in Chiayi); 4) Molecular signatures of genetic bottlenecks associated with recent introductions are evident in both populations; 5) The two sampled populations, Taoyuan and Chiayi, are highly genetically differentiated at both the nuclear or mtDNA genomes. In addition to these general findings, we found numerous triploid individuals in ants of the polygyne (**P**) but not the monogyne (**M**) form, and distinct geographic distribution of each of the two social forms between the two populations.

Colony social organization and patterns of intracolony worker relatedness

The estimate of relatedness among workers within monogyne colonies was not significantly different from 0.75, the predicted value for a nest headed by one singly-mated queen. These data also are consistent with data from earlier studies of monogyne *S. invicta* colonies in both the U.S.A. (Ross and Fletcher, 1985b) and South America (Ross et al., 1997) and suggest that the social and mating biology of **M** queens is virtually invariant, with **M** nests representing closed societies headed by one singly-mated reproductive queen.

In contrast, colony genetic structure of **P** social form does differ between native South America and introduced populations in the U.S.A. (Ross et al., 1996). **P** nests of *S. invicta* in the native range contain relatively few queens which are close relatives and relatedness among nestmate workers is significantly greater than zero whereas **P** nests in the U.S.A. contain high numbers of unrelated queens and relatedness among nestmate workers is not significantly greater than zero. These differences in queen number and relatedness between the two ranges are consistent with predictions based on differences in the population densities of ants in these two areas (Ross et al., 1996), which are substantially higher in the U.S.A. compared to South America (Porter et al., 1997). One consequence of the higher population densities in the U.S.A. is that nesting sites are more often saturated such that opportunities for independent founding by queens are more limited in the introduced range (Ross et al., 1996). These constraints on independent founding lead to increased selection pressure on queens to seek adoption into existing colonies and on resident workers of colonies to accept such queens. As queen number within these nests increases, relatedness among nestmate workers is expected to decrease.

Relatedness among workers within **P** nests in Taiwan is small yet significantly greater than zero and intermediate to the values observed for native and U.S.A. populations. The social organization of **P** nests in Taiwan likely reflects the fact that nesting sites are much less saturated compared to sites in the U.S.A. since the population densities of ants in Taiwan currently are much lower than those in the U.S.A. However, while such a difference between the two introduced ranges are evident now, it is unlikely to persist especially if *S. invicta* continues to spread in Taiwan so that habitats become saturated. If *S. invicta* continues to spread so that habitats become more saturated, future studies of the social evolution of **P** populations in Taiwan could shed additional light on whether such a change in habitat availability does in fact lead to a shift in social organization towards a higher queen number within nests and lower relatedness among nestmates.

Patterns of isolation by distance

The lack of isolation by distance among colonies of the **M** form within Taoyuan was not unexpected given that most **M** queens take part in aerial mating flights, often dispersing several kilometers or more before landing and attempting to found a new nest (Toom et al., 1976; Ross and Fletcher, 1985b; Ross et al., 1999). Such long-distance dispersal is predicted to erase any signature of nuclear differentiation among closely spaced nests.

The lack of detectable isolation by distance within the **P** form at both studied sites was somewhat unexpected considering the reproductive habits of **P** queens, which often mate in or near their natal nests and form new colonies by budding or by entering into nearby existing nests (Shoemaker and Ross, 1996; Ross et al., 1997; Ross and Shoemaker, 1997). One explanation for the lack of detectable isolation by distance is simply that males disperse rather long distances to mate with **P** queens. Under this scenario, one would predict that long distance male-mediated gene flow would result in homogenization of allele frequencies at nuclear loci, yet significant microgeographic mtDNA structure would remain espe-

cially if queen dispersal is limited. However, no such pattern was found: Significant isolation by distance was absent for both the nuclear and mtDNA markers. An additional explanation for these results is that the signature of isolation by distance predicted to emerge solely from the natural dispersal biology of these ants is confounded by the fact that these ants are frequently inadvertently transported relatively great distances by humans. Such frequent inadvertent human transport may be a common characteristic of invasive ants found in close proximity to human habitats and thus play a role in shaping the genetic structure in introduced areas. Finally, it is possible that the lack of a detectable signal of isolation by distance is due to the extremely recent introduction of fire ants into Taiwan, with insufficient time having passed for populations to achieve migration/drift equilibrium.

Gene flow between sympatric social forms

The pronounced mtDNA differentiation between sympatric social forms in Taoyuan is consistent with results from earlier studies in both South America and the U.S.A. showing that interform gene flow mediated by queens is rare or absent. Queens of each social form rarely succeed in becoming reproductives in colonies of the alternate form as a result of behavioral decisions (attributed largely to differences in genotypes of queens at the single gene, *Gp*-9) made by both queens and workers (Shoemaker and Ross, 1996; Ross and Shoemaker, 1997; Keller and Ross, 1998; DeHeer et al., 1999; Goodisman et al., 2000; Ross and Keller, 2002). The contrasting weak or nonexistent interform divergence at nuclear genes also is consistent with studies of *S. invicta* in the U.S.A. showing that queens of the **P** form predominantly mate with males of the **M** form where the two forms co-occur (Ross and Shoemaker, 1993; Ross and Keller, 1995; Shoemaker and Ross, 1996). In the U.S.A., most males in **P** colonies are sterile diploid males, which means that **P** queens rely largely on immigrant **M** males for mating opportunities (Ross and Fletcher, 1985a; Ross and Shoemaker, 1993). Such interform matings act as a homogenizing force only for nuclear markers. The similar frequency of triploid workers in **P** nests in Taiwan and the U.S.A. suggests many diploid males also occur in Taiwan as well (Ross et al., 1993, 1999; Krieger and Keller, 1997; Krieger et al., 1999), which means that **P** queens here must also mate with **M** males. The finding of contrasting patterns of differentiation between the two genomes parallels results from earlier studies involving *S. invicta* in both the U.S.A. and South America (Shoemaker and Ross, 1996; Ross et al., 1997; Shoemaker et al., 2006). One final caveat, however, is that we cannot completely rule out the possibility that the lack of detectable nuclear differentiation is due to insufficient time having passed for divergence to occur despite restricted gene flow between the two forms.

Understanding the distributions of the two social forms in the two infested areas in Taiwan, each of which exhibits rather distinct dispersal strategies, is important for developing effective control strategies of fire ants in these infested areas (Porter et al., 1991). The distributional pattern of social forms in Taoyuan suggests that the expansion of this population has involved persistent outward movement of ants from the core infestation coupled with frequent long distance movement of **M** reproductives into outlying areas (Fig. 1b). Based on these results, we recommend that bait broadcasting and surveillance efforts in Taoyuan should include not only the currently infested areas, but also should include an area extending well beyond the infested areas. In contrast to the distributional pattern of social forms in Taoyuan, the large infested area in Chiayi consists mostly of **P** nests (92 % of colonies collected; Fig. 1c). However, despite this difference, we suggest a similar eradication strategy should be employed. This is because even though comprehensive bait broadcasting within the entire area may be more effective than for Taoyuan because of the low queen vagility and relatively slow rate of reinvasion by budding of polygynous queens (Porter et al., 1991), some **M** colonies do occur in Chiayi and there also is potential for additional **M** colonies to arise locally from the **P** social form, a possibility that is especially more likely in areas of low density (Ross and Shoemaker, 1997; DeHeer and Tschinkel, 1998; DeHeer et al., 1999).

Genetic bottlenecks in Taoyuan and Chiayi populations

Simulations and statistical tests using the program BOTTLENECK provide evidence of a significant genetic bottleneck associated with the introduction of fire ants into Taoyuan, with only one exception (Table 3). However, this single exception is based on a sign test, which has relatively low statistical power when the number of loci is below 20 such as our study (Cornuet and Luikart, 1996). In Chiayi, significant excess in heterozygosity using the infinite alleles model (IAM) was observed for all tests but not for the two-phase model (TPM; Table 3). Because Luikart and Cornuet (1998) suggest that implementing the test under the IAM model may be more reliable than other models, these results, coupled with finding that allelic frequencies fit a normal L-shaped distribution, provide evidence for a genetic bottleneck. Interestingly, estimates of N_A and H_E (Table 2) are remarkably similar for introduced populations from Taiwan and the U.S.A., and considerably lower than those from the native South American range. While the geographic origin of ants in Taiwan is unknown, the similar genetic diversity measures between the introduced ranges raises the issue that the introduction of ants into Taiwan has left only a modest footprint on patterns of diversity if these ants originated from the U.S.A.

Table 3. Tests for genetic bottlenecks using the infinite allele model (IAM) and two-phase model (TPM; 90 % stepwise mutation model and 10 % multistep mutation model [Garza and Williamson, 2001]) implemented in the program BOTTLENECK ($\alpha = 0.05$).
*Significance at given statistic level.

Population	Simulated model	Significance test	Standardized differences test	Wilcoxon test
Taoyuan	IAM	0.148	0.003*	0.015*
	TPM	0.186	0.035*	0.023*
Chiayi	IAM	0.037*	0.022*	0.007*
	TPM	0.521	0.293	0.281

Genetic differentiation between Taoyuan and Chiayi populations

All analyses of population differentiation using the microsatellite and mtDNA data indicate that the two geographic populations are strongly differentiated and thus likely originated from separate invasions. While multiple lines of evidence support this notion, the strongest evidence is the confinement each of the three mtDNA variants to only one of the two geographic populations. Interestingly, all three variants found in Taiwan are the same three variants commonly found in ants from the U.S.A. (Shoemaker et al., 2006), China (GenBank accession numbers DQ831670-DQ831672), and Australia (CCY and DDS, unpublished results). The occurrence of these three variants in all recently invaded areas suggests that ants from Taiwan could have originated from any of these other areas. We currently are generating the necessary baseline data for ants from all of these introduced regions as well as from populations in their native range to infer the source population and invasion history of ants in Taiwan and these other areas.

From the perspective of managing fire ants in Taiwan, treating each infested area as a separate management unit would appear more reasonable and means that scientists probably should focus on separate plans for elimination of two isolated populations (Hampton et al., 2004). On the other hand, because these two areas are at high risk of connectivity via long distance human-mediated dispersal, care must be taken to prevent reinvasion into these areas even after successful eradication. Stringent inter-county quarantine measures, such as movement control, should be established to monitor the risk of transportation of fire ant-infested goods to prevent jump dispersal between the two areas and decrease probability of successful eradication (Drees and Gold, 2003).

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