

# Propagule pressure and colony social organization are associated with the successful invasion and rapid range expansion of fire ants in China

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## Abstract

We characterized patterns of genetic variation in populations of the fire ant *Solenopsis invicta* in China using mitochondrial DNA sequences and nuclear microsatellite loci to test predictions as to how propagule pressure and subsequent dispersal following establishment jointly shape the invasion success of this ant in this recently invaded area. Fire ants in Wuchuan (Guangdong Province) are genetically differentiated from those found in other large infested areas of China. The immediate source of ants in Wuchuan appears to be somewhere near Texas, which ranks first among the southern USA infested states in the exportation of goods to China. Most colonies from spatially distant, outlying areas in China are genetically similar to one another and appear to share a common source (Wuchuan, Guangdong Province), suggesting that long-distance jump dispersal has been a prevalent means of recent spread of fire ants in China. Furthermore, most colonies at outlier sites are of the polygyne social form (featuring multiple egg-laying queens per nest), reinforcing the important role of this social form in the successful invasion of new areas and subsequent range expansion following invasion. Several analyses consistently revealed characteristic signatures of genetic bottlenecks for *S. invicta* populations in China. The results of this study highlight the invasive potential of this pest ant, suggest that the magnitude of international trade may serve as a predictor of propagule pressure and indicate that rates and patterns of subsequent range expansion are partly determined by the interplay between species traits and the trade and transportation networks.

**Keywords:** fire ants, genetic structure, invasive species, jump dispersal, microsatellites, mitochondrial DNA, pest management, social form, *Solenopsis invicta*

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## Introduction

Invasive species pose major threats to worldwide biodiversity, public health, agriculture and the economy, and,

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not surprisingly, their negative impacts are likely to continue to increase with increasing global human activities (Sakai *et al.* 2001). Because invasive species often are characterized by small founder population sizes, they often remain undetected until the severity of their impacts is overwhelming (Hulme 2006; Hulme *et al.* 2008). Successful strategies for alleviating the harmful effects of invasives partly rely on reconstructing the invasion history, tracing patterns of ongoing migration and developing effective integrated management plans

that are compatible with the biology of the target invasive species in newly colonized areas (Strayer *et al.* 2006). However, inference of invasion history and migration routes of such species can be misleading or unclear if historical data provide the only supporting source of information. Further, observational field data are often insufficient to discern an invading organism's reproductive biology or mode of dispersal, which is highly relevant to the development of effective management schemes. Population genetic approaches employing highly polymorphic genetic markers potentially offer a powerful means to more fully resolve many of these issues (i.e. understanding the invasion route, demographic history, population connectivity or life history traits; Dlugosch & Parker 2008; Ficetola *et al.* 2008; Fonseca *et al.* 2010). From a more basic perspective, genetic data obtained from native and invasive populations also may be of additional utility for increasing our understanding of the evolutionary mechanisms underlying invasion success (Herborg *et al.* 2007; Darling *et al.* 2008).

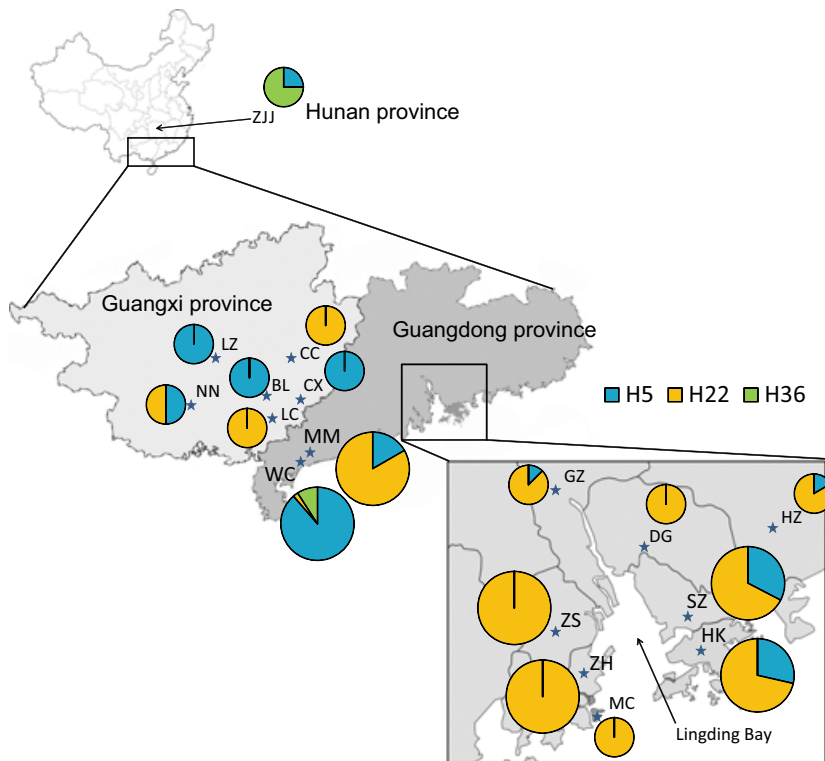
Several ant species are considered to be among the most damaging invaders worldwide (five ant species are listed among the 100 worst alien invasive species worldwide; ISSG website, [http://www.issg.org/worst100\\_species.html](http://www.issg.org/worst100_species.html)). Although not universal, the success of many invasive ants has been correlated with changes in behaviour, mating system, colony social organization, genetic structure or pressure from natural enemies (Suarez & Tsutsui 2008; Suarez *et al.* 2008; Helanterä *et al.* 2009).

One of the most notorious invasive ant species is the red imported fire ant, *Solenopsis invicta*. This ant was inadvertently introduced into the southern USA from South America in the 1930s and subsequently spread rapidly throughout the southern states. More recently, well-established populations of *S. invicta* have been reported in other regions of the world, including Australia, Taiwan and China (McCubbin & Weiner 2002; Huang *et al.* 2004; Zhang *et al.* 2007). As a result of its significant pest status, an enormous amount of research has been conducted to increase our understanding of the basic biology of *S. invicta*, both in its native and introduced USA ranges (reviewed in Tschinkel 2006). Included among this work are a number of population genetic studies of *S. invicta* relevant to understanding the biology of the species in the USA and more recently invaded areas. For instance, two previous population genetic studies of *S. invicta* in Australia and Taiwan revealed that patterns of genetic structure in these recently invaded areas were similar to the patterns found in the USA, with minor differences likely reflecting the recency of introductions into the former areas (Henshaw *et al.* 2005; Yang *et al.* 2008).

More recently, Ascunce *et al.* (2011) conducted a detailed genetic study reconstructing the worldwide invasion history of *S. invicta*. One major result from that study was the finding that the immediate source of fire ant infestations in countries of the Pacific Rim most likely was the USA. The repeated successful introductions from the USA may result from particular population traits that preadapted fire ants as a colonizer or may be a consequence of higher propagule pressure from the USA relative to other regions (Ascunce *et al.* 2011).

Ascunce *et al.* (2011) also demonstrated that fire ants in mainland China generally belong to one of two distinct genetic clusters (China1 and China2), with one comprised of populations from Hong Kong, Shenzhen and Wuchuan and the other of populations from Macao (Fig. 1). These authors suggested that these two distinct genetic clusters reflect two separate invasions of *S. invicta* into China. However, the fact that Wuchuan is geographically distant from the other two populations in the China1 cluster (approximately 450 km, Fig. 1) with no record for ants occurring in intervening areas raises suspicions that fire ants in the Wuchuan area are the result of a separate third introduction. Further support for this alternate hypothesis comes from the fact that Hong Kong and Shenzhen populations of *S. invicta* are in close proximity to international commerce centres in south-eastern China, whereas Wuchuan is not but is instead close to another international hub (i.e. Zhanjiang) that primarily coordinates trade for south-western China. The first objective of the present study was to employ extensive genetic data to test our prediction that fire ants in Wuchuan result from a separate third introduction of fire ants into China from the USA.

The second objective of this study was to test the prediction that the US source population or region of ants for the presumable third introduction into China represents an area in the USA with the highest level of exports destined for China (estimated from data on the overall amount of total exports from each state within the USA to China). Historical records indicate that fire ants, like many other invasive species, likely are transported as cargo stowaways (Krushelnycky *et al.* 2005; Wetterer & Snelling 2006; Zhang *et al.* 2007), suggesting increasing volume and frequency of international trade will lead to higher propagule pressure and invasion potential. Thus, we predict that the volume of trade from fire ant-infested areas potentially may serve as a general predictor of the potential of different fire ant populations serving as the source of recent or new outbreaks of fire ants, such as those found in Wuchuan (Levine & D'Antonio 2003; Westphal *et al.* 2008; Simberloff 2009).



**Fig. 1** Map showing the distribution of collection sites of *Solenopsis invicta* (as indicated by star symbols) in three provinces (Guangdong, Guangxi and Hunan) in China. Pie diagrams show the frequencies of mtDNA haplotypes (H5, H22 and H36) at each collection site, with larger pie diagrams corresponding to main sites (which are also indicated in bold).

The presence of fire ants at numerous small and distant outlier sites located away from the core infested areas in China (Zhang *et al.* 2007) suggests that the rate of subsequent expansion of the range of *S. invicta* in China is greater than expected if ants disperse by natural means only (Fig. 1), and more likely result from unintentional human transport. Dispersal strategies of *S. invicta* differ substantially between the monogyne (single egg-laying queen) and polygyne (multiple reproductive queens per colony) social forms. Monogyne queens participate in large aerial mating flights and often disperse several kilometres or more before attempting to found nests independently whereas polygyne queens either disperse much shorter distances and attempt to enter established nests after these short flights or disperse on foot to initiate a new nest located several metres away from their natal nest (Tschinkel 1998). Despite the ability of monogyne queens to disperse several kilometres or more, the absence of monogyne colonies along sampled transects between distant outlier and core infestation sites in China suggests long-range jump dispersal of fire ants has been a common means of spread. A third objective of this study was to test the prediction that fire ants at these numerous distant outlier sites in China indeed result from unintentional human transport, as previously found for fire ants in the USA (Tschinkel 2006). Furthermore, as part of this latter objective, we test the

prediction that fire ant colonies at outlier sites and the source population(s) of these outlier sites are comprised largely of the polygyne social form, as previous studies suggest the inadvertent transport and establishment of ants of this form occur more frequently than their monogyne counterparts (Tschinkel 1998; DeHeer *et al.* 1999).

A final objective of this study was to test for the characteristic signature of genetic bottlenecks (e.g. losses of both allelic richness and heterozygosity) in *S. invicta* populations at the main study sites in China. Invasive species generally are predicted to suffer from reductions in genetic diversity during founding events, possibly reducing adaptive potential and increasing the risk of extinction (Kolbe *et al.* 2004; reviewed in Dlugosch & Parker 2008). However, multiple introductions of a species into a new area from diverse sources may ameliorate such costs and increase the probability of establishment and survival. Despite the fact invasive *S. invicta* populations in China result from multiple introductions from different regional sources, we predict that the genetic diversity within each invasive *S. invicta* population is low. The rationale for this prediction is that all fire ant populations in China likely are derived from relatively few original founders, all of which come from source populations or areas that are minimally genetically divergent from each other (Shoemaker *et al.* 2006).

We tested the above predictions by conducting a detailed population genetic study of *S. invicta* in China using a large set of samples from numerous sites throughout China not reported in Ascunce *et al.* (2011) and a large number of informative genetic markers (67 microsatellite loci and mitochondrial sequences) combined with a diverse suite of genetic analyses. The results from this study provide a comprehensive overview of the population genetic structure of *S. invicta* in China as well as baseline information for integrated pest management of this ant species and for studies of evolutionary changes associated with very recent fire ant invasions.

## Materials and methods

### *Historical records of fire ant invasion into China and collection of study samples*

*Solenopsis invicta* was first reported in Hong Kong (HK) and Wuchuan (WC), Guangdong Province, in September 2004 and then reported in Shenzhen (SZ), Zuhai (ZH) and Macao (MC), Guangdong Province, in early 2005 (Zeng *et al.* 2005). Since then, sites near these outbreak populations [Dongguan (DG), Huizhou (HZ), Guangzhou (GZ), Zhongshan (ZS) and Maoming (MM), Guangdong Province] have been infested successively by *S. invicta*. More recently, this pest ant has been discovered in multiple areas much further away from each

other and from the original sites. These areas include Luzhai (LZ), Chanchuan (CC), Cenxi (CX), Beiliu (BL), Nanning (NN) and Luchuan (LC) in Gaungxi Province, as well as a park in Zhangjiajie (ZJJ), Hunan Province (Zhang *et al.* 2007; also see Fig. 1).

For the present study, samples of fire ants (a minimum of 30 adult workers per nest) were collected from colonies at each of the 17 geographic sites mentioned above (see Table 1). Collected ants were preserved in 95% alcohol during field collection and immediately stored at  $-20^{\circ}\text{C}$  upon returning to the laboratory pending molecular analyses (additional details regarding geographic distribution, GPS coordinates and number of colonies collected at each site are presented in Fig. 1 and Table 1). Although several sites are represented by a small number of sampled colonies, especially those from Guangxi Province, we note that these may encompass most if not the entire set of nests in these areas, given that we could not find other nests there and that these appear to be areas of recent introduction(s) from other sites. Nonetheless, because of the substantial variation among sites in the number of sampled colonies, we excluded sites with fewer than nine colonies from some of our genetic analyses to avoid potential bias in the results owing to small sample sizes. Sites with a minimum of nine colonies are considered hereafter as main sites: These include sites HK, SZ, ZH, ZS, MM and WC (see Table 1).

**Table 1** Geographic information, abbreviation, sample size and proportion of polygyne colonies of each collection site in this study

Site ID	GPS coordinate (Longitude/Latitude)	Province/SAR	Abbreviation	N	Social form (% of Polygyne*)
Hong Kong <sup>†</sup>	114°11'/22°39'	Hong Kong SAR	HK	42	21.4
Shenzhen <sup>†</sup>	114°07'/22°33'	Guangdong	SZ	46	39.2
Zuhai <sup>†</sup>	113°34'/22°17'	Guangdong	ZH	14	0.0
Zhongshan <sup>†</sup>	113°22'/22°31'	Guangdong	ZS	9	0.0
Macao	113°54'/22°19'	Macao SAR	MC	5	0.0
Wuchuan <sup>†</sup>	110°47'/21°26'	Guangdong	WC	44	68.2
Maoming <sup>†</sup>	110°53'/21°40'	Guangdong	MM	10	100.0
Huizhou	114°22'/23°05'	Guangdong	HZ	6	0.0
Dongguan	113°45'/23°02'	Guangdong	DG	7	0.0
Guangzhou	113°14'/23°08'	Guangdong	GZ	8	0.0
Luzhai	109°74'/24°29'	Guangxi	LZ	3	100.0
Chanchuan	108°90'/24°77'	Guangxi	CC	1	100.0
Cenxi	110°00'/22°55'	Guangxi	CX	2	100.0
Beiliu	110°21'/22°42'	Guangxi	BL	7	57.1
Nanning	108°19'/22°48'	Guangxi	NN	2	100.0
Luchuan	110°16'/22°19'	Guangxi	LC	2	100.0
Zhangjiajie	110°29'/29°08'	Hunan	ZJJ	4	75.0

N, number of colonies collected; SAR, special administration region.

\*The proportion of monogyne: 1-% of polygyne.

<sup>†</sup>Sites with a minimum of nine colonies are treated as main sites.

### Trade patterns between USA and China

A framework that categorizes the pathways of the invasive species has been established to predict the risk of introduction by such species and to develop preventive methods such as quarantine measurements (Hulme *et al.* 2008). Among six principle pathways reflecting a gradient of human involvement, the 'stowaway' seems more appropriate to describe the introduction of *S. invicta* given its tramp species biology and past interception records (Krushelnycky *et al.* 2005; Wetterer & Snelling 2006; Zhang *et al.* 2007). The overall amount of total exports from the USA to China (as expressed by millions of US\$) can be used as a predictor of the potential of a given area to serve as the source for fire ant populations in China. Data were obtained from the US-China Business Council statistics for the amount of overall export (millions of US\$) into China by each state in the USA from 2000 to 2005 (the year of official report of *S. invicta* in China is 2005).

### DNA extraction and determination of social form

Two separate sets of DNA extractions were performed on ants from each colony: The first set of extractions was performed on a single worker ant per colony whereas the second set was performed on pools of 10–15 workers. All extractions were performed using the Puregene DNA extraction kit (Gentra Systems Inc., USA) following the suggested protocol for extracting DNA from animal tissues. The extractions from single ants were used as template DNA for mitochondrial DNA sequencing and microsatellite genotyping and the bulk-extracted DNA samples were used as the template for determination of social form, first using a diagnostic *Gp-9* polymerase chain reaction (PCR) assay developed by Valles & Porter (2003) and then using a second series of PCR assays that more reliably distinguish variation within the class of alleles associated with the expression of polygyny (*b*-like alleles) not detected by the first assay (Mescher *et al.* 2003; Gotzek *et al.* 2007; Shoemaker & Ascunce 2010). More specifically, Gotzek *et al.* (2007) demonstrated that all *b*-like alleles in polygyne queens consistently contain three diagnostic amino acid residues: possession of only one or two of these critical residues is not sufficient for polygyny. The Valles & Porter (2003) assay appears to be reliable in distinguishing social forms in USA populations of *S. invicta* where few *Gp-9* allelic variants exist. However, this assay cannot reliably do so in the native South American range because many *Gp-9* variants exist in South American populations of fire ant, and this assay does not provide information on variation at all three diagnostic amino acid residues (Gotzek *et al.* 2007). In contrast, the sec-

ond set of assays we employed has been shown to consistently and accurately identify the variable nucleotides at all three sites characteristic of the *B*-like and *b*-like *Gp-9* allele classes, allowing for accurate determination of colony social form (Shoemaker & Ascunce 2010), which is especially relevant given the unknown origin of all sampled nests in China. For each set of 96 PCR reactions, we included template DNA from a single monogyne individual (*BB*) and a single polygyne individual bearing a *b*-like allele (*Bb* or *Bb'*) as controls.

### Mitochondrial DNA (mtDNA) sequencing

Polymerase chain reactions for the mtDNA included primers C1-J-2195 and DDS-COII-4 (Ross & Shoemaker 1997) designed to specifically amplify an approximately 910 bp fragment corresponding to portions of the cytochrome oxidase subunit I (*cox-1*) and subunit II (*cox-2*) genes. PCR reaction conditions and thermal cycling profile were identical to those described in Ahrens *et al.* (2005). PCR products were purified and then used as templates for sequencing reactions performed using the two primers above and the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). Sequencing reactions were purified using CleanSEQ magnetic beads (Agencourt Bioscience, MA, USA) and run on an ABI 3730 sequencer at the National Taiwan University Core Laboratory for Sequencing or the University of Florida Biotechnology Facility (ICBR).

### Microsatellite genotyping

The same set of template DNA samples used for mtDNA sequencing also served as template for microsatellite PCRs. A total of 67 microsatellite loci were amplified and scored from these ants (Krieger & Keller 1997; Chen *et al.* 2003; Ascunce *et al.* 2009, 2011; Ross *et al.* 2010). One primer of each locus primer pair was labelled at the 5' end with one of four fluorescent dyes (FAM, PET, NED or VIC; Applied Biosystems). Primer pairs were combined in multiplex reactions by taking into account PCR thermal cycling profiles, dye labels, and expected size and yield of the PCR products. The complete set of 67 loci was amplified in 22 separate 12 µL multiplex-PCR reactions, each containing two to five pairs of primers, Hot-Start *Taq* 2× Mastermix (Denville Scientific, NJ, USA), 0.06–0.4 µM of each primer, 1–2 µL of total genomic DNA (10–20 ng) and water. Further details regarding primer sequences, PCR cocktails and thermal cycling profiles are described in detail elsewhere (Ascunce *et al.* 2009; Ross *et al.* 2010). Resulting PCR amplicons were diluted (100:1 to 200:1) and pooled into a total of six plates, each one representing a different injection on a capillary DNA sequencer.

GeneScan 600 LIZ size standard (0.1  $\mu$ L) was added to all pool-plex dilutions, which subsequently were run on an ABI-3730XL-96 capillary sequencer (Applied Biosystems) at the University of Florida Biotechnology Facility (ICBR). Microsatellite genotypes were scored using GENEMARKER software (SoftGenetics, PA, USA).

#### MtDNA sequence analyses

Sequences were aligned with previously published sequences in GenBank (accession numbers AY249097, AY950731 and AY249114; Shoemaker & Ross 1996) for determining haplotype identity. MtDNA haplotype frequencies at all collection sites were estimated using ARLEQUIN 3.0 (Excoffier *et al.* 2005). Pairwise  $F_{ST}$  values between sites were based on mtDNA haplotype frequencies and estimated using ARLEQUIN 3.0. Hierarchical  $F_{ST}$  values were calculated at two levels (among sites and between social forms within sites) using the analysis of molecular variance (AMOVA) approach implemented in ARLEQUIN. Statistical significance of differentiation at each level was assessed by means of permutation tests (20 000 data permutations).

#### Microsatellite analyses

Preliminary analyses of our data revealed that 15 of the 67 microsatellite loci were monomorphic within *S. invicta* at all 17 collection sites in China. We therefore performed the majority of subsequent genetic analyses on the genotypic data for the remaining 52 variable microsatellite loci.

We tested for the presence of null alleles or scoring errors owing to allele dropout using the program MicroChecker (van Oosterhout *et al.* 2004). Departures from Hardy–Weinberg equilibrium (HWE) at each locus in each main site were assessed using exact probability tests with 10 000 permutations (option 1) implemented in GENEPOP on the web (Raymond & Rousset 1995; <http://genepop.curtin.edu.au/>). Summary statistics for each locus, including mean number of alleles ( $N_A$ ), number of private alleles ( $N_{PA}$ ), observed ( $H_O$ ) and expected heterozygosities ( $H_E$ ), and  $F_{IS}$  for each site, were calculated using GENALEX 6.1 (Peakall & Smouse 2006). Significance of  $F_{IS}$  values was determined by bootstrapping over loci (10 000 replications) to obtain 95% confidence intervals. Allelic richness ( $A_R$ ) was estimated by correcting for variation in sample sizes with the rarefaction method using the program HP-RARE (Kalinowski 2005).

#### General population genetic analyses

Linkage disequilibrium (LD, expressed as  $D'$ ) between pairs of loci within each main site was estimated using

PowerMarker (Liu & Muse 2005), and the significance levels of  $D'$  were obtained using both permutation and Markov chain Monte Carlo approaches without adjusting for multiple comparisons. For each main site, the finding of more than 5% of total pairwise locus comparisons displaying significant  $D'$  values ( $P < 0.05$ ) was interpreted as more LD than would be expected by chance in a population lacking such disequilibrium (Schug *et al.* 2007).

Pairwise  $F_{ST}$  values between all main sites were estimated using FSTAT (Goudet 2001), and the significance of the measured genetic differentiation was assessed using exact tests as implemented in GENEPOP on the web. Pairwise genetic distances (Nei's unbiased  $D$ ) between sites were calculated using MSA (Dieringer & Schlötterer 2002).

The software STRUCTURE 2.3 (Pritchard *et al.* 2000) was used to estimate the number of distinct genetic clusters represented by all sampled individuals in the present study, as well as subsets of the entire dataset, and to probabilistically partition each individual's genome into membership fractions (ancestry) in each defined cluster. We first performed simulations using STRUCTURE on subsets of the data consisting of all individuals from each site to identify potential cryptic genetic structure within sampled sites. For these and subsequent STRUCTURE analyses, we used only the multilocus genotypic data for the 52 variable microsatellite loci. Simulations were performed for values of  $K$  (number of inferred clusters) ranging from one to five using 50 000 Markov chain Monte Carlo generations and 100 000 generations in the data collection phase. Ten independent runs were conducted for each  $K$ . Next, STRUCTURE simulations were performed on the entire dataset of individuals from all 17 sites using similar simulation parameters as above, with  $K$  varying from one to 20. Finally, we ran separate STRUCTURE simulations ( $K$  values from one to five) on all samples within each genetic cluster (if any) identified in the latter runs to learn whether lower-level genetic structure occurred within any of the major clusters (STRUCTURE generally detects genetic structure only at the highest level; Hubisz *et al.* 2009). All simulations were performed using the admixture and correlated allele frequencies models with no prior information. Selection of  $K$  for STRUCTURE simulations was based on the combination of results of the  $\Delta K$  method developed by Evanno *et al.* (2005), and the estimated posterior probabilities of the data for each  $K$ . DISTRUCT (Rosenberg 2004) were used to perform graphic visualization of the STRUCTURE results.

We used principal component analysis (PCA), which plots relationships between populations on  $n$  principal coordinates based on allele frequencies across all loci, to visualize the clustering patterns among main sites, as

implemented in the program GENALEX (Peakall & Smouse 2006).

We performed analyses of molecular variance (AMOVA), as implemented in ARLEQUIN, within and among three regional groups, each of which comprised sites that were geographically close to one another (HK/SZ, ZH/ZS and MM/WC). Statistical significance of the differentiation at each level was established by means of 20 000 data permutations.

#### *Identification of putative source population of fire ants in Wuchuan*

The most likely source of ants in WC resulting from a putative third invasion in China was inferred by estimating the assignment likelihood values ( $L_i \rightarrow l$ , the likelihood of assigning a given individual  $i$  to population  $l$ ), as implemented in GENECLASS 2 (Piry *et al.* 2004), with multiple collection sites in various states in the USA (two from each of California, Louisiana, Georgia and Mississippi; one from each of Florida and Texas) serving as reference sites. Microsatellite genotypic data for ants from these USA sites were obtained from Asuncion *et al.* (2011). All assignment likelihood tests were performed under the Bayesian criterion (Rannala & Mountain 1997). Also, estimates of pairwise  $F_{ST}$  served as a supplementary method to interpret the potential source population by estimating the genetic differentiation between the ants in WC and those in each USA reference site. The reference site displaying both the highest likelihood and the lowest  $F_{ST}$  values was inferred as the most likely source.

#### *Identification of putative sources of colonies from outlier areas*

The proportion of estimated membership coefficients (degree of admixture,  $Q$ ) of each individual genotype to distinct genetic clusters, as inferred from STRUCTURE, was used to infer the putative geographic source of sampled outlier colonies located away from the main sites. Individuals with  $Q$  estimates  $\geq 0.70$  for a single cluster were assigned to that cluster ( $Q$  estimates based on the selected  $K$  value). Individuals were assigned to two clusters if  $Q$  was ambiguous (e.g. 0.5 for each cluster). The assignment likelihood values (Piry *et al.* 2004) were also used to identify the most likely source site of outlier nests, with all nonfocal sites serving as reference populations. The most likely source was inferred as the site with the highest likelihood value. Estimates of  $F_{ST}$  were not included in this analysis as this method is prone to biases if sample sizes are too small as is the case for some outlier sites.

#### *Tests for genetic bottlenecks*

Three approaches were used to test for the characteristic signatures associated with genetic bottlenecks: heterozygote excess (implemented in BOTTLENECK; Piry *et al.* 1999), the  $M$  ratio test (implemented in M\_P\_VAL in conjunction with CRITICAL\_M; Garza & Williamson 2001) and, more directly, comparisons of genetic diversity in terms of allelic richness and expected heterozygosity. Populations that have experienced a recent population bottleneck are predicted to have greater heterozygosities ( $H_E$ ) than expected at mutation-drift equilibrium ( $H_{EQ}$ ) because alleles, especially rare alleles, are generally lost faster than heterozygosity in bottlenecked populations (Cornuet & Luikart 1996; Piry *et al.* 1999).  $H_{EQ}$  was simulated using BOTTLENECK with 10 000 replications under the two-phase model (TPM) consisting of 20% multi-state change and a variance of 12 as suggested by Piry *et al.* (1999). The significance of heterozygosity excess was tested by the Wilcoxon signed-rank test, which, in theory, has relatively high power when large numbers of loci are employed, as is the case for the present study (Luikart *et al.* 1998). For the second method we calculated and used the  $M$  ratio (number of alleles divided by the allelic size range) as an indicator of bottlenecks, with the expectation that numerous alleles within the expected size range are missing because of their loss during and following the bottleneck (Garza & Williamson 2001). The observed  $M$  ratio for each site was compared to the lower 5% tail of the critical ratio ( $M_C$ ) distribution generated by CRITICAL\_M, which requires three parameters to be set:  $\theta = 4 N_e \mu$  (where  $N_e$  = effective population size and  $\mu$  = mutation rate of  $5 \times 10^{-4}$ ),  $\Delta g$  (mean size of non-one-step mutations) and  $ps$  (the per cent of one-step mutations). We used the parameter values  $\theta = 10$ ,  $\Delta g = 3.5$  and  $ps = 90\%$  as recommended by Garza & Williamson (2001). Sites with an observed  $M$  ratio under the  $M_C$  threshold were considered to show evidence of having experienced a recent bottleneck. Finally, we also compared genetic diversity, as measured by allelic richness ( $A'_R$ ) and expected heterozygosity ( $H'_E$ ) at identical sets of microsatellite loci, among sites in China, a single site from the immediate putative source (Mississippi, USA; Asuncion *et al.* 2011), and a single site in the native South American range which represents the presumed source for ants found in the USA (Formosa, Argentina; Caldera *et al.* 2008). Notably, we estimated these two values ( $A'_R$  and  $H'_E$ ) using data generated for all 67 microsatellite loci to avoid any potential bias associated with removing loci invariant in China but not elsewhere (see Table 2).

**Table 2** Basic genetic profiles and tests for genetic bottlenecks for all main sites of *Solenopsis invicta* in China

	Basic genetic profile											Tests for bottleneck		
	$N$	$N_A$	$A_R$	$A'_R$	$N_{PA}$	$H_O$	$H_E$	$H'_O$	$H'_E$	$F_{IS}$	HWE	% LD significant pair	Wilcoxon test ( $P$ )	$M$ ratio
HK	42	3.192	2.883	2.460	2	0.473	0.476	0.367	0.370	0.007	NS	9.6	<0.001	0.71 <sup>†</sup>
SZ	46	3.385	2.947	2.512	4	0.479	0.491	0.371	0.381	0.024	NS	13.0	<0.001	0.68 <sup>†</sup>
ZH	14	2.558	2.459	2.133	3	0.448	0.427	0.345	0.332	-0.049	*	10.3	<0.001	0.57 <sup>†</sup>
ZS	9	2.423	2.423	2.104	1	0.404	0.409	0.313	0.312	0.014	*	22.0	<0.001	0.55 <sup>†</sup>
WC	44	3.135	2.706	2.324	2	0.428	0.437	0.332	0.339	0.022	NS	10.0	<0.01	0.68 <sup>†</sup>
MM	10	2.788	2.754	2.364	0	0.456	0.462	0.354	0.386	0.014	NS	2.9	<0.001	0.66 <sup>†</sup>

$N$ , sample size;  $N_A$ , average number of alleles;  $A_R$ , Allelic richness;  $N_{PA}$ , number of private alleles;  $H_O$  and  $H_E$ , observed and expected heterozygosity.  $A'_R$ ,  $H'_O$  and  $H'_E$ , (allelic richness, observed and expected heterozygosity) estimated using all 67 microsatellite loci (see text for more detail);  $F_{IS}$ , inbreeding coefficient; HWE, Hardy–Weinberg equilibrium; LD, linkage equilibrium.

\*Significant deviation from HWE.

<sup>†</sup>Below the critical value.

## Results

### Trade patterns between USA and China

Table S1 (Supporting information) shows the top ten states in the USA exporting goods to China between 2000 and 2005 (the year of official report of *Solenopsis invicta* in China is 2005). Four of these states (California, Texas, Louisiana and Georgia) are currently infested by fire ants. Of these states, both California and Texas exported twofold to tenfold more goods than Louisiana and Georgia (Table S1, Supporting information), suggesting the former two states represent higher risks for serving as potential sources for *S. invicta* in China.

### Distribution of social forms

Results of the two diagnostic *Gp-9* PCR assays were completely concordant and revealed that some proportion of polygyne colonies occur at all main sites except ZH and ZS (Table 1). These results generally are consistent to the previous findings of Shao *et al.* (2008) and Huang *et al.* (2009). This social form was completely absent at sites (GZ, DG and HZ) that are considered the result of range expansion from SZ (Table 1), whereas a high proportion of polygyne nests (80% across all sites) were found at most other outlier sites (Table 1).

### MtDNA genetic structure/haplotype distribution

Analyses of the mtDNA sequence data revealed only three haplotypes (designated as H5, H22 and H36; see Yang *et al.* 2008) among all surveyed individuals (Fig. 1). These variants are the same three haplotypes commonly found in other invasive populations from

the USA, Australia and Taiwan (Ascunce *et al.* 2011). Considering the main sites only (sites where a minimum of nine colonies were collected), geographically close sites tend to have similar mtDNA haplotype distributions (exact test, all  $P > 0.19$ ), with the exception of MM and WC (exact test,  $P < 0.001$ ) from western Guangdong (Fig. 1). While haplotypes H5 and H22 are commonly found at most collection sites, only seven sampled individuals (four and three from WC and ZJJ, respectively) bear the H36 haplotype.

### General analyses of microsatellite data

We genotyped a total of 212 individual ants (a single worker ant per colony) at all 67 microsatellite loci. The proportion of missing data for the 52 polymorphic loci generally was quite low (<0.2%) with the single exception that involved locus *Sol-20* (up to 7% missing data). All subsequent genetic analyses were therefore conducted both with and without *Sol-20*. Importantly, we found that both sets of genetic analyses generated identical results. Therefore, we only present results that included all 52 polymorphic microsatellite loci. Using Micro-Checker, we found no evidence of scoring errors or allelic dropouts for remainder of loci, suggesting our scoring of genotypes was reliable. Only 4.8% (15 of 312) of the tests for deviation from HWE were significant, most of which were attributable to cases involving MM, ZH and ZS, for which sample sizes were quite small (this likely accounts for the observation that ZH and ZS also do not conform to HWE at the population level; Table 2). Two loci, *Sol-20* and *Sol-42*, displayed significant departures from HWE in at least one of the main sites (heterozygote deficiency in all cases, exact test, all  $P < 0.02$ ), which is consistent with previous findings by



Ross *et al.* (1999) and Shoemaker *et al.* (2006). However, because exclusion of these two loci did not affect the patterns or significance of the results, we performed subsequent analyses using genotypic data generated from all 52 loci.

Linkage disequilibrium (LD, expressed as  $D'$ ) between all possible pairs of microsatellite loci at each main site was assessed using exact tests employing both MCMC and permutation algorithms (using a conservative  $P$ -value cut-off of 0.05). Results using both algorithms were nearly identical and showed that >5% of the pairwise comparisons were statistically significant for all but one main site (MM, Table 2). These results suggest LD is common in invasive Chinese populations. However, given that the significant LD observed could not be consistently attributed to the same locus pairs, it seems unlikely that it is simply the result of physical linkage. Furthermore, STRUCTURE simulation results show that all individuals at each main site consistently belong to a single genetic cluster, suggesting that the observed LD is not due simply to cryptic population structure.

Our study populations generally exhibited low genetic variation, with  $H_E$  and allelic richness ( $A_R$ ) ranging from 0.409 to 0.491 and from 2.423 to 2.947, respectively, over the 52 polymorphic microsatellite loci (Table 2). The highest number of alleles, highest allelic richness and highest number of private alleles (alleles that are unique to a given site) were found at site SZ. The small numbers of private alleles found in HK, SZ, WC and MM (average 1.56 with average allele frequency 0.02) potentially result sampling error (i.e. the confinement of these low-frequency alleles to single sites likely results from insufficient sample sizes needed for detecting very rare alleles).  $F_{IS}$  values consistently were near zero and statistically insignificant at every main site, as expected if inbreeding is rare (Table 2).

#### Nuclear (microsatellite) genetic structure

Population genetic differentiation, as expressed by pairwise  $F_{ST}$  estimates, was significant between most pairs of sites. Three trends in divergence were observed that describe the overall patterns of between-site differentiation (Table 3, lower diagonal): (i) low levels of differentiation (0.0001–0.0081) occur between sites in close geographic proximity (e.g. HK and SZ), with the exception of WC and MM; (ii) moderate levels of differentiation occur between WC and all the other main sites excluding ZH and ZS (range 0.0234–0.0612); (iii) high levels of differentiation occur between ZH or ZS and each of remaining sites (HK, SZ, WC and MM; all pairwise  $F_{ST}$  estimates are >0.13 and highly significant, with all  $P < 0.01$ ). The overall estimates of Nei's unbiased  $D$  between pairs of sites were in complete agreement with

**Table 3** Nei's unbiased genetic distance (upper diagonal) and  $F_{ST}$  (lower diagonal) of all pairwise comparisons between main sites

	HK	SZ	ZH	ZS	WC	MM
HK	—	0.0183	0.1933	0.1742	0.0675	0.0388
SZ	0.0081*	—	0.1792	0.1697	0.0639	0.0336
ZH	0.1585**	0.1436**	—	0.0310	0.2347	0.2353
ZS	0.1417**	0.1325**	0.0001*	—	0.2182	0.2224
WC	0.0427*	0.0385*	0.2026**	0.1897**	—	0.0786
MM	0.0234*	0.0168*	0.1877**	0.1771**	0.0612*	—

\* $P < 0.05$ .

\*\* $P < 0.01$ .

the patterns found from  $F_{ST}$  values in suggesting the presence of three different general levels of divergence (Table 3, upper diagonal).

The clustering method implemented in STRUCTURE gave higher posterior probabilities, as calculated from Bayes' Rule, for single genetic clusters ( $K = 1$ ) than for two or more clusters at each main study site. These results are consistent with a lack of significant cryptic lower-level genetic structure within our main sites. STRUCTURE simulations using all sampled individuals from all sites revealed that individuals from main sites were assigned with highest probability to one of two distinct genetic clusters ( $K = 2$ ), one comprising individuals from ZH, ZS and MC, and a second comprising ants from HK, SZ, WC and MM (referred to hereafter as clusters China\_A and China\_B, respectively; Fig. 2a). We next conducted separate STRUCTURE runs on individuals representing each of these two clusters to determine whether additional cryptic structure exists. While no additional structure was evident within the China\_A cluster ( $K = 1$ ), simulations did suggest that individuals within the China\_B cluster could be further partitioned into two genetic clusters ( $K = 2$ ), one of which comprises mostly individuals from main sites HK, SZ and MM (referred to hereafter as cluster China\_B1), and a second of which comprises individuals from WC (referred to hereafter as cluster China\_B2; Fig. 2b).

Genetic structure also was assessed by principal components analyses (PCA), the first of which was performed to resolve the relationships among the main sites only. Ordination of the main sites according to the first two component axes, which explains almost 60% of the variance, is shown in Fig. 3. The PCA strongly supports the STRUCTURE results by showing identical grouping patterns of sites, with one group consisting of sites ZH and ZS and the second comprising all remaining sites except WC. The individual-based PCA analyses were consistent with the site-based PCA above:

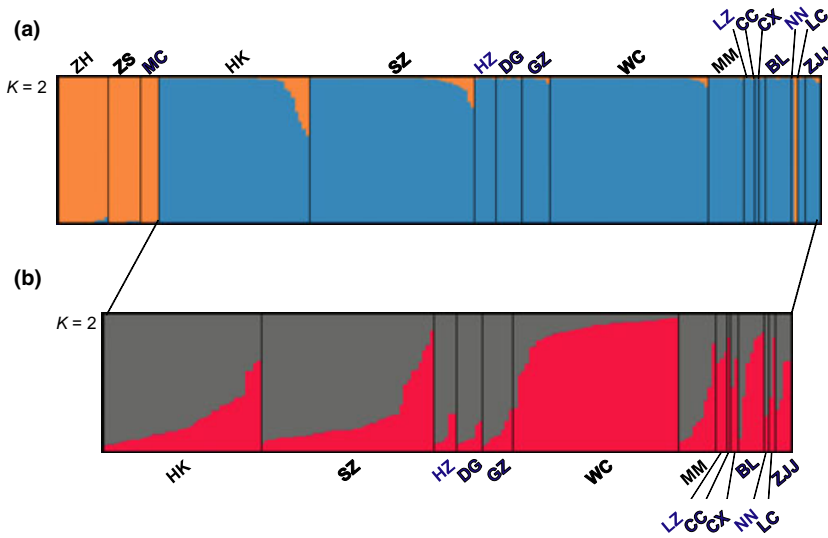


Fig. 2 Membership of (a) all sampled individuals and (b) individuals that were assigned to the cluster China\_B only in each hypothetical genetic cluster estimated by Bayesian simulation implemented in STRUCTURE. Sample site codes coloured in blue denote outlier sites.

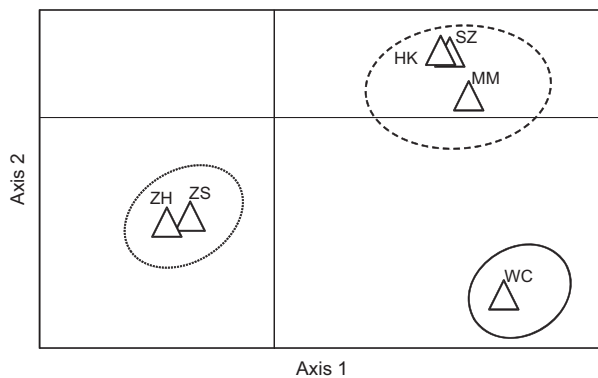


Fig. 3 Principal components analyses (PCA) showing clustering of sampling sites (main sites only) based on microsatellite data. Three major clusters were identified (denoted as PCA I, II and III; see also Table S2, Supporting information).

Considering individuals from the main sites only, three groups (PCA I, II and III) were discernable, with PCA I comprising individuals from ZS and ZH, PCA II comprising those from HK, SZ and MM, and PCA III largely comprising those from WC (although some overlap among PCA II and III is evident; Fig. S1, Supporting information). Analyses considering individuals from outlier areas revealed that these individuals generally fell into one of three groups: PCA I included all individuals from MC and a single individual from NN, PCA II included nearly all individuals from HZ, DG and GZ, and PCA III included most outliers from Guangxi Province (Fig. S1, Supporting information).

Hierarchical analyses of molecular variance (AMOVA) were employed to explore the distribution of nuclear genetic variation within sites, between sites within predefined groups and between predefined groups of our

samples. AMOVA performed on regional groups formed by combining neighbouring sites revealed significant genetic differentiation between regional groups ( $P < 0.0001$ , Fig. S2, Supporting information) but not between sites within groups ( $P = 0.26$ , Fig. S2, Supporting information). These results provide additional support to the earlier analyses indicating very low differentiation among sites within regional groups (with the notable exception of the group with MM and WC). Given that multiple analyses show more genetic affinity of MM with HK and SZ than with WC, we conducted two separate AMOVAs using different predefined groups consisting of MM/WC vs. HK/SZ and MM/HK/SZ vs. WC to verify this finding. These AMOVAs revealed significant between-group genetic variation when grouping MM with HK and SZ but not when grouping MM with WC (see Figs. S2, Supporting information).

#### Identification of the source population of a putative third invasion in China

Results from the above analyses indicate the existence of a third genetic cluster comprised almost entirely of individuals from WC, likely reflecting another independent invasion of *S. invicta* into China. Ten collection sites distributed throughout most of heavily infested states in the USA were considered as potential source populations for this recently invaded population. Although estimates of assignment likelihood values ( $L_i \rightarrow I$ ) and pairwise  $F_{ST}$  values marginally differ among all potential source populations, the Texas population shows the highest assignment likelihood value and the lowest pairwise  $F_{ST}$  value with WC, suggesting Texas may be the most likely source population (Table 4). This conclusion is supported by the trade data between USA and China

indicating that Texas is the top exporter of merchandize to China compared with the other fire ant-infested states in the southern USA.

Sources of outlier colonies

We performed several analyses to determine the source populations (sites) of ants collected at outlier sites (see Table S2, Supporting information). STRUCTURE analyses revealed that all individuals from MC as well as single individual from NN had high membership coefficients in the China\_A genetic cluster (0.99 on average), whereas the remaining individuals from all other outlier sites (nests) had high membership coefficients in the China\_B cluster (Fig. 2a; Table S2, Supporting information). Furthermore, all individuals from HZ, DG and GZ could be assigned with high average membership coefficient (0.87) to cluster China\_B1 (one of two lower-level genetic clusters found within China\_B), but most individuals from outlier nests collected from Guangxi Province were assigned to the China\_B2 cluster (Fig. 2; Table S2, Supporting information).

Results from the GENECLASS 2 analyses were equally revealing (Table S2, Supporting information). ZH or ZS were identified as the most likely source populations for individuals from MC, whereas individuals from outlier sites HZ, DG and GZ are most likely derived from either SZ or HK, which together form the China\_B1 cluster. Moreover, for ants from Guangxi Province, site WC generally was inferred as the most likely source; however, in some cases, outlier sites could not be ruled out as putative sources of other outlier sites (e.g. LZ was identified as the source of CC\_1 with highest assignment likelihood; see Table S2, Supporting information).

In summary, all three relevant analyses yielded consistent results regarding the identification of the putative geographic source populations (sites) of the outlier colonies (sites), and suggested that site WC was a common source of many of these (Fig. 4). We also found several sites at which individuals were identified as derived from two different sources (e.g. NN, Figs. 2 and 4; Table S2, Supporting information), suggesting the possibility of multiple introductions into outlier sites.

Tests for genetic bottlenecks

The Wilcoxon signed-rank test performed using the TPM model revealed a significant excess of heterozygosity at every main site (Table 2). Furthermore, the observed *M* ratio values for each of these sites were well within the lower 5% tail of the critical ratio (*M<sub>C</sub>*) distribution (Table 2). These results suggest that all main sites experienced recent bottlenecks. Estimates of both allelic richness and expected heterozygosity

Table 4 Pairwise estimate of *F<sub>ST</sub>* (values in parentheses) and mean assignment likelihood (*L<sub>i → j</sub>*) of each main site to each potential source population in USA

	Potential source populations									
	CA_RMV	CA_OC	TX	FL	EL	M3	PE	PMS	MS	WLA
HK	46.27 (0.097)	45.20 (0.083)	<b>37.18 (0.053)*</b>	39.49 (0.066)	39.32 (0.065)	43.15 (0.090)	40.71 (0.068)	38.93 (0.060)	39.39 (0.068)	40.05 (0.072)
SZ	47.42 (0.084)	46.28 (0.074)	<b>38.54 (0.041)*</b>	40.36 (0.055)	39.85 (0.046)	43.72 (0.072)	41.84 (0.052)	39.66 (0.044)	39.83 (0.050)	41.27 (0.061)
ZH	48.50 (0.171)	50.06 (0.166)	<b>41.72 (0.121)*</b>	43.45 (0.133)	42.73 (0.124)	45.88 (0.142)	44.10 (0.133)	42.42 (0.125)	42.80 (0.131)	46.87 (0.163)
ZS	47.47 (0.168)	49.38 (0.161)	<b>40.38 (0.110)*</b>	41.38 (0.119)	40.45 (0.113)	44.65 (0.133)	42.68 (0.126)	41.76 (0.116)	43.22 (0.126)	45.34 (0.148)
WC	43.72 (0.112)	48.22 (0.136)	<b>38.27 (0.081)</b>	39.51 (0.091)	38.88 (0.087)	43.03 (0.110)	40.54 (0.089)	38.90 (0.085)	39.23 (0.088)	40.01 (0.088)
MM	44.94 (0.099)	45.82 (0.088)	38.79 (0.060) <sup>†</sup>	38.35 (0.062)	39.25 (0.065)	44.66 (0.095)	40.44 (0.068)	39.46 (0.055)	38.94 (0.060)	38.90 (0.066)

*L<sub>i → j</sub>* values are expressed by log scale; values in parentheses indicate the pairwise estimate of *F<sub>ST</sub>*.

Maximum *L<sub>i → j</sub>* and minimum *F<sub>ST</sub>* (in parentheses) are noted in bold.

CA\_RMV: Rancho Mission Viejo, California; CA\_OC: Orange County, California; TX: Austin, Texas; FL: Gainesville, Florida; EL: Hammond, Louisiana.

M3: Eastville, Georgia; PE: Eastville, Georgia; PMS: Pascagoula, Mississippi; MS: Hurlley, Mississippi; WLA: DeQuincy, Louisiana.

\*Two sites form the same genetic cluster, and one might be the result of range expansion from the other or vice versa.

<sup>†</sup>The deduced source of MM is most likely SZ/HK site complex (see text more details).

(expressed as  $A'_R$  and  $H'_E$  in Table 2) in introduced populations from China were only slightly lower than the estimates for Mississippi ( $A'_R = 3.2$ ;  $H'_E = 0.413$ ), whereas both estimates for the two introduced areas were substantially lower than those for Formosa, Argentina ( $A'_R = 7.8$ ;  $H'_E = 0.542$ ).

## Discussion

### *Multiple introductions of Solenopsis invicta into China*

A recent study by Ascunce *et al.* (2011) suggested two separate introductions of fire ants occurred into China, which have given rise to the distinct populations on each side of Lingding Bay (e.g. Hong Kong and Macao). Our genetic study extends this earlier work and confirms our prediction of a third independent introduction of fire ants into China corresponding to the large population at Wuchuan (WC). The fact that recently invaded areas in China (e.g. Lingding Bay and Wuchuan) are at or near important international commerce centres in south-eastern China reinforces the importance and role of an interconnected global transportation network in the inadvertent movement of fire ants. Also, the common finding of multiple introductions into recently invaded areas (e.g. Taiwan, China, Australia) raises concerns that the mixing of individuals from different sources may result in unique genotypes with high invasive or adaptive potential (Reusch *et al.* 2005; Suarez & Tsutsui 2008) or, for species such as fire ants with complementary sex determination (Ross & Fletcher 1985, 1986; Hedrick *et al.* 2006), may reduce the genetic load associated with producing sterile diploid males through increased genetic variation of sex-determining locus (Cook 1993). Thus, management efforts that focus on suppression of local populations also should monitor the connectivity between introduced populations. This latter task particularly is critical for the *S. invicta* populations across Lingding Bay, where two genetically distinct clusters currently are separated by <10 km of uninhabited territory.

### *Propagule pressure as a potential predictor for source population*

Several analyses of our genetic data indicate that the immediate source of the third introduction appears to be the USA, more specifically, an area at or near our sampling site (Austin, Table 4) in Texas. These results coupled with the fact that Texas also ranks first among fire ant-infested states in the southern USA in the exportation of goods to China lend support to the prediction that volume of trade may represent a general

predictor of the potential for a given area or region to serve as the source population for an invasive species, especially those that are transported as stowaways. While the amount of trade is not the direct causal determinant of establishment probability, it does reflect the intensity of anthropogenic activities that in turn may affect the invasion process, especially propagule pressure (Pyšek *et al.* 2010). Clearly other factors not captured in trade statistics also are important in shaping the invasion success of a given species (e.g. density and distribution of a given species within potential source areas, probability of survivorship during the transportation, suitability of new environment for species' establishment and spread) given the fact that the invasion process is comprised of multiple interrelated steps through which successful invaders must overcome before successful establishment (Sakai *et al.* 2001). Along these lines, while California would seem to be a more plausible source for ants in WC based on the trade volume and traffic with China, the low densities and sporadic distribution of *S. invicta* in California (Jetter *et al.* 2002) presumably would decrease the probability of inadvertent transport associated with human activity.

### *Movement and spread of S. invicta in China: a local bridgehead effect*

Our data support our prediction of numerous instances of long-distance jump dispersal of *S. invicta* within China. First, when considering only the main sites, only one pair of geographically adjacent sites, MM and WC, are highly genetically differentiated. Despite the close geographic proximity of these two sites (<30 km), MM ants have greater genetic affinity with ants from sites SZ and HK than ants from site WC. The reduced genetic diversity in MM combined with the fact that all microsatellite alleles found at this site are a subset of those in SZ or HK suggests that MM likely is the result of long-distance dispersal from an area near SZ or HK. Second, multiple lines of evidence suggest that two outlier sites, NN and ZJJ, were colonized by ants from at least two different source populations (Fig. 4; Table S2, Supporting information). Lastly, virtually every genetic analysis performed assigned with high probability individuals from the majority of outlier sites in Guangxi Province to WC, which is 80–350 km distant from these outlier sites.

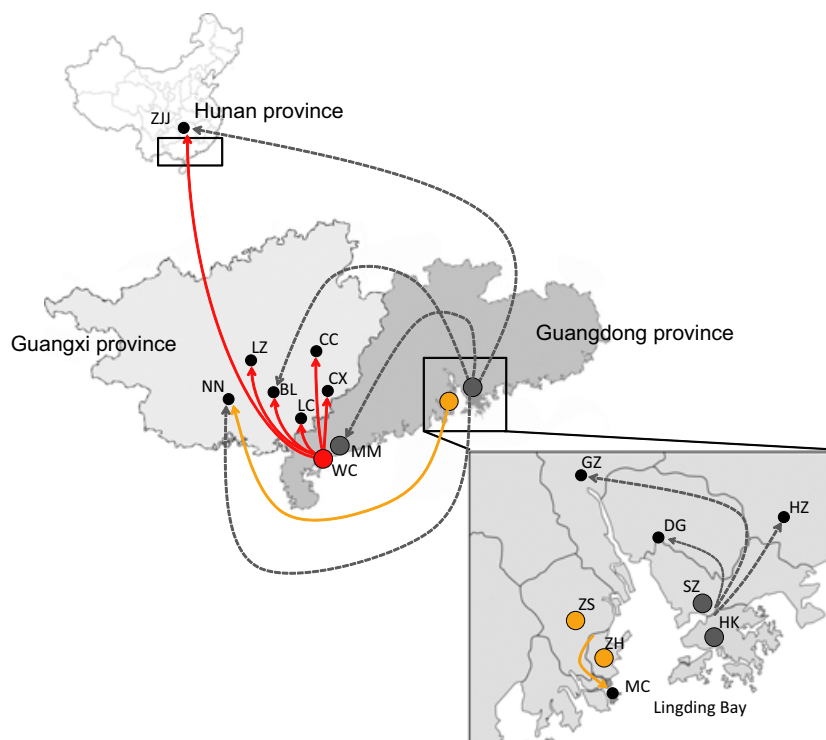
Our prediction that most colonies at outlier sites in Guangxi and Hunan Provinces are the result of jump dispersal of ants of the polygyne social form (which features multiple egg-laying queens per nest) was also supported by the genetic data. Under natural conditions, spread of this form is primarily through colony

budding, a process whereby a small group of queens and workers travel on foot to establish a new colony nearby their natal colony (Vargo & Porter 1989). While sexuals of this social form are capable of participating in aerial mating flights (Goodisman *et al.* 2000), their movement tends to be fairly local such that natural dispersal alone likely cannot account for the rapid widespread expansion or long-distance movement of polygyne fire ants in China (Porter *et al.* 1988; Porter 1993). On the other hand, the propensity of polygyne ants to spread to distant locales by unnatural means (i.e. human-mediated movement), which can be attributed partly to high nest densities and the high probability that transported fragments contain at least one queen and dozens of workers (King *et al.* 2009; Yang *et al.* 2009), has been documented in other recently invaded areas including Australia and Taiwan (Yang *et al.* 2009; Jane Oakey, personal communication). While it is unclear how often polygyne colonies survive such long-distance transportation, the documented invasion history of polygyne fire ants in the USA suggests that these ants clearly can establish themselves over broad areas and that these areas may also serve as additional potential sources for subsequent colonization of other areas (Tschinkel 2006; King *et al.* 2009). Such long-distance movement of largely polygyne fire ants within infested countries is analogous to the patterns of global invasion and spread of *S. invicta* (Ascunce *et al.* 2011). Repeated introductions of an invasive organism from a

single source population which is itself established by recent invasion have been termed the invasive bridgehead effect (Lombaert *et al.* 2010). Ascunce *et al.* (2011) demonstrated that the original source of fire ants in recently invaded areas can be traced to the southern USA; our results suggest a similar pattern once fire ants invade a new area as nearly all outlier colonies are polygyne and originate from a common source population (WC). Hence, for socially polymorphic ant species such as *S. invicta*, the composition of source populations in terms of the social forms present may be a predictor for invasion success not only during the global spread of fire ants but also during the postestablishment population expansion phase within invaded areas. In summary, our results indicate that long-distance dispersal, likely facilitated by human-aided transport primarily of the polygyne social form, has been a prevailing means of spread of *S. invicta* throughout south-eastern China.

#### *Loss of genetic diversity and invasion success of S. invicta*

Loss of allelic diversity as a result of a reduction in population size is a typical consequence of the introduction of species into new environments (Dlugosch & Parker 2008; Ficetola *et al.* 2008). Results of three separate analyses (heterozygosity excess, *M* ratio and comparison of genetic diversity indices) revealed the predicted pat-



**Fig. 4** Inferred routes of colonization of *Solenopsis invicta* within China. The colours of filled circles for the main sites correspond to each of the three major genetic clusters identified by STRUCTURE (see Fig. 2). All other collection sites (outlier sites) are represented by black-filled circles.

terns characteristic of genetic bottlenecks for populations of *S. invicta* at each main site in China. These results are likely quite robust given the large number of polymorphic microsatellite loci employed for the present study (Luikart *et al.* 1998; Piry *et al.* 1999; Spencer *et al.* 2000; Garza & Williamson 2001; Williamson-Natesan 2005). The minor differences in genetic diversity estimates ( $A'_R$  and  $H'_E$ ) between populations from China and the USA are not surprising when one considers that the initial introduction of fire ants into the USA from South America likely led to a substantial loss of many rare alleles (Shoemaker *et al.* 2006; Caldera *et al.* 2008). This initial introduction presumably resulted in an allelic frequency spectrum in the USA skewed towards relatively high frequency alleles (higher proportion of alleles with high frequencies) so that the subsequent loss of rare alleles likely was relatively minor during secondary introductions from the USA into China.

The finding of a consistent reduction in genetic diversity associated with all three independent introductions from the same source population likely reflects the fact that the numbers of original founders into each area were quite small (at the very least, the numbers of lineages surviving from the initial invasion through subsequent population growth are relatively small; Ciosi *et al.* 2008). Despite evidence for reduced genetic variation within invasive *S. invicta* populations compared with native populations, the consequences of such reduced genetic variation, if any, are not clear. In many haplodiploid social insects with complementary sex determination, such as *S. invicta*, a reduction of genetic diversity at the sex locus (loci) leads to higher proportions of queens that produce diploid males. The production of diploid males often represents a significant mortality factor because of their inviability and/or sterility (Ross & Fletcher 1985; Hedrick *et al.* 2006) and increases extinction proneness (Zayed & Packer 2005). Nonetheless, the genetic load associated with producing a high proportion of diploid males in USA polygyne fire ant populations (Ross & Fletcher 1985; Ross *et al.* 1993) seems of little consequence given its stunning success as an invader. Similar patterns are also evident in introduced *S. invicta* populations in China as well as other newly introduced populations (Australia and Taiwan, Henshaw *et al.* 2005; Yang *et al.* 2008), suggesting that the loss of substantial genetic diversity may not be a significant barrier to invasion success of this ant.

## Conclusions

Our genetic results provide support for the widespread notion that the introduction routes and subsequent spread of invasive species are linked to local and global trade and transportation networks. Thus, pathway risk

assessment integrating knowledge of the volume, sources and destinations of traded goods represents a potentially informative predictor for propagule pressure and for potential hotspots for inadvertent species introductions. Several additional patterns of genetic variation we detected also have implications for development of effective pest management strategies of *Solenopsis invicta* within China. For example, long-distance dispersal, likely facilitated by human-aided transport primarily of the polygyne social form, has been a prevailing means of spread of *S. invicta* throughout south-eastern China, which raises suspicions that additional infestations are likely to occur and will be discovered with increasing surveillance efforts. Indeed, the failure to recognize the ability of fire ants to spread by human means during the shipment and movement of nursery stock is partly responsible for the poor success of initial quarantine efforts to prevent the outward spread of fire ants after their initial invasion into the USA (Tschinkel 2006). Lastly, although clear signatures of recent genetic bottlenecks in introduced fire ant populations in China are evident, the consequences of loss of genetic diversity, if any, are not apparent given its successful establishment over a wide area in China in a relatively short time.

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### Data accessibility:

MtDNA sequences: GenBank accessions HM241155–HM241157.  
 Microsatellite data deposited in the Dryad repository: doi:10.5061/dryad.2j5r8v6v.

### Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1** Top US state exporters of goods to China from 2000 to 2005 (expressed as millions of US\$).

**Table S2** Assignment of colonies collected from outlier sites using different genetic analyses.

**Fig. S1** Principal components analyses (PCA) showing clustering of individuals based on microsatellite data.

**Fig. S2** Results from analysis of molecular variance (AMOVA) for different pre-defined groups as follows: (a) Main sites that are in close geographic proximity; (b) Same as (a) but data for individuals from sites ZH and ZS excluded; (c) Sites assigned to China\_B1 and B2 clusters; \* $P < 0.05$  (see text for various  $P$  values).

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