Global Invasion History of the Fire Ant *Solenopsis invicta*

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The fire ant *Solenopsis invicta* is a significant pest that was inadvertently introduced into the southern United States almost a century ago and more recently into California and other regions of the world. An assessment of genetic variation at a diverse set of molecular markers in 2144 fire ant colonies from 75 geographic sites worldwide revealed that at least nine separate introductions of *S. invicta* have occurred into newly invaded areas and that the main southern U.S. population is probably the source of all but one of these introductions. The sole exception involves a putative serial invasion from the southern United States to California to Taiwan. These results illustrate in stark fashion a severe negative consequence of an increasingly massive and interconnected global trade and travel system.

Invasive species pose major threats to agriculture, natural environments, and public health (*1*–*4*). Such species can displace native fauna, reduce biodiversity, serve as pathogen vectors, or impair ecosystem services (*3*, *4*). Given that most invasions are shaped by trends in human transport, their frequency of occurrence will probably increase with increasing global trade and travel (*4*–*7*). An important task in developing strategies to prevent or mitigate future invasions is to reconstruct the history and routes of introduction of exotics. Such knowledge facilitates the design of monitoring or quarantine programs targeting source areas or key transportation routes and provides necessary information for identifying effective biological control agents (*3*, *8*). Knowledge of the source of invading populations, and the genetic data on which this information is based, also inform hypotheses concerning the environmental and evolutionary factors responsible for successful biological invasions (*3*, *8*).

Fire ants (*Solenopsis invicta*) were inadvertently introduced into the United States early in the past century as stowaways in cargo shipped from their native South American range (*9*). They rapidly spread throughout the southern United States and, more recently, have been introduced to California and other regions of the world, including the Caribbean, Australia, New Zealand, Taiwan, Hong Kong, Macao, and China [collectively referred to as newly invaded areas (NIAs)]. The economic impact of fire ant infestations is enormous, with current estimated costs of control, medical treatment, and damage to property in the United States alone greater than $6 billion annually (*10*). Moreover, models of future range expansion based primarily on historical temperature and precipitation data show the potential for this ant to become established over almost half of terrestrial land masses (*11*). Clearly, a firm understanding of the patterns of global invasion by *S. invicta* is needed to assist efforts to curtail or reduce the impact of future introductions.

We sampled 2144 *S. invicta* colonies from 75 geographic sites distributed throughout the native, southern United States, and NIA ranges (table S1 and Fig. 1). We extracted DNA from a single individual per colony and generated genotypes at 66 nuclear microsatellite markers. We also sequenced portions of the mitochondrial DNA (mtDNA) genome and the nuclear gene *Gp-9* for individuals from large subsets of these colonies. We identified 322 unique mtDNA haplotypes, the majority of which (311) were confined to ants from the native range. Among the remainder, only three haplotypes were found in any NIAs (table S2). Although these three variants are effectively absent from most of the native range, occurring only at low frequencies (<5%) in eight populations in northeastern Argentina, the likely source area for *S. invicta* in the United States (*12*), they are the dominant haplotypes in the southern United States (Fig. 1 and table S2). This limitation of NIA haplotype variation to the three most common U.S. haplotypes suggests that the southern United States is the primary source of the NIA populations. Results from analyses of the highly variable nuclear gene *Gp-9* parallel the mtDNA results (fig. S1).

Bayesian genetic clustering analyses implemented in the program STRUCTURE (*13*) were run using the microsatellite genotypes. Almost all individuals were assigned with high probability (membership coefficients > 0.85) to one of two distinct clusters ($K = 2$), one consisting of ants from non-native areas (NIAs and the southern United States) and the other of ants from South America (Fig. 1A). The exception to this pattern involves three sites in northeastern Argentina: Clorinda, Herradura, and Formosa (called the ForA group henceforth), where individuals consistently had significant membership in both clusters (Fig. 1A), as expected if this area served as the original source (*12*). STRUCTURE simulations with higher values of K support these findings (figs. S2 and S3), as do simulations limited to the subset of individuals from just the introduced areas and the native ForA group (fig. S4). The consistent distinction in microsatellite variation between ants from introduced and native areas is concordant with the mtDNA and *Gp-9* results in suggesting that the United States is the immediate source of all NIA ants. Several additional analyses of the microsatellite data further substantiate this conclusion (figs. S5 to S7).

Although these results point to the United States rather than South America as the source of ants in NIAs, a result in keeping with the presumed disparity in propagule pressure related to the differing scales of the respective global transportation networks, they do not distinguish between the possibilities that NIA populations result from separate, independent introductions from the United States or, instead, from one or more serial introduction events, whereby ants in some NIAs are derived from another NIA. Results of STRUCTURE simulations using microsatellite data from invasive areas only (southern United States + NIAs) provide initial evidence that most or all NIA populations are independently derived from the United States (Fig. 1 and fig. S8). These simulations identified eight genetic clusters, distinctive sets of which correspond to each major geographic region (the United States; Taiwan; mainland China, Hong Kong, and Macao; and Australia); the sole

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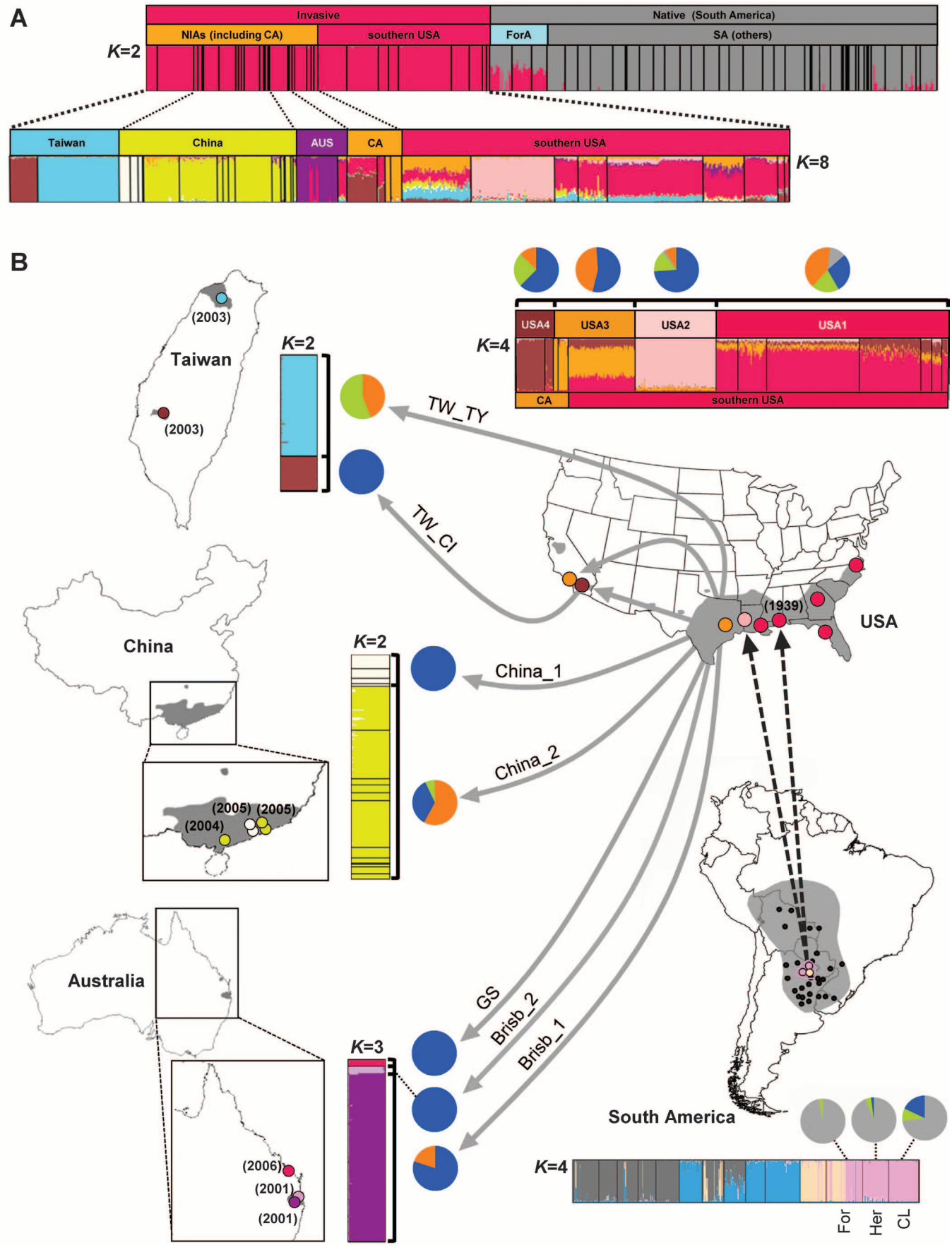
Fig. 1. Proposed global invasion pathways for *Solenopsis invicta* based on 67 nuclear markers and mtDNA sequences. (**A**) Assignment of 2144 individuals to $K = 2$ nuclear genetic clusters and 938 individuals from introduced populations to $K = 8$ clusters inferred from STRUCTURE simulations. (**B**) Genetic clusters for the United States, Taiwan, China (including Hong Kong and Macao), Australia, and South America, analyzed separately. Clusters for each introduced population are demarcated in brackets, with $K = 4$ for the United States, $K = 2$ for Taiwan, $K = 2$ for China, and $K = 3$ for Australia. Current native and introduced ranges of *S. invicta* are in gray. Small circles indicate sample sites, with the color of each circle corresponding to the cluster to which sampled individuals were assigned (black circles in the native range represent sites not belonging to the ForA group). The first years of detection in the southern United States and at each NIA site also are indicated. Pie diagrams indicate mtDNA haplotype frequencies for individuals assigned to each of the introduced or native populations. The three common mtDNA haplotypes in NIAs and the United States are indicated in orange (H5_AT1), blue (H22_AT1), and green (H36_AT1); the remaining haplotypes (pooled) are shown in gray. Native-range haplotype frequencies are shown only for sites that are the presumed source of the U.S. populations, the ForA group (For, Formosa; Her, Herradura; CL, Clorinda). Gray arrows depict nine inferred routes of invasion into NIAs (labeled by predominant STRUCTURE cluster); black dashed arrows represent two separate invasions from South America to the United States (*22*). AUS, Australia; CA, California; SA, South America.



exception concerns a genetic cluster well represented in both Taiwan and California (fig. S8). STRUCTURE simulations run separately on each regional NIA support the existence of two or more distinct genetic clusters within each, with individual clusters corresponding almost perfectly to single sample sites or groups of sites (Fig. 1). These NIA clusters are also well represented in the southern United States. These results suggest two or more recent invasions from the United States into each major regional NIA (*14*, *15*). In total, we identified nine putative introductions into NIAs, each represented by a distinct genetic cluster (two each for Taiwan, China, and California, and three for Australia; Fig. 1). This conclusion that NIA ants are directly derived from the United States is well supported by additional population genetic analyses (figs. S5, S9, and S10, and table S3).

Finally, we inferred the sources of the nine NIA introductions using a model-based Bayesian inference framework [approximate Bayesian computation (ABC)] that assumes complex demographic scenarios such as those characterizing colonization events (*16*). The final selected scenarios based on the cumulative results under three scenarios and various parameter settings (fig. S11) (*13*) are presented in Fig. 1. In virtually every simulation using site data, a scenario of separate introductions from the United States into each NIA was supported with very high probability relative to the alternative serial introduction scenario (table S4A). The only exception involves a putative serial introduction whereby fire ants in southern Taiwan are derived from a California population, which itself is derived from an earlier introduction from the southern United States. Results for ABC analyses using STRUCTURE-defined genetic clusters within geographic regions are similar, but in a few instances suggest more specific U.S. source locations for some of the NIA populations (Fig. 1 and table S4B).

Our study indicates that fire ants have been introduced on no fewer than nine separate occasions to California, Asia, and Australia from the southern United States, where *S. invicta* populations previously were confined for decades. We consider this a minimum estimate, because our NIA sample collections were not geographically exhaustive, and analyses of a few individuals obtained in Trinidad and New Zealand (from intercepted colonies) suggest that these ants also originated in the United States (fig. S2). Although we find little evidence for serial introductions among regional NIA populations, long-distance human-mediated transport of *S. invicta* after an initial introduction probably explains the dispersion of a single genetic cluster (China_2) across several hundred kilometers in China (Fig. 1). Such long-distance transport evidently was responsible for much of the early spread of the ant within the southern United States (*17*).

Repeated introductions of an invasive organism from a single source population which was itself established by recent invasion has been termed the invasive bridgehead effect (*18*–*20*). Although it is predicted to be common (*7*), empirical demonstrations of the effect are limited (*18*, *19*). Nonetheless, its occurrence has implications both for understanding evolutionary shifts associated with introductions and for developing effective management strategies. For instance, our finding of repeated successful introductions of *S. invicta* from the southern United States suggests that particular population traits associated with its success there may have pre-adapted these ants for ready colonization of other areas. Alternatively, the repeated introductions could reflect higher propagule pressure from the United States relative to native areas, given that the probability of introductions increases with escalating traffic flow in global transportation networks (*7*). The utility of data such as ours for identifying and modifying specific means of conveyance responsible for fire ant invasions remains unclear. This is because fire ants, like many other invasive insects, are probably transported as cargo stowaways, the invasion potential of which is defined more by the tempo and mode of transport (a proxy for propagule pressure) than by any specific attributes of the commodity (*21*). Nonetheless, because invasions tend to originate from locations with high cargo movement volume that are highly connected to the transport network, the integration of knowledge of invasion routes with details of transport networks may usefully inform the deployment of such measures as targeted surveillance and incursion response actions designed to limit the risk of spread of stowaways within transport networks (*7*).

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23. We thank numerous individuals responsible for fire ant collections (see table S1) and the University of Florida Interdisciplinary Center for Biotechnology Research and Genetics Institute Fisher Bioinformatics Cluster for technical and computer support. We are grateful to S. Porter and D. Gotzek for reviewing an earlier draft of the manuscript. This work was supported by the National Research Initiative of the USDA National Institute of Food and Agriculture (grant no. 2006-35302-16561: Agreement No. 58-6615-7-137); the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Taiwan [grant no. 98AS-9.1.2-BQ-B1(1)]; and the National Science Council Executive Yuan, Taiwan (grant no. NSC97-2917-I-002-103). Sequence data have been deposited in GenBank (accession nos. HM231195-96, HM231200, and HM241155-65).

Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6020/1066/DC1
Materials and Methods
Figs. S1 to S12
Tables S1 to S7
References

6 October 2010; accepted 13 January 2011
10.1126/science.1198734

Cascading Effects of Bird Functional Extinction Reduce Pollination and Plant Density

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Reductions in bird numbers could hamper ecosystem services such as pollination, but experimental proof is lacking. We show that functional extinction of bird pollinators has reduced pollination, seed production, and plant density in the shrub *Rhabdothamnus solandri* (Gesneriaceae) on the North Island ("mainland") of New Zealand but not on three nearby island bird sanctuaries where birds remain abundant. Pollen limitation of fruit set is strong [pollen limitation index (PLI) = 0.69] and significant on the mainland but small (PLI = 0.15) and nonsignificant on islands. Seed production per flower on the mainland is reduced 84%. Mainland sites have similar adult densities, but 55% fewer juvenile plants per adult, than island sites. Seed addition experiments near adult *R. solandri* plants on the mainland found strong seed limitation 5 years after sowing for *R. solandri* but not for two other co-occurring woody species. This demonstrates a terrestrial trophic cascade.

Bird species have declined in range and density worldwide, raising concerns that the ecological services they provide, such as pollination and dispersal, may fail (*1*–*5*), with cascading impacts on biodiversity. However, there are few documented cases where failure of