BIODIVERSITY RESEARCH

Genetic relationships between native and introduced populations of the little fire ant *Wasmannia auropunctata*

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ABSTRACT

Native to much of Central and South America, the little fire ant *Wasmannia auropunctata* has been rapidly spreading throughout the world. In its introduced range, *W. auropunctata* is frequently linked with drastic reductions of ant diversity; anecdotal reports of damaging attacks on vertebrates are also common. As it poses an ever-increasing threat to biodiversity, *W. auropunctata* has emerged as a model system for the study of ecological differences between native and invasive ant populations. These studies have been hampered by a lack of information on the genetic relatedness between native and introduced populations. By investigating the genetic structure of *W. auropunctata* populations, we provide a framework for conducting phylogenetically independent tests of differences between these ants in their native and invasive ranges. Phylogenetic analyses, based on the *cox1/cox2* region of mtDNA, revealed at least three separate source populations of *W. auropunctata* distributed across two large clades. Much of the Caribbean region, presumably part of the native range, is inhabited by a clade of ants sharing very similar or identical mtDNA haplotypes, suggesting the possibility of multiple introductions or high levels of gene flow across that area. Most invasive populations in the Pacific were closely related to these ants. The invasive populations in Gabon and New Caledonia arise from another, relatively distantly related clade. Phylogenetically independent contrasts confirm McGlynn's (1999) observation that invasive *W. auropunctata* populations are smaller than native populations. Given the complex phylogeographical structure of *W. auropunctata* populations, future comparative work should correct for phylogenetic effects.

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INTRODUCTION

Invasive species may seriously alter the structure of native ecosystems, carrying substantial economic and environmental costs. Ants, which drive numerous ecological processes, are a particularly destructive class of invaders, whose effects range from displacement of native fauna, to restructuring plant communities, to damaging economic infrastructure and agriculture (Holway *et al.*, 2002). At the same time, substantial progress has been made in understanding the causes of invasive ant behaviour, in part because invasive ant colonies interact with their environment through relatively easily observed worker behaviour (Mikheyev & Mueller, 2006). For example, decreases in diversity at recognition alleles accompanying ant introductions repeatedly led to decreased intraspecific aggression, creating ecologically dominant supercolonies in the invasive range (Holway *et al.*,

1998; Tsutsui *et al.*, 2001; Giraud *et al.*, 2002). Other, less understood, correlates of ant invasiveness include smaller body size and omnivory (McGlynn, 1999; Holway *et al.*, 2002). However, despite potentially complex relationships between native and invasive populations, studies of invasive ant biology rarely, if ever, use phylogenetic contrasts to evaluate the strength of their correlations. Thus, the statistical validity of such conclusions becomes difficult to evaluate.

Knowledge of genetic relationships between source and introduced populations of invasive species is crucial for understanding the biology of invasion. For instance, relationship networks between native and introduced populations can help ascertain transmission routes and modes of introduction (Amsellem *et al.*, 2000; Jousson *et al.*, 2000; Suarez *et al.*, 2001; Hanfling *et al.*, 2002; Schaffelke *et al.*, 2002). Additionally, identification of the geographical location from where an invasive species originated

allows the selection of appropriate biological control agents (Jouvenaz, 1983) and to examine ecological and evolutionary changes that accompany an invasion (Tsutsui *et al.*, 2000; Krieger & Ross, 2002; Maron *et al.*, 2004).

In cases where an invader is localized to a small geographical area, the source may be relatively clear, especially in the presence of adequate historical records (Allen *et al.*, 1974; Duncan *et al.*, 2003). However, if the native range of an invasive species covers a large area, or if there are multiple introduced populations, the elucidation of source populations requires molecular markers, especially in species with difficult taxonomy (Suarez *et al.*, 2001; Tsutsui *et al.*, 2001; Collins *et al.*, 2002; Milne & Abbott, 2004).

The little fire ant *Wasmannia auropunctata* is native to the entire Neotropical region, as well as some temperate regions, ranging from mid-Mexico and the Caribbean to northern Argentina. Over the past 100 years, *W. auropunctata* spread widely across the Pacific and into Central Africa [see table 2 in Wetterer & Porter (2003)]. It is a pest in both its native and introduced ranges, frequently harassing plantation and garden workers with its painful and irritating sting (Spencer, 1941; Fabres & Brown, 1978). Unlike the better-studied invasive ants such as the red imported fire ant *Solenopsis invicta* and the Argentine ant *Linepithema humile*, which tend to invade mainland temperate regions, *W. auropunctata* has a long history of colonizing tropical habitats, especially islands [reviewed by Holway *et al.* (2002) and Wetterer & Porter (2003)]. However, like *S. invicta* and *L. humile*, *W. auropunctata* invasions often have negative impacts on the native ant fauna (Clark *et al.*, 1982; Lubin, 1984; Jourdan, 1997; Roque-Albelo *et al.*, 2000; Le Breton *et al.*, 2003; Walker, 2006). Additionally, some reports suggest that *W. auropunctata* attacks native reptiles, birds, and mammals (Roque-Albelo & Causton, 1999; Wetterer *et al.*, 1999; Jourdan *et al.*, 2001; Walsh *et al.*, 2004). Thus, determining the source population(s) of *W. auropunctata* invasions has become increasingly important as the range of this pest continues to expand and it increasingly endangers isolated island ecosystems and protected mainland habitats.

As the threats to conservation posed by *W. auropunctata* have increased, it has also become the target of comparative studies examining similarities and differences between populations in invasive and native ranges (Le Breton *et al.*, 2004; Errard *et al.*, 2005; Fournier *et al.*, 2005a). However, over the past 100 years, *W. auropunctata* has spread widely (Holway *et al.*, 2002; Wetterer & Porter, 2003) (Fig. 1). This, coupled with the large native range, makes it difficult to assign *a priori* the source population for any of the invasions, without relying on genetic data. In order to circumvent these complications, we attempted to establish genetic relationships between invasive and native *W. auropunctata* populations using mitochondrial markers. We then demonstrated the utility of this phylogeny for investigating the evolution of invasive ant traits by conducting phylogenetically independent test of McGlynn's (1999) observation that invasive *W. auropunctata* populations are smaller in size than native populations. To the best of our knowledge, this is the first phylogenetically corrected study of trait differences between native and invasive populations in insects.

METHODS

We sampled ants from 22 sites in the native range and 10 sites in the introduced range of *W. auropunctata* (Fig. 1). Most of the samples came from sporadic collections made by large number of workers (see Acknowledgements) over the past decade and were either point-mounted or stored in alcohol. Because the relationships of the ants within each collection were unknown and likely not comparable between samples, generally only one individual from each site was sequenced.

DNA extraction and amplification

We attempted to resolve relationships among *W. auropunctata* populations by using the mtDNA region between and including parts of cytochrome oxidase subunits I and II, which includes a spacer region, as well as a t-RNA leucine sequence. Initially we attempted to amplify this region with 'universal' primers that have been successfully used by Wetterer *et al.* (1998) to amplify this sequence in *W. auropunctata*. However, amplification using the universal primer pair was unreliable, so internal *Wasmannia*-specific primers were designed based on the few successfully amplified sequences. The sequences of the primers were WaF: 5′-TANCAATTCTTATATTCATTATCTGAGAAGC-3′ and WaR: 5′-TTANTATGNATTTCATCTGTAAGGTAAAG-3′, anchored in *cox1* and *cox2*, respectively. DNA from individual workers was extracted, amplified, purified, and sequenced as in Mikheyev *et al.* (2006). The specific temperature reaction conditions involved an initial denaturing step at 94 °C for 2 min, followed by 30 cycles at 94 °C for 10 s, 60 °C for 20 s, and 72 °C for 10 s. Information from both the forward and the reverse sequences were combined to improve sequence accuracy. The sequences are deposited in GenBank under accession numbers EF409383-EF409414.

Phylogenetic analysis

An unidentified non-*auropunctata* species of *Wasmannia* from Ecuador, as well as a *Mycocepurus goeldii* sequence from GenBank (AF016017), were used as outgroups. The 10–17 bp intergenic spacer region, which could not be aligned between ingroup and outgroup taxa was eliminated from the analysis. After GTR + G was selected as the best model of evolution using MODELTEST (Posada & Crandall, 1998), phylogenies were constructed using MRBAYES (3.1.1) (Huelsenbeck & Ronquist, 2001) and GARLI (0.951) (Zwickl, 2006). The data were not partitioned into codon positions. Other than the specification of the nucleotide substitution model, all MRBAYES and GARLI parameters were left as defaults. The Bayesian analysis was run for 693 000 generations before convergence was obtained, as measured by the average standard deviation of split frequencies of four concurrent runs dropping below 0.01, and the first 50% of the generations were discarded. A phylogenetic reconstruction using the codon-based model implemented in MRBAYES was also carried out, but produced qualitatively the same results, and is not discussed here. Additionally, as traditional phylogenetic methods may not be appropriate for intraspecific genealogies (Posada

Figure 1 *Wasmannia auropunctata* native range and sampling map. The black region represents the approximate native range of *W. auropunctata*. Circles represent sites sampled for the present study.

& Crandall, 2001), we constructed a median-joining network using NETWORK 4.1 (Bandelt *et al.*, 1999).

Head width analysis

The Bayesian phylogeny was used to test, in a phylogenetically corrected framework, McGlynn's (1999) observation that invasive populations of *W. auropunctata* are smaller. Head width is correlated with body weight and other morphometric parameters and is a standard proxy for body size (Hölldobler & Wilson, 1990). Therefore, we followed McGlynn's (1999) example in using this metric for comparing interpopulation size variation. Three *W. auropunctata* workers from each of the populations included in the phylogeny (except the Cuban population, where only one worker was available) were point-mounted and their head widths were measured with an ocular micrometer. Ants were labelled as native or introduced according to the review by Wetterer & Porter (2003), except in the case of Cocos Island, Costa Rica, which likely harbours an introduced population (Solomon & Mikheyev, 2005). The analysis was performed using averages of all the workers in a population sample. Given McGlynn's (1999) previous findings that invasive ants were smaller than native ants, one-tailed *P*-values were used. A Wilcoxon signed rank test was used when comparing phylogenetically independent contrasts computed using CAIC's (2.6.9) BRUNCH algorithm (Purvis & Rambaut, 1995). A Spearman rank correlation was used to analyse phylogenetically independent contrasts obtained in PHYLIP (3.66), with the degrees of freedom reduced to account for polytomies (Purvis & Garland, 1993; Felsenstein, 1995).

RESULTS

Phylogenetic analysis

Both the Bayesian and the maximum likelihood trees gave the same overall topology (Fig. 2). The relationships between popu-lations in the native and introduced ranges suggested that there have been at least three different source populations. Mitochondrial DNA reveals a complex genetic structure in the *W. auropunctata* populations with at least two distinct clades (Fig. 2). The two clades overlap in Brazil and in Ecuador. Aside from differences in the coding region, clade A differed from clade B by one indel in the spacer region. As seen in Fig. 3, the relationships evident in the median-joining network are largely the same as those of the traditional phylogenetic methods. Although the network shown has been generated using tolerance (ε) set to zero, greater values (up to three) do not substantially alter the network depicted despite increasing the number of median vectors.

Head width analysis

Introduced *W. auropunctata* population were slightly (~4%) smaller than native populations ($t_{28} = 2.7$, $P = 0.007$). In addition, the native populations were more variable in head size than the introduced populations ($F_{20,9} = 5.3$, two-tailed $P = 0.014$). Because the introduced populations were generally very closely related, and only three phylogenetically independent comparisons could be made in CAIC ($P = 0.091$), which is the lowest value obtainable using a Wilcoxon test given the sample size. Felsenstein's phylogenetically independent contrasts were significant ($N = 13$, $r_s = 0.55$, $P = 0.02$).

DISCUSSION

Both *W. auropunctata* clades (Fig. 2) have given rise to invasive populations, with at least three separate source populations being detected by the phylogenetic analysis. Lineages from clade A appear to have colonized many of the Pacific islands, whereas those from clade B invaded Gabon and New Caledonia. Cocos Island appears to have been invaded separately from the other Pacific islands. Given the Costa Rican government's repeated attempts at colonizing the island (Weston Knight, 1990), it is
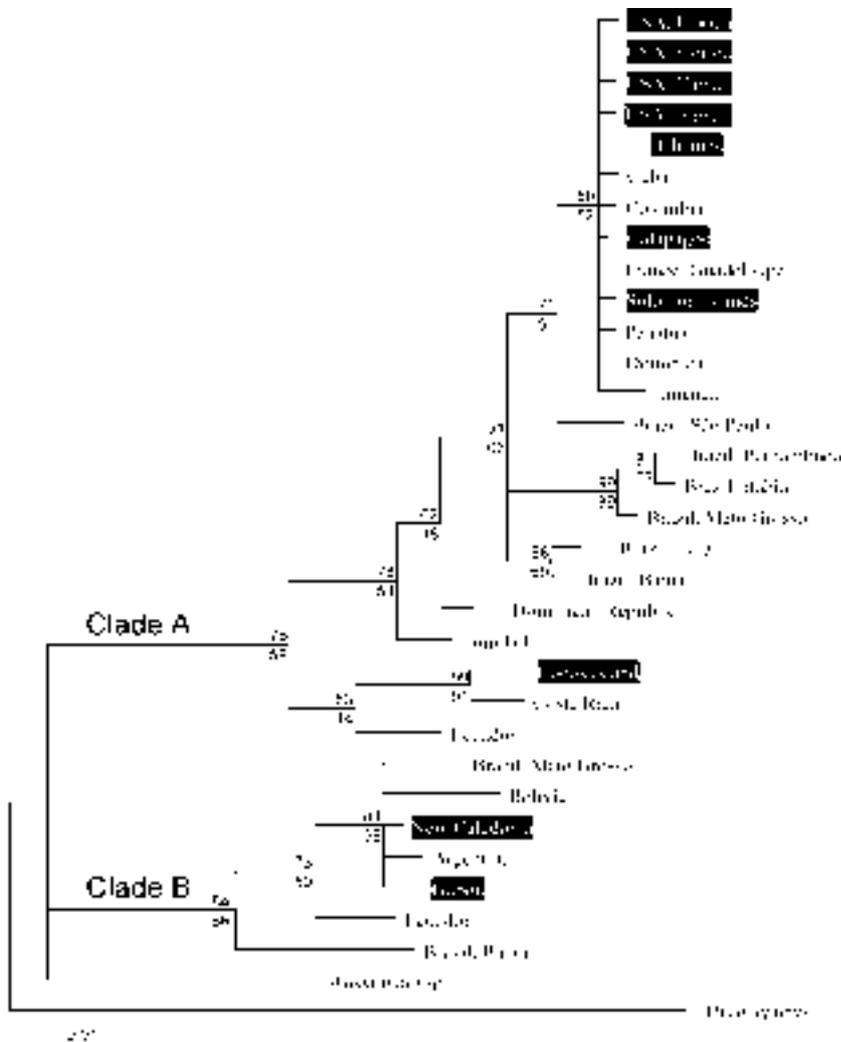
Figure 2 Bayesian tree of the phylogenetic relationships between *Wasmannia auropunctata* populations, with introduced populations highlighted in black. All nodes with > 50% support have posterior probabilities and likelihood bootstrap support values indicated, respectively, above and below the nodes. Note that the branch length of the *Mycocepurus* outgroup is not to scale.

likely that the source population originated from the Costa Rican mainland (Solomon & Mikheyev, 2005), which is also suggested by phylogenetic patterns (Fig. 2). The distribution of invasive populations from clade B, in Gabon and New Caledonia, is puzzling given that most all other Pacific islands have been colonized by the Caribbean clade. Both Gabon and New Caledonia are former colonies of France and likely have been historically linked through trade. Possibly, the relatively recent New Caledonian population arose from the older Gabonese population (Wetterer & Porter, 2003).

The existence of multiple introductions and the probable extensive transport of ants between invasive populations strongly imply that multiple introductions to any site might be responsible, as has been the case for the red imported fire ant (Ross & Fletcher, 1985), and invasive species in general (Sakai *et al.*, 2001). Growing evidence suggests that interspecific hybridization may occasionally produce highly competitive invasive hybrids (Ellstrand & Schierenbeck, 2000). Together with extensive ant transport, the existence of two clades of *W. auropunctata*, possibly representing two different species in the native range, makes interspecific hybridization in the invasive range also a distinct possibility.

Curiously, the unresolved clade that occupies many of the Caribbean islands and surrounding area is more closely related to ants of the Brazilian mainland than to ants from the Dominican Republic and Trinidad. The presence of closely related populations of *W. auropunctata* throughout the Caribbean region suggests the possibility of recent multiple introductions, perhaps from a Brazilian source. Alternatively, ancestral Dominican and Trinidadian populations may have been replaced by recent introductions from elsewhere. Such introductions may have either displaced or hybridized with native *W. auropunctata* populations, just as the introduction of Africanized honey bees has largely supplanted European mtDNA haplotypes in the New World (Clarke *et al.*, 2001). Given that *W. auropunctata* disperse slowly without human aid (Walsh *et al.*, 2004; Walker, 2006), the possibility that the Caribbean region enjoyed naturally high levels of genetic exchange and that the Dominican Republic and Trinidad were independently colonized from other mainland sources appears less likely. A detailed genetic study with highly polymorphic markers, such as microsatellite markers developed for this species (Fournier *et al.*, 2005b), may help resolve the two possibilities by elucidating fine-scale differences and similarities between the Caribbean populations.
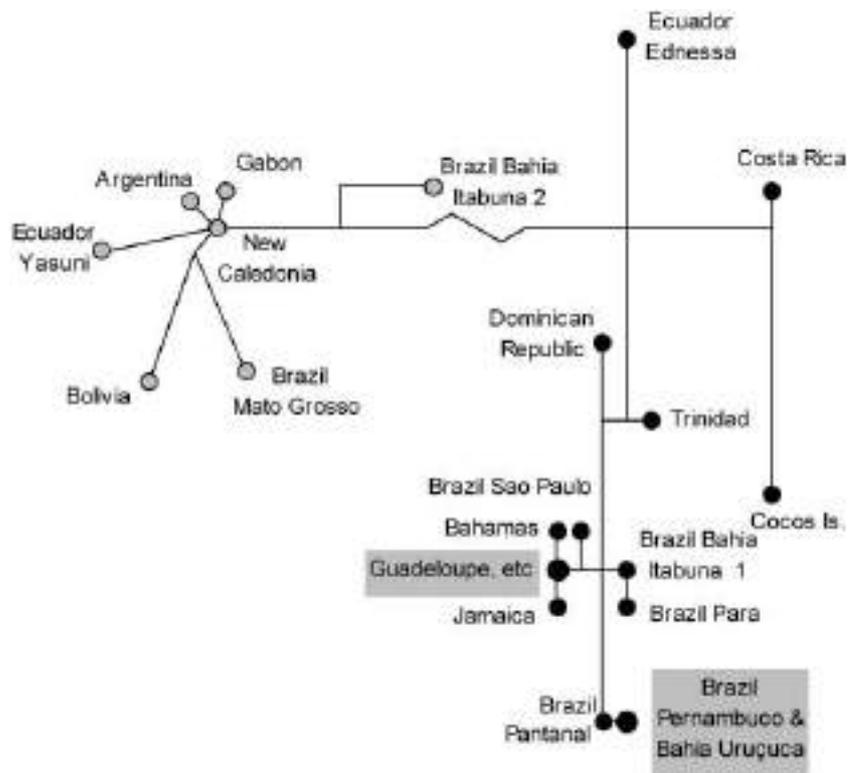
Figure 3 Median-joining network of the *Wasmannia auropunctata* populations. The length of the edges joining nodes is proportional to the number mutations between them, except in the case of the edge joining clades A (grey) and B (black), which has been scaled down by a factor of 2.5. Median vectors lie at the unlabelled junctions of the edges. Two of the nodes (grey underlay) represent more than one population. Specifically, the node 'Guadeloupe, etc.' also encompasses Cuba, Dominica, Florida, Galápagos, Hawaii, Panama, and the Solomon Islands.

The complex phylogeographical structure of *W. auropunctata* populations means that phylogenetic correction is essential for making meaningful comparisons between native and invasive populations. As several source populations exist (Figs 2 and 3), one cannot arbitrarily assume that a population within the native range is necessarily closely related to any given invasive population. Also, given the genetic diversity of *W. auropunctata* lineages that occur in the native range, and the virtual impossibility of elucidating the exact source of any introduced population, studies considering only a single source and a single invasive population may underestimate the range of variation present in the native range, with the possibility of drawing incorrect conclusions. By showing the patterns of genetic variation and relatedness in native and invasive populations, our work allows for the design of future, more rigorous, phylogenetically controlled studies.

Although we have shown that McGlynn's (1999) observation that invasive *W. auropunctata* populations have smaller workers than those in the native range holds after phylogenetic correction, the reason for this phenomenon remains unclear. McGlynn suggested that differences in size may be linked to fighting ability, by decreasing colony investment per individual worker, possibly accelerating colony growth. Alternatively, the worker size differences may be correlated with life-history differences, such as higher levels of polygyny in the introduced range (McGlynn, 1999). The reason for worker size differences between native and invasive populations remains to a large extent unclear. However, given the apparent prevalence of the pattern, it may reflect important general differences in selective pressures on ants in their native and invasive ranges.

Although the large native range and multiple sites of introduction make *W. auropunctata* a challenging study system, these same features make it an interesting case study for invasion biology. Its tendency to colonize isolated islands allows for natural replication of invasion events, provided that the invasion history can be phylogenetically accounted for. Likewise, the multiple origins of the invasion provide an opportunity to examine parallel changes in invasive populations. Finally, more work is needed to understand why only a few of the populations in *W. auropunctata*'s large native range appear to have become invasive. The global range of *W. auropunctata* has greatly expanded in the past several decades (Wetterer & Porter, 2003) and the number of introduced population will likely only continue to increase. The present phylogenetic framework supplies the foundation to understand these novel introductions and to help combat the future spread of this damaging ant.

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