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Chloroplast and microsatellite DNA diversities reveal the introduction history of Brazilian peppertree (*Schinus terebinthifolius*) in Florida

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Abstract

Brazilian peppertree (*Schinus terebinthifolius*) is a woody perennial that has invaded much of Florida. This native of northeastern Argentina, Paraguay, and Brazil was brought as an ornamental to both the west and east coasts of Florida at the end of the 19th century. It was recorded as an invader of natural areas in the 1950s, and has since extended its range to cover over 280 000 ha. Our goals were to understand the history of this invasion, as one step toward understanding why this exotic was so successful, and ultimately to improve development of biological control agents. We sampled plants from the native and exotic ranges, particularly Florida, and genotyped these individuals at nuclear and chloroplast loci. Nuclear microsatellite and cpDNA loci reveal strong genetic population structure consistent with limited dispersal in the introduced and native ranges. Bayesian clustering of microsatellite data separates the east and west coast plants in Florida into distinct populations. The two chloroplast haplotypes found in Florida are also concordant with this separation: one predominates on the east coast, the other on the west coast. Analysis of samples collected in South America shows that haplotypes as distinct as the two in Florida are unlikely to have come from a single source population. We conclude that the genetic evidence supports two introductions of Brazilian peppertree into Florida and extensive hybridization between them. The west coast genotype likely came from coastal Brazil at about 27° south, whereas the east coast genotype probably originated from another, as yet unidentified site. As a result of hybridization, the Florida population does not exhibit low genetic variation compared to populations in the native range, possibly increasing its ability to adapt to novel environments. Hybridization also has important consequences for the selection of biocontrol agents since it will not be possible to identify closely co-adapted natural enemies in the native range, necessitating more extensive host testing.

Keywords: Brazilian peppertree, exotic, genetic diversity, introductions, invasive, population genetic structure, *Schinus terebinthifolius*

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Introduction

Invasive exotic species are an increasingly important problem in natural ecosystems (Mack *et al*. 2000; Sakai *et al*. 2001). Pimentel *et al*. (2000) estimated that 50 000 nonindigenous species have been introduced into the United States alone, causing annual economic losses totalling up to $120 billion in agriculture, forestry, and other segments of the economy (Pimentel *et al*. 2005). Invasive species also cause huge losses to biodiversity (Wilcove *et al*. 1998). In the United States, Hawaii and Florida stand out as the most susceptible to exotic species invasion with over 4000 and 925 introduced species, respectively (Cox 1999).

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Hypotheses explaining why certain alien species are successful invaders can be broadly divided into those that focus on the invaded habitat and those that focus on the invader (Hierro *et al*. 2005). Despite the different perspectives, however, the hypotheses are complementary. Some habitats may be vulnerable to invasion because they have vacant or under-utilized niches, low species richness, increased human disturbance regimes, or be inherently less competitive than the community from which the invader originated (Elton 1958; Lonsdale 1999; Callaway & Ascheborg 2000; Levine 2000; Daehler 2003). Invaded habitats also may lack the natural enemies including seed predators, herbivores, pathogens, and competitors that control the invasive species in its native habitat (Keane & Crawley 2002).

Characteristics of particular species such as high intrinsic population growth rates, high phenotypic plasticity, and a superior ability to utilize local resources compared to native species may pre-adapt them to becoming invasive (Kolar & Lodge 2001; Sakai *et al*. 2001). Characteristics that contribute to the invasive nature of an exotic species may also have evolved in response to selection pressures exerted by the novel habitat (Blossey & Notzold 1995; Sakai *et al*. 2001; Lee 2002; Allendorf & Lundquist 2003). Due to founder events, introduced species intuitively would often seem to have low genetic diversity that would limit their ability to adapt to novel conditions. Yet, invasive species provide some of the best examples of rapid evolutionary change (Lee 2002; Allendorf & Lundquist 2003; Stockwell *et al*. 2003). This potential paradox may be explained by the growing evidence that successful invasive species are often introduced multiple times (Kolar & Lodge 2001). In addition to obvious demographic benefits, multiple introductions can increase genetic variation by increasing population size or by mixing genetically distinct source populations or species (Ellstrand & Schierenbeck 2000; Allendorf & Lundquist 2003; Kolbe *et al*. 2004; Petit *et al*. 2004). In plants, there are many examples of hybridization between species that has led to invasiveness (Huxel 1999; Mooney & Cleland 2001; Schaal *et al*. 2003; Petit *et al*. 2004). Hybridization also occurs between distinct source populations within a single species; however, in these cases it is inherently harder to show that hybridization causes invasiveness (Kolbe *et al*. 2004). Ellstrand & Schierenbeck (2000) predict that intentionally introduced species, which are often introduced several times, are more likely to be involved in hybridization events than species introduced a single time.

In addition to having implications for the demography and evolution of invasive species, their introduction history can have important management implications for biocontrol (Roderick & Navajas 2003). Strict host specificity has been emphasized in the biological control of weeds to avoid the possibility of negative impacts on nontarget plants (McEvoy 1996; Pearson & Callaway 2003). Although host specificity has most often been considered at the subgenus or species level, there is increasing evidence of subspecific genotype specificity in insect herbivore/plant associations (Karban 1989; Nissen *et al*. 1995; Kaltz & Shykoff 1998). For example, variability in establishment of *Spurgia esulae* (Diptera: Cecidomyiidae) and *Aphthona* spp. (Coleoptera: Chrysomelidae) on invasive leafy spurge (*Euphorbia esula* L.) is associated with differences in genotype of the plant (Lym *et al*. 1996; Lym & Carlson 2002). Variability in insect populations also influences host compatibility. Goolsby *et al*. (2003) found that a Queensland population of an eriophyid mite collected for biological control of *Lygodium microphyllum* performed poorly on *L. microphyllum* from Florida, and suggested that the mite may occur in biotypes specific to different *L. microphyllum* populations. If an invasive species is an intra- or interspecific hybrid however, it will no longer have closely co-adapted natural enemies and so may require more extensive testing with biocontrol agents sampled from the different source regions. Application of molecular techniques for genetically characterizing target weed populations and biological control agents provides opportunities for more precise 'biotype matching' and for ascertaining the introduction history of the invasive species (Nissen *et al*. 1995).

Brazilian peppertree (*Schinus terebinthifolius* Raddi; Anacardiaceae) is native to Paraguay, northeastern Argentina, and Brazil and has been introduced into subtropical areas worldwide (Ewel *et al*. 1982). In Florida and Hawaii, Brazilian peppertree was introduced as an ornamental, and has since become one of the most widespread and destructive invasive plant species in these states (Smith 1985; Ferriter 1997; Schmitz *et al*. 1997). Historical accounts suggest that Brazilian peppertree was introduced at least twice into Florida around 1898. The tree was popularized on the west coast of Florida by an enthusiastic amateur botanist Dr George Stone, who lived in Punta Gorda (Austin 1978; Morton 1978). According to Nehrling (1944), Dr Stone brought Brazilian peppertree seeds 'somewhere from Brazil'. He distributed these seedlings freely to friends and had them planted along city streets in Punta Gorda. At about the same time, seeds of unknown provenance were sent to the Plant Introduction Service in Miami and some seedlings were distributed locally (Morton 1978). Following its arrival in Florida, there was a lag of 50–60 years before the species was recorded as an invader of native vegetation (Ewel *et al*. 1982). Since that time, Brazilian peppertree has spread to cover more than 280 000 ha in south Florida alone (Ferriter 1997; Schmitz *et al*. 1997).

Our objectives in this study were (i) to characterize genetic variation in exotic and native populations of Brazilian peppertree, (ii) to determine if the genetic data are concordant with the historical accounts of introduction into south Florida, and (iii) if evidence of multiple introductions exists, to determine how differentiated the source

populations may have been. Our data will provide the first step in identifying the best areas to seek closely co-adapted natural enemies that could be used in a Brazilian peppertree biological control program (Habeck *et al*. 1994; Hight *et al*. 2002; Cuda *et al*. 2004).

Materials and methods

Study species

In Brazil, five varieties have been recognized: *Schinus terebinthifolius* var. *terebinthifolius*, *S. terebinthifolius* var. *raddianus*, *S. terebinthifolius* var. *rhoifolius*, *S. terebinthifolius* var. *acutifolius*, and *S. terebinthifolius* var. *pohlianus* (Barkley 1944). The Florida population of Brazilian peppertree has not been described at the varietal level in part because many of the morphological characteristics used to distinguish them are broadly overlapping and it is unclear how well differentiated these varieties are in the native range (Ferriter 1997). In Florida, Brazilian peppertree grows to about 13 m in height, sprouts easily from the root base, and is often multistemmed (Ewel *et al*. 1982). Plants of this dioecious species may flower and fruit within 3 years of germination. Brazilian peppertree flower mainly in the fall and are pollinated by a variety of native flies and wasps (Ewel *et al*. 1982). Seed set is from November to early April, although drupes have been observed on the plant almost throughout the year (Ewel *et al*. 1982). The plants produce fruit when very few other plants are fruiting, especially so in late winter months, and the red drupes are widely consumed and dispersed by birds and mammals such as raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*) (Ewel *et al*. 1982; Ewel 1986). Brazilian peppertree has invaded a range of habitats in Florida, from abandoned fields, to native sawgrass marshes, mangrove, and pineland ecosystems (Doren & Jones 1997). Brazilian peppertree often gains a foothold in native communities by being an efficient colonizer of disturbances due to hurricanes and human habitat alteration (Ewel 1986; Horvitz & Koop 2001). In its native habitat, Brazilian peppertree is reported to occur as scattered individuals in a variety of habitats (Ewel *et al*. 1982). In Florida and Hawaii, however, it forms dense, monospecific stands that preclude understorey plant growth (Ewel 1986). Brazilian peppertree may contain allelopathic compounds that further inhibit the growth of competing vegetation (Morton 1978; Morgan & Overholt 2005). It also seems to tolerate a wide range of salinity, flooding, and burning regimes (Doren & Jones 1997).

Sample collections

We sampled 139 individuals from six south Florida cities located on the west coast (Punta Gorda, Sanibel, and Naples) and east coast (Miami, Florida City, and Key Largo) (Fig. 1);

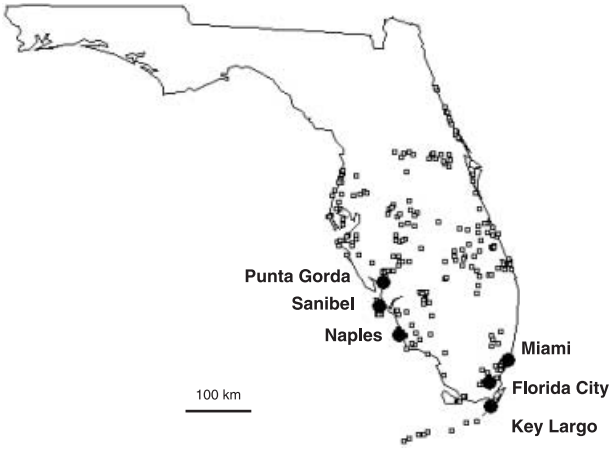

Fig. 1 Sampling sites within Florida. Six closed circles show locations of cities; 215 open squares show localities of individual plants.

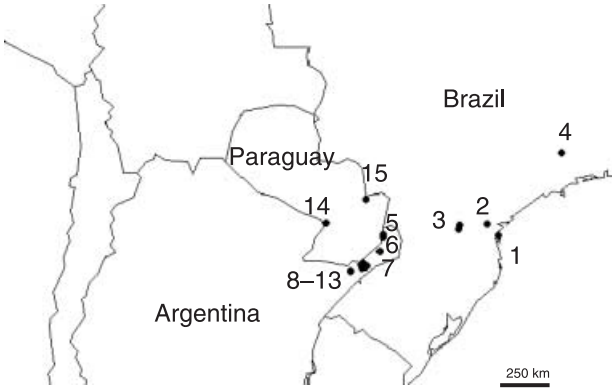

Fig. 2 Sampling localities in Brazil, Argentina, and Paraguay. Numbers refer to: 1, Balneário Camboriú; 2, Curitiba; 3, Irati; 4, Palmital; 5, AR13; 6, AR12; 7, AR11; 8–13, AR1,2,4–7; 14, Paraguay 1; 15, Paraguay 2.

these cities delineate the suggested introduction sites. We also sampled an additional 215 individuals from across the species range in Florida. In addition, 57 individuals were sampled from three other areas where Brazilian peppertree has been introduced: US Virgin Islands (St Tomas and St John), Texas, and Hawaii (Molokai and Hawaii). In the native range, a total of 82 individuals were sampled from nine sites in northwestern Argentina, 26 individuals were sampled from four sites in southeast Brazil, and two individuals were sampled in Paraguay (Fig. 2). For all analyses that use separate localities within South America (e.g. F_{ST}), we used nine sites for which we genotyped 10 or 12 individuals at the microsatellite loci and 10 sites for which we sequenced cpDNA from at least six individuals. Within these sites and in Florida, sampled individuals were at least 10 m apart. Leaf tissue was stored in 100% ethanol and DNA was extracted following the methods of Kim *et al*. (1997).

Microsatellite and cpDNA typing

All individuals were genotyped at five Brazilian peppertree microsatellite loci grouped into two multiplexes (Williams *et al*. 2002). Polymerase chain reactions (PCR) (10 μL) contained 50 ng DNA, 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1% Tween, 200 μM each dNTP, and 0.4 U *Taq* DNA polymerase. Multiplex A consisted of three loci with the following final primer concentrations: *StAAT25* (0.20 μM), *StAAT47* (0.25 μM), *StAAT55* (0.07 μM), and multiplex B consisted of two loci: *StAAT1* (0.20 μM), and *StAAT17* (0.15 μM). Reactions were cycled in a Hybaid PxE thermal cycler using the simulated tube function. Cycling parameters were one cycle at 94 °C for 2 min, followed by 30 cycles of 15 s at 94 °C, 15 s at 50 °C (multiplex A) or 55 °C (multiplex B), 30 s at 72 °C, and a final cycle at 72 °C for 5 min. Genotypes were scored using an ABI 310 Genetic Analyser and GENEMAPPER 3.0 (PE Biosystems).

An intergenic region in the cpDNA was amplified using primers trnS and trnG described in Hamilton (1999), and 716 base pairs were sequenced using BigDye Terminator Cycle Sequencing Kit version 1.1 (PE Biosystems). Sequences were analysed using an ABI 310 Genetic Analyser. Sequences were aligned manually using BIOEDIT (Hall 1999). We sequenced 50 individuals from across Florida and only found two haplotypes that could be distinguished by the number of repeats in a mononucleotide repeat. We subsequently designed primers around this microsatellite and screened the remaining 304 individuals in Florida. We completely sequenced all individuals sampled from Texas, Hawaii, and the US Virgin Islands and 87 individuals sampled in Argentina, Brazil, and Paraguay.

Statistical analysis

Analyses of microsatellite genotype data. Loci were tested for deviation from Hardy–Weinberg equilibrium (HWE) and genotypic linkage equilibrium using GENEPOP version 3.3 (Raymond & Rousset 1995). When loci deviated significantly from HWE we used the program MICRO-CHECKER (Van Oosterhout *et al*. 2004) to determine the most probable technical cause of the departure: heterozygote deficiencies at microsatellite loci are often due to null alleles, preferential amplification of smaller alleles in heterozygotes (short allele dominance) (Wattier *et al*. 1998), or difficulty in scoring heterozygotes due to stutter. Alternatively, heterozygote deficits result from population substructuring (Wahlund effect) and inbreeding. In contrast to technical causes, biological effects are expected to produce significant deficits in all loci. When MICRO-CHECKER indicated the presence of null alleles, we calculated the null frequency using the method of Brookfield (1996) and corrected allele frequencies accordingly. We calculated the number of alleles, observed heterozygosity (H_O), and Nei's gene diversity (H_E), using MICROSATELLITE ANALYSER (MSA 2.65) (Dieringer & Schlötterer 2002). We calculated the inbreeding coefficient F_{IS} and allelic richness (A_R) (corrected for sample size) for each locality using FSTAT version 2.9.3 (Goudet 2001). To statistically compare levels of A_R between sampling localities, we used Wilcoxon rank sum tests. To compare F_{IS}, H_O, and A_R between the six cities in south Florida and the nine sampling locations in South America, we averaged these values across loci within a sampling location and then permuted these values between the two groups (Florida and South America) 5000 times using FSTAT. We calculated the level of genetic differentiation between sampling localities using theta (Weir & Cockerham 1984) as an estimate of F_{ST} using FSTAT. We chose to use F_{ST} rather than R_{ST} because R_{ST} has been shown to have a larger standard error when using a small number of loci and samples (Gaggiotti *et al*. 1999). We determined statistical significance of pairwise F_{ST} values by permuting genotypes among populations 10 000 times using MSA.

We used a Bayesian-clustering method implemented in the program STRUCTURE version 2.1 to determine the number of possible introductions of Brazilian peppertree into Florida and to estimate the degree of hybridization between separate introductions (Pritchard *et al*. 2000; Pritchard & Wen 2003). STRUCTURE can cluster similar multilocus genotypes (microsatellite genotypes in this study) into K populations (K = the number of potential populations) without using the geographical locations of individuals. The model assumes linkage equilibrium (LE) and Hardy–Weinberg equilibrium (HWE). Departures from LE and HWE lead to samples being subdivided in a way that maximizes LE and HWE. The membership of each individual in a subpopulation is estimated as (q), the ancestry coefficient, that varies on a scale of 0–1.0, with 1.0 indicating full membership in a population. Individuals can be assigned to multiple clusters (with values of q summing to 1.0) indicating they are admixed. In this study, we categorized individuals with $q \geq 0.90$ as having pure ancestry within a population and individuals with $q < 0.90$ as being hybrids. Using only individuals sampled from the six south Florida cities, we first ran the MCMC (Monte Carlo Markov chain) for 10^6 iterations following a burn-in period of 10^5 iterations. We then ran three independent runs for $K = 1$ to 7 using the correlated allele frequencies model and assuming admixture (the default values). The model gave virtually identical results when we assumed no admixture. The three independent simulations for each K were highly concordant. We then repeated this analysis (using the same parameter values) using all individuals sampled in Florida for $K = 1$–10.

Analyses of cpDNA sequence data. For the cpDNA haplotype data, we calculated gene diversity within a locality using

ARLEQUIN version 2.000 (Schneider *et al*. 2000). We used an analysis of molecular variance (AMOVA) to partition genetic variation in haplotype frequencies within and between populations. We also used ARLEQUIN to calculate pairwise values of F_{ST} and Nei's average number of differences between populations (D) (Nei & Li 1979). Relationships among haplotypes were visualized by constructing a statistical parsimony network using the method of Templeton *et al*. (1992) and implemented in the program TCS (Clement *et al*. 2000).

The presence of spatial genetic structure in South America was tested by correlating pairwise F_{ST} values (for microsatellite and cpDNA data) and Nei's average number of differences between populations (D) (cpDNA data) with log-transformed geographical distance. We used a Mantel test implemented in the program IBD version 1.4 (Bohonak 2002) to ascertain the significance of these relationships.

Results

Within Florida

Within Florida, all five microsatellite loci were polymorphic with an average of four alleles each (range 2–7 alleles). Observed heterozygosity averaged 0.46 (range 0.24–0.63) (Table 1). All individuals produced PCR products at all loci with the exception of locus *StAAT47*, which did not amplify with repeated attempts in 18 individuals, strongly suggesting the presence of a null allele at this locus. Across Florida, all five loci exhibited significant heterozygote deficits (F_{IS} range: 0.15–0.51) ($P < 0.0001$ in all cases) and most pairs of loci (6 of 10) exhibited significant genotypic linkage disequilibrium ($P < 0.0001$ in all cases), suggesting the presence of population substructure.

Similarly, within south Florida cities all loci were polymorphic with the exception of *StAAT17*, which was monomorphic in Punta Gorda (Table 1). Average observed heterozygosity (0.46) and the number of alleles (3.4) were similar to the overall values. Genotypic linkage disequilibrium was not detected in the 60 pairwise locus comparisons within south Florida cities.

There was evidence of population structure among the south Florida cities, for which multilocus F_{ST} was 0.18 (95% CI 0.11–0.25) and F_{IS} was positive 0.09 (95% CI 0.02–0.23). Within cities six tests for heterozygote deficits were significant at the nominal 0.05 level (Table 1). MICRO-CHECKER indicated that the most likely cause of heterozygote deficits within cities was the presence of null alleles. The estimate of F_{ST} changed very little (0.17) after correcting allele frequencies, but lowered F_{IS} to –0.007. Correcting for null alleles in the subsequent analyses did not change our results and so we present uncorrected values only. Average allelic richness across loci within the cities ranged from 2.6 to 3.6 (based on 12 individuals). Allelic richness was similar between east and west coast cities (4.2 vs. 3.6, respectively). Twenty-one of the 22 alleles found in Florida were present in the six south Florida cities. Only three of the 21 alleles present in the cities were not shared between coasts and these were all found on the east coast. Pairwise F_{ST} values were significantly different between east coast cities and west coast cities. All comparisons of cities within coasts were insignificant, except between Punta Gorda and the other two west coast cities (Sanibel and Naples) (Table 2).

Chloroplast haplotype data also revealed population structure in south Florida. Two cpDNA haplotypes were found in Florida (A and B) at a frequency of 60% and 40%, respectively (Tables 3 and 4). Genetic variation was significantly partitioned among the south Florida cities ($F_{ST} = 0.28$, $P < 0.0001$). Pairwise F_{ST} values were not significantly different between Naples and the east coast cities. Sanibel and Punta Gorda, two other west coast cities, were significantly different from all other cities except each other (Table 2).

Bayesian clustering analyses of microsatellite genotypes, implemented in STRUCTURE, indicated that the most likely number of populations (K) in south Florida was two (Fig. 3A). The eastern cities of Miami, Florida City, and Key Largo cluster together, whereas Punta Gorda, Naples, and Sanibel form the other cluster (Fig. 4A). Individuals in the east coast cluster averaged 84% east and 16% west ancestry, whereas in the west coast cluster individuals averaged 71% west and 29% east ancestry. The relatively higher level of east coast ancestry on the west coast than vice versa was due to a higher level of admixture in Naples than either Punta Gorda or Sanibel (Fig. 4A). The distribution of the two cpDNA haplotypes is concordant with the groupings revealed by STRUCTURE; haplotype B is more common on the east coast (40 of 66 individuals) and haplotype A is more common on the west coast (59 of 73 individuals) ($\chi^2_1 = 25.04$, $P < 0.001$). Among the south Florida cities, the average proportions of east and west ancestry (q) were strongly correlated (Spearman rank correlation, $r_s = 0.89$, $n = 12$, $P < 0.0001$) with the proportions of the B and A haplotypes, respectively, providing further support of an association between the haplotypes and clusters delimited by STRUCTURE.

Clustering analysis of all individuals within Florida gave more ambiguous results. The highest log-likelihood value of the data conditional on K[$\ln(X \mid K)$] occurred at $K = 5$, or five populations. However, $\ln(X \mid K)$ increases very little from $K = 2$ to 5, but increases greatly from $K = 1$ to 2 (Fig. 3B). Pritchard & Wen (2003) point out that in such a case the true number of populations is probably closer to the lowest number that captures the major structure in the data. Therefore, we compared the geographical distribution of individuals for $K = 2$ to 5 and found that two

Table 1 Genetic diversity at microsatellite loci within sampling sites. N_A, number of alleles at a locus; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient; A_R, allelic richness corrected for sample size using 10 individuals

	Florida	South America	Miami	Florida City	Key Largo	Naples	Sanibel	Punta Gorda	US Virgin Islands	Texas	Hawaii	Irati	AR1	AR2	AR4	AR6	AR7	AR11	AR12	AR13
N	354	108	32	12	22	23	20	30	10	32	15	12	10	10	10	10	10	10	10	10
StAAT1																				
N_A	7	13	6	4	6	5	3	5	4	5	1	5	5	5	5	5	4	7	6	4
H_E	0.74	0.85	0.63	0.63	0.74	0.70	0.66	0.67	0.44	0.72	0	0.59	0.81	0.80	0.81	0.81	0.59	0.85	0.82	0.50
H_O	0.63	0.68	0.66	0.58	0.73	**0.48***	0.85	0.67	**0.1***	0.59	0	0.42	1.00	0.80	0.90	1.00	0.70	0.90	0.80	0.40
F_{IS}	0.15	0.20	−0.04	0.08	0.01	0.33	−0.30	0.01	0.78	0.18	NA	0.30	−0.26	0.00	−0.13	−0.26	−0.19	−0.07	0.03	0.21
StAAT17																				
N_A	5	25	4	4	3	3	2	1	2	3	1	8	5	7	6	6	5	5	5	4
H_E	0.38	0.93	0.62	0.62	0.56	0.20	0.14	0.00	0.52	0.15	0	0.86	0.77	0.74	0.79	0.80	0.71	0.78	0.69	0.71
H_O	0.32	0.44	0.66	**0.33***	0.55	0.22	0.15	0.00	0.5	0.13	0	**0.58***	**0.40***	**0.30***	**0.30***	0.90	0.70	**0.40***	**0.20***	**0.10***
F_{IS}	0.14	0.53	−0.06	0.47	0.03	−0.08	−0.06	NA	0.04	0.17	NA	0.33	0.50	0.61	0.64	−0.13	0.02	0.50	0.72	0.87
StAAT25																				
N_A	4	9	4	4	4	4	4	4	3	4	2	5	2	3	4	3	3	2	2	3
H_E	0.69	0.69	0.55	0.66	0.49	0.59	0.67	0.27	0.65	0.6	0.19	0.78	0.10	0.57	0.73	0.67	0.28	0.34	0.48	0.35
H_O	0.59	0.44	0.56	0.58	0.50	0.61	0.65	0.27	0.5	0.47	0.2	0.92	0.10	0.50	0.30	0.70	0.30	**0.00***	0.50	0.40
F_{IS}	0.15	0.37	−0.02	0.12	−0.02	−0.03	0.03	0.00	0.24	0.23	−0.08	−0.19	0.00	0.14	0.60	−0.05	−0.08	1.00	−0.05	−0.14
StAAT47																				
N_A	2	5	2	2	2	2	2	2	2	2	1	2	1	1	1	1	1	1	1	1
H_E	0.48	0.21	0.48	0.34	0.51	0.36	0.51	0.21	0.48	0.46	0	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H_O	0.24	0.02	**0.28***	0.42	0.48	0.27	**0.06***	**0.08***	0.3	0.29	0	**0.00***	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F_{IS}	0.51	0.91	0.42	−0.22	0.07	0.25	0.89	0.64	0.39	0.38	NA	1.00	NA	NA	NA	NA	NA	NA	NA	NA
StAAT55																				
N_A	4	7	2	4	3	3	3	3	2	3	2	2	1	2	3	3	2	2	2	2
H_E	0.62	0.44	0.29	0.47	0.47	0.66	0.67	0.66	0.27	0.64	0.24	0.52	0.00	0.10	0.59	0.28	0.34	0.48	0.52	0.27
H_O	0.50	0.38	0.22	0.58	0.36	**0.43***	0.60	0.77	0.1	0.59	0.13	0.50	0.00	0.10	0.70	0.30	0.40	0.50	0.70	0.30
F_{IS}	0.19	0.14	0.25	−0.25	0.23	0.35	0.10	−0.17	0.64	0.07	0.45	0.04	NA	0.00	−0.20	−0.08	−0.20	−0.05	−0.37	−0.13
Mean																				
N_A	4.4	11.8	3.6	3.6	3.6	3.4	2.8	3	2.6	3.4	1.4	4.4	2.8	3.6	3.8	3.6	3	3.4	3.2	2.8
H_E	0.58	0.62	0.52	0.54	0.55	0.50	0.53	0.36	0.47	0.51	0.09	0.61	0.34	0.44	0.58	0.51	0.38	0.49	0.50	0.37
H_O	0.46	0.39	0.47	0.50	0.52	0.40	0.46	0.36	0.30	0.41	0.07	0.48	0.30	0.34	0.44	0.58	0.42	0.36	0.44	0.24
F_{IS}	0.22	0.37	0.08	0.09	0.06	0.20	0.13	0.02	0.38	0.20	0.22	0.21	0.11	0.24	0.26	−0.15	−0.10	0.27	0.13	0.36
A_R	3.51	6.25	3.11	3.49	3.31	2.97	2.75	2.49	2.60	2.96	1.39	4.25	2.80	3.60	3.80	3.60	3.00	3.40	3.20	2.80

*Deviation from Hardy–Weinberg expectations ($P < 0.05$).

Table 2 Matrix of pairwise F_{ST} between south Florida cities for microsatellite loci (above the diagonal) and a cpDNA locus (below diagonal)

City	Florida City	Key Largo	Miami	Naples	Punta Gorda	Sanibel
Florida City		0.010	−0.004	**0.171**	**0.390**	**0.135**
Key Largo	−0.056		0.010	**0.116**	**0.332**	**0.138**
Miami	−0.049	−0.040		**0.166**	**0.377**	**0.172**
Naples	0.006	−0.020	−0.011		**0.100**	0.033
Punta Gorda	**0.683**	**0.545**	**0.513**	**0.417**		**0.106**
Sanibel	**0.503**	**0.386**	**0.375**	**0.255**	−0.003	

Values in bold are significant after Bonferroni adjustment.

Table 3 Sampling localities for *Schinus terebinthifolius* and the frequency of cpDNA haplotypes. Latitude and longitude are presented for sampling localities in the native range only

				Haplotype								
	Latitude	Longitude	*N*	A	B	C	D	E	F	G	H	I	J
Florida			354	212	142								
Florida City			12	4	8								
Key Largo			22	9	13								
Miami			32	13	19								
Naples			23	12	11								
Punta Gorda			30	29	1								
Sanibel			20	18	2								
Texas			32	25	7								
Hawaii			15	15									
US Virgin Islands			10	1	9								
South America			87	2	0	16	46	12	7	1	1	1	1
Argentina													
AR1	27°50′	56°12′	7				2		5				
AR2	27°30′	55°41′	7				6		1				
AR4	27°40′	55°38′	8				8						
AR5	27°34′	55°22′	2						1	1			
AR6	27°34′	55°22′	7				7						
AR7	27°25′	55°36′	10				10						
AR11	27°39′	55°25′	7				7						
AR12	26°48′	54°45′	7			7							
AR13	26°05′	54°35′	7			5	1				1		
Brazil													
Irati	25°47′	50°66′	12					12					
Curitiba	25°42′	49°29′	6			4	2						
Balneário Camboriú	26°59′	48°38′	2	2									
Palmital	21°45′	45°24′	3				3						
Paraguay 1	25°20′	57°31′	1									1	
Paraguay 2	24°08′	55°25′	1										1

populations gave a biologically informative pattern. The two populations delimited by STRUCTURE appeared to be roughly separated by several bodies of water: the Everglades in the south, Lake Okeechobee further north, and a series of lakes and rivers running through central Florida from Jacksonville south to Lake Okeechobee. Individuals in the eastern cluster averaged 68.2% east and 31.8% west ancestry, while in the western cluster individuals averaged 67% west and 33% east ancestry. Using this geographical separation, haplotype B is more common on the eastern side of Florida (94 of 140 individuals) and haplotype A is more common on the western side (144 of 214 individuals) ($\chi^2_1 = 40.4$, $P < 0.001$). At higher values of *K*, individuals from different geographical regions began to be apportioned

Table 4 Description of cpDNA haplotypes for *Schinus terebinthifolius*. Polymorphic sites and their position in base pairs are indicated. Entire sequences are in GenBank Accession nos AY928398–AY928407

	Position (bp)												
Haplotype	11	204	219	225	227	244	354	384	447	552	582	657	676
A	T	A_{11}	G	T	—	C	A	A	T	A	T	T	C
B	G	A_{14}	G	G	—	C	A	A	C	A	T	T	C
C	T	A_{14}	G	T	—	A	T	A	C	C	G	T	T
D	T	A_{13}	G	T	—	A	T	A	C	C	G	T	T
E	T	A_{14}	C	T	—	A	T	A	C	C	G	T	T
F	T	A_{12}	G	T	—	A	T	A	C	C	T	T	C
G	T	A_{11}	G	T	—	A	T	A	C	C	T	T	C
H	T	A_{15}	G	T	—	A	T	A	C	C	G	T	T
I	T	A_{14}	G	T	C	A	T	—	C	C	G	T	T
J	T	A_{12}	G	T	—	A	T	A	C	C	T	G	C

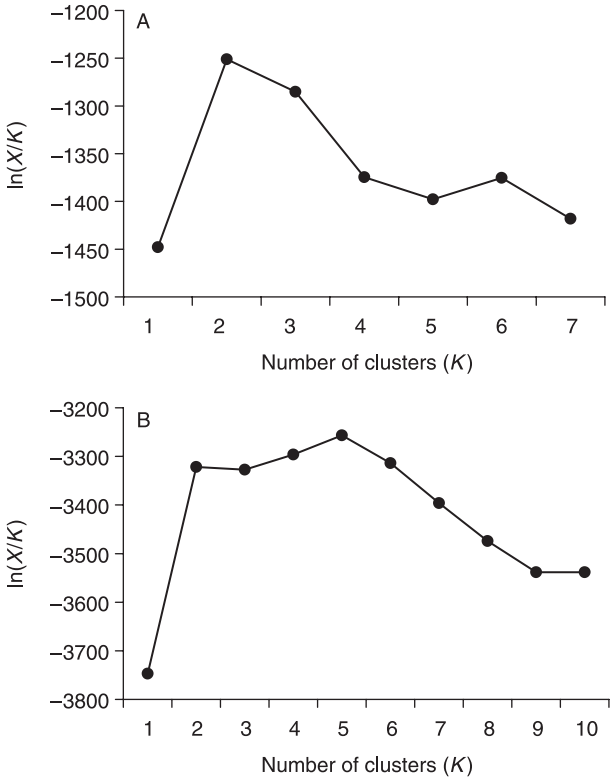

Fig. 3 Log-likelihood probability of the number of inferred clusters (*K*) (A) among six south Florida cities and (B) for all individuals in Florida estimated using STRUCTURE (Pritchard *et al*. 2000).

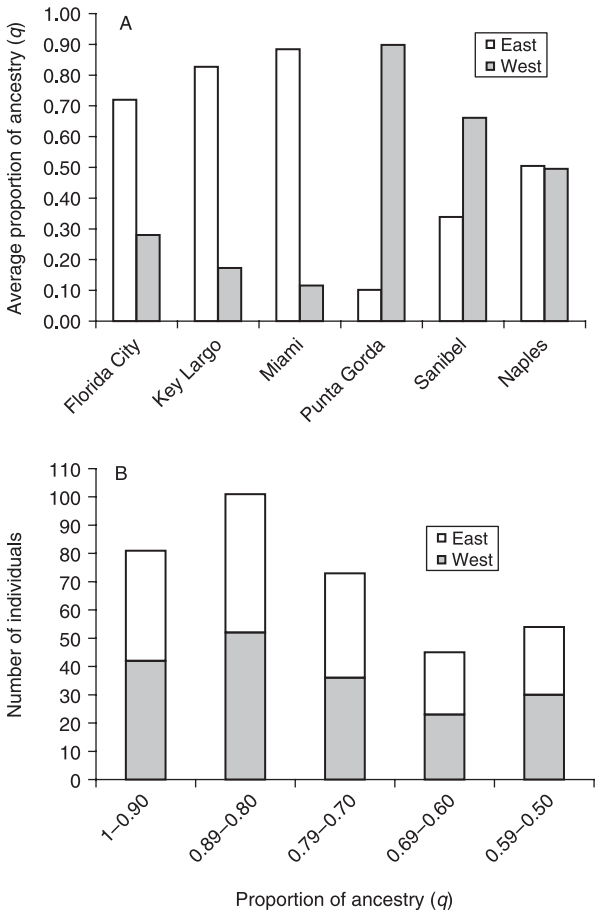

Fig. 4 The level of hybridization between east and west coast introductions of *Schinus terebinthifolius* (A) within six south Florida cities (presented as average individual ancestry coefficients *q*) and (B) as categories for all individuals in Florida. Hybridization was determined by calculating the proportion of ancestry (*q*) from the two clusters defined by STRUCTURE for each individual (see text). Individuals with *q* above 0.90 have high ancestry within a cluster while values lower than these indicate increasing levels of admixture between the clusters.

equally between clusters, further indicating that these values were less likely to represent true clusters.

We compared ancestry coefficients (*q*, from STRUCTURE) of individual plants to examine hybridization between the two assigned ancestral populations of the east and west coasts. Across Florida, most (75.7%) individuals were categorized as hybrids with less than 90% pure ancestry (268 of

354) (Fig. 4B). Of the 86 individuals that were categorized as pure (≥ 90% ancestry), 44 belonged to the eastern cluster and 42 belonged to the western cluster. Considering pure individuals to be only those that also contained a concordant haplotype (as defined above) reduced the numbers of pure individuals to 73 (34 east and 41 west) or 20.6% of all individuals.

Comparison of Florida with other exotic populations

At the microsatellite loci, Texas, Hawaii, and the US Virgin Islands contained a total of 18 alleles, all of which were found in Florida. Texas and the US Virgin Islands were polymorphic for all loci, while Hawaii was monomorphic at three of the five loci (Table 1). All loci were in Hardy–Weinberg equilibrium within these areas, except *StAAT1* in the US Virgin Islands. Within Texas, *StAAT47* gave evidence of a null allele since 11 of the 32 individuals failed to amplify at this locus even after repeated attempts. Allelic richness among the four non-native areas differed significantly (Kruskal–Wallis, $H = 11.86$, d.f. = 3, $P = 0.008$, based on 10 individuals each). Pairwise tests indicated that this difference was due to Hawaii having lower allelic richness than the other three sites (Mann–Whitney, $P = 0.01$ for all cases) (Table 1). These differences were not significant, however, after correction for multiple tests. In the cpDNA data, 'A' was the most common haplotype, present in 72% of all individuals ($n = 57$ individuals), whereas haplotype B was found in 28% of individuals. Haplotype A was the most common haplotype in Texas (in 80% of individuals) and the only haplotype found in Hawaii (Table 3). Haplotype B was the most common haplotype in the US Virgin Islands, found in 90% of individuals (Table 3). The partitioning of genetic variation among Florida, Texas, Hawaii, and the US Virgin Islands was pronounced for microsatellite loci ($F_{ST} = 0.14$) and for the cpDNA locus ($F_{ST} = 0.21$).

Comparison of Florida exotics to South American native populations

Brazil and Argentina had a total of 59 alleles (range 5–25 alleles per locus). Nine of the tests for heterozygote deficits in the nine localities had significant heterozygote deficits at the nominal 0.05 level (Table 1). Several loci were monomorphic within localities: *StAAT47* was monomorphic in eight of nine localities for the same allele, *StAAT55* was monomorphic in two localities, and *StAAT25* was monomorphic in one locality (Table 1). There was only one case of significant genotypic linkage disequilibrium after a correction for multiple tests (*StAAT1* and *StAAT25* in AR4).

Fifteen of the 22 total alleles found in Florida also were found in the South American samples. Six of the seven alleles unique to Florida also were found in the other three non-native areas (US Virgin Islands, Texas, and Hawaii). Allelic richness for the total South American sample was higher than the total sample from Florida (Mann–Whitney, $W = 40$, d.f. = 1, $P = 0.01$, mean $A_R = 11.8$ vs. 4.2, respectively, $n = 107$ individuals). Average allelic richness ($n = 10$ individuals), F_{IS}, and H_O among the six south Florida cities (mean = 3.0, 0.09, 0.45, respectively) was not significantly different, however, from the nine localities in Argentina and Brazil (mean = 3.4, 0.16, 0.40) ($P > 0.15$ in all cases) (Table 1). F_{ST} and F_{IS} among the nine localities in South America (F_{ST} = 0.19, 95% CI 0.15–0.24; $F_{IS} = 0.16$; 95% CI −0.06–0.39) were similar to these values among the south Florida cities. Similar to Florida, MICRO-CHECKER indicated that the heterozygote deficits observed were most likely due to null alleles. Adjusting allele frequencies for null alleles changed F_{ST} slightly (0.17) and lowered F_{IS} to 0.04 (95% CI −0.07–0.21).

The South American samples contained nine cpDNA haplotypes (A, C–J) (Tables 3 and 4). Haplotype B, the less common Florida haplotype, was not found in these samples. Haplotype A, the more common Florida haplotype, was found in two individuals from Balneário Camboriú, Santa Catarina in southeast Brazil. Haplotype D was the most common and widespread haplotype in Brazil and Argentina, occurring in 53% of all sampled individuals (Table 3). Gene diversity among the six south Florida cities (mean = 0.38) was generally higher than among the localities in South America (mean = 0.13); all of the Florida cities contained two haplotypes while many (6 of 10) sites within South America contained only one haplotype, although this difference is not significant (Mann–Whitney, $W = 65$, d.f. = 1, $P = 0.13$) (Table 3). Genetic variation at the cpDNA locus was much more strongly subdivided among sampling sites in the native range ($F_{ST} = 0.77$, $P < 0.0001$) than in Florida.

There was a positive relationship between pairwise F_{ST} values and geographical distance for both marker types, suggesting that Brazilian peppertree is structured in an isolation-by-distance pattern in its native range (Fig. 5A, B). This pattern remains for both markers when only considering the sampling localities in Argentina (microsatellites: $y = 0.08x + 0.09$, $R^2 = 0.28$, $P = 0.002$; cpDNA: $y = 0.66x - 0.29$, $R^2 = 0.46$, $P = 0.009$). The positive relationship between Nei's number of pairwise differences between populations (D) and geographical distance also indicates that more similar haplotypes tend to cluster together ($y = 1.16x - 0.28$, $R^2 = 0.26$, $P = 0.03$).

A parsimony network suggests that the A and B haplotypes found in the non-native populations are distinct from the three common haplotypes (C, D, and E) in South America (Fig. 6). Haplotypes found within a locality in South America tend to be similar and differ by one to three nucleotide positions (Table 3 and Fig. 6). The A and B haplotypes found in the non-native range differ from each other at six nucleotide positions (Table 4 and Fig. 6).

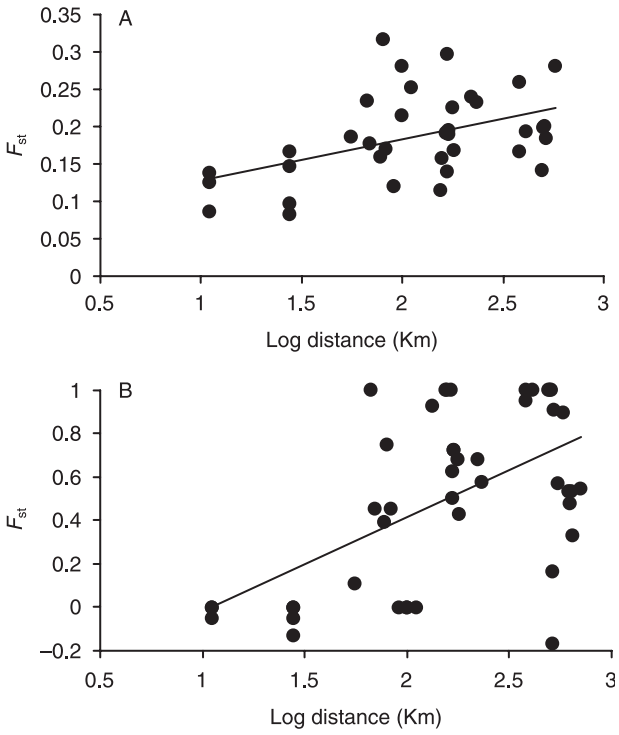

Fig. 5 The relationship of pairwise F_{ST} values between sampling sites in South America and pairwise log geographical distance for (A) microsatellite loci ($y = 0.06x - 0.07$, $R^2 = 0.21$, $P = 0.01$) and (B) a cpDNA locus ($y = 0.42x - 0.36$, $R^2 = 0.32$, $P = 0.003$).

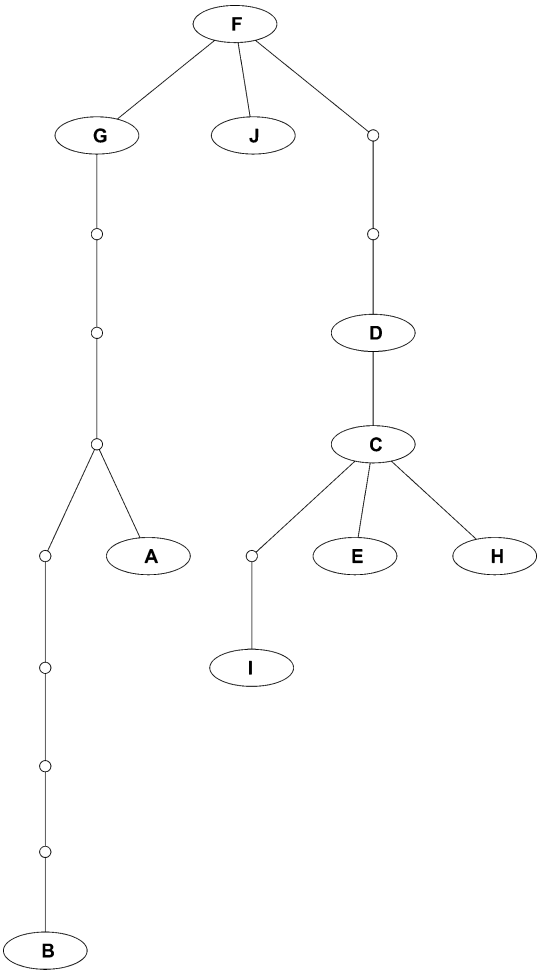

Fig. 6 Parsimony network of *Schinus terebinthifolius* chloroplast diversity. Each connecting line indicates one nucleotide difference and unlabelled nodes are inferred intermediates.

Discussion

Both microsatellite genotypes and cpDNA haplotypes indicate that Brazilian peppertree was introduced into south Florida from two distinct source populations. Hybridization between the two introductions has been extensive, yet not so great as to mask the signal of two separate introductions. The Bayesian modelling approach in STRUCTURE separates south Florida cities into two clusters that are concordant with the historical introduction points (Punta Gorda and Miami). Similarly, STRUCTURE indicates that all individuals within Florida are best defined by two clusters. There are only two cpDNA haplotypes within Florida and these are also concordant with the clusters indicated by the microsatellite data. Haplotype A is associated with the west coast introduction whereas haplotype B is associated with the east coast introduction. The two genetic data sets are in agreement on this east–west separation; the proportion of east–west ancestry calculated from nuclear genotype data is strongly correlated with the proportion of the A or B haplotype. Pairwise F_{ST} values among south Florida cities also indicate this geographical break. The eastern cities are significantly differentiated from the western cities, but cities are similar to each other within a region for both the microsatellite loci and cpDNA locus.

The distribution and relationships of haplotypes within South America also support two separate introductions to Florida. In South America, haplotype diversity within a locality is low and is highly structured among localities. At a single sampling location there is either a single haplotype or two haplotypes that are very similar to each other. In contrast, haplotypes A and B are distinct from each other at six positions. Since the positive relationship between Nei's genetic distance and geographical distance indicates that similar haplotypes tend to occur close together, we conclude that haplotypes A and B are unlikely to co-occur in South America. The presence of haplotype A in Balneário Camboriú suggests this is a possible source region and should be more intensively sampled. We expect that the other source population exists (or existed) some distance from those sampled, and will be found to contain haplotype B and the seven microsatellite alleles not yet found in South American samples.

In exotic and native ranges, both nuclear and chloroplast DNA detected strong population structure in Brazilian peppertree. This suggests restricted dispersal, which is supported in the native range by increasing genetic differentiation with increasing geographical distance between sites in both genetic data sets. Restricted dispersal is consistent with what is known about how insect pollination and animal seed dispersal correlate with mean F_{ST} estimates in plants (Hamrick & Godt 1996). Levels of subdivision at microsatellite loci are very similar between Florida sites and sites in the native range, but haplotypes are much more strongly subdivided within the native range, suggesting seed dispersal is more limited there. Two mechanisms that would disperse haplotypes in Florida are not as likely to operate in the native range. First, humans have moved Brazilian peppertree for ornamental plantings. Second, long-distance dispersal by large numbers of migrating American robins (*Turdus migratorius*) occurs in certain years (Ewel *et al*. 1982). Both may have dispersed haplotypes further than is typical in the native range. These also would have dispersed nuclear genotypes, but the signal of two distinct introductions is still strong enough that F_{ST} values are similar to those occurring in the native range.

Genetic diversity within introduced species is often low because the population is founded with just a few individuals from the native range (Sakai *et al*. 2001). Recently however, there have been examples discovered of exotic populations that have high genetic diversity as a result of multiple introductions and hybridization (e.g. Allendorf & Lundquist 2003; Walker *et al*. 2003; Kolbe *et al*. 2004; Suehs *et al*. 2004). In the case of Brazilian peppertree in Florida, hybridization between two separate and distinct introduced populations has resulted in genetic variation similar to that found within a population in the native range. The original introductions of Brazilian peppertree on the east and west coasts of Florida may have been small founding populations that contained relatively low levels of genetic variation. Since these two populations have come into contact and hybridized extensively, genetic diversity is now similar to levels that are common within the native range. The low genetic diversity observed in Hawaii, variation that is a subset of that found in Florida, suggests that only a few individuals from one of the source populations in Florida were introduced onto those islands. In contrast, Texas and the US Virgin Islands contain both haplotypes A and B and genetic diversity at microsatellite loci is similar to that found in Florida. Similarity of Florida cpDNA haplotypes and microsatellite alleles with these three other non-native areas suggests that these areas were colonized by individuals originating in Florida.

There is increasing evidence that hybridization plays an important role in species invasions (Ellstrand & Schierenbeck 2000; Petit *et al*. 2004); however, it is currently unknown whether this applies to Brazilian peppertree in Florida. From the initial introductions of Brazilian peppertree into Florida at the end of the 19th century, there was a lag period of over 50 years before researchers noticed that it had spread into the Everglades. A lag phase often precedes invasion by introduced species (Mack *et al*. 2000). A recorded lag may be an artefact of failure to detect individuals in the initial phase of exponential growth when the population is still small or very localized (Crooks & Soulé 1999), or it may be an artefact of an unrecognized, cryptic invasion (Saltonstall 2002). Alternatively, the lag may represent a period during which novel genotypes arise through hybridization and the expansion phase may represent the success of a well-adapted genotype (Ellstrand & Schierenbeck 2000).

Consistent with the high level of genetic population structure within Florida, dispersal may be relatively limited and could in part be responsible for the lag period. Of particular interest here is that the invasive phase for Brazilian peppertree in Florida may have occurred after an intraspecific hybridization event. Increased genetic variability or hybridization may not be a precondition for invasiveness in this species. For example, Brazilian peppertree in Hawaii has very low variation and likely originated from a single source and yet it has been a very successful invader on those islands. On the other hand, some communities like Hawaii seem to be especially favourable to invasion (Cox 1999; Lonsdale 1999) and so increased genetic variation or hybridization may not be as important for invasion success as in communities less susceptible to invasion. Nonetheless, hybridization may have played a role in Brazilian peppertree's ability to invade novel habitats such as mangroves and pinelands in Florida.

Classical biological control aims to lessen the damage caused by invasive organisms through the introduction of highly specialized natural enemies and may provide the best hope for combating widely established invasive plants like Brazilian peppertree (Strong & Pemberton 2000; Myers & Bazely 2003). Biological control agents with the highest potential for effective control are likely to be those that share an evolutionary history with their hosts. The Florida population of Brazilian peppertree is predominantly an intraspecific hybrid, making it impossible to locate natural enemies with which it shares an evolutionary history. Our results suggest that studies of potential biocontrol agents must examine their performance against east and west coast types and hybrids. Three insect species are in quarantine and under consideration as biocontrol agents: the thrips *Pseudophilothrips ichini* Hood (= *Liothrips ichini*) (Thysanoptera: Phlaeothripidae), the sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae), and the tortricid leaf roller *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae) (Cuda *et al*. 2004, 2005; Martin *et al*. 2004). Additional candidates may be introduced as they are identified during exploration trips

in South America. We suggest that all be tested against the entire range of Florida genotypes.

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