GEOGRAPHIC DISTRIBUTION OF GENETIC VARIATION AMONG NATIVE AND INTRODUCED POPULATIONS OF CHINESE TALLOW TREE, *TRIADICA SEBIFERA* (EUPHORBIACEAE)¹

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- Premise of the study: Invasive plants often display genetically determined variation in patterns of growth and resource allocation between native and introduced genotypes, as well as among genotypes within different regions of the introduced range. We examined patterns of genetic variation within and among native and introduced populations of the tetraploid Chinese tallow tree (*Triadica sebifera*, Euphorbiaceae) to determine whether nonselective evolutionary processes or the introduction history could contribute to previously observed phenotypic differences between native and introduced populations as well as among introduced populations.
- Methods: We used six microsatellite markers to study 12 native populations in China, 51 introduced populations in the southeastern USA, and one introduced population in Australia.
- Key results: Genetic diversity was greater within and among native populations than introduced populations. Within the southeastern USA, populations in Georgia and South Carolina differed substantially in their genetic composition and had greater genetic diversity than the rest of the southeastern USA. Greater genetic similarity between some populations in the native range and introduced range indicate a common provenance for Georgia and South Carolina populations that could have come from any of several western or southern Chinese populations and a different provenance for other southeastern USA populations and the Australian population, which were most similar to more northeastern Chinese populations.
- Conclusions: Differences among introduced populations in potentially adaptive traits (e.g., herbivore tolerance, herbivore resistance, growth rates) may result in part from the introduction history, in particular from differences present among source populations in the native range.

Key words: Euphorbiaceae; genetic diversity; introduction history; invasive species; microsatellites; polyploid; population genetic structure; *Sapium sebiferum*.

Native and introduced genotypes of invasive plants often differ in genetically determined patterns of growth and resource allocation, with introduced genotypes being larger, growing faster, displaying lower defenses, and having higher reproductive rates than native genotypes (Siemann and Rogers, 2001, 2003b; Blair and Wolfe, 2004; Maron et al., 2004; Buschmann et al., 2005). In addition, substantial differences in density, morphology, physiology, resistance and tolerance to pests, and invasiveness often occur among populations within the introduced range (Warwick et al., 1987; Amsellem et al., 2000; Siemann and Rogers, 2001; Bartlett et al., 2002; Sexton et al., 2002; Siemann and Rogers, 2003a; Maron et al., 2004; Kliber and Eckert, 2005; Maron et al., 2007). Adaptive responses to

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new selective pressures in the introduced range could lead to phenotypic shifts between native and introduced genotypes (Blossey and Nötzold, 1995; Sakai et al., 2001), and in a similar fashion, differences in the selective regime in disparate areas of the introduced range could lead to adaptive differentiation among nonnative populations (Siemann et al., 2006; Keller and Taylor, 2008), as has been found for several herbaceous plants in response to climate (Barrett et al., 2008; Montague et al., 2008; Colautti et al., 2010). Alternatively, the introduction history, such as the number of independent introductions, number of individuals introduced, and identity of founders, and nonselective evolutionary processes, such as genetic drift and intraspecific hybridization among multiple introductions, could contribute to genetic differentiation of introduced populations from native populations as well as among introduced populations (Amsellem et al., 2000; McCauley et al., 2003; Saltonstall, 2003; Durka et al., 2005; Williams et al., 2005). In particular, the introduction history could play a large role if different pools of genetic material with different phenotypic traits are introduced into disparate parts of the introduced range. The importance of the introduction history in leading to differences in reproductive traits, morphology, and invasiveness in the introduced range is increasingly being recognized for herbaceous and aquatic plants (e.g., Maron et al., 2004; Brown and Eckert, 2005; Kliber and Eckert, 2005; Lui et al., 2005; Maron et al., 2007; Keller et al., 2009; Xu et al., 2010) but has not yet been demonstrated in longer-lived plants such as trees, potentially

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only because few invasive tree species have been examined in this context.

Genetically determined phenotypic differences between native and introduced genotypes as well as among introduced genotypes have been found for Chinese tallow tree [Triadica sebifera (L.) Small, formerly Sapium sebiferum Roxb.; Euphorbiaceae] (Siemann and Rogers, 2001; Rogers and Siemann, 2002, 2004, 2005; Zou et al., 2006, 2007, 2008a). The introduction history of Chinese tallow tree includes variation in timing of introduction to different parts of the southeastern United States, where it is now abundant in various natural and anthropogenically disturbed habitats (Hunt, 1947; Scheld and Cowles, 1981; Bruce et al., 1995; Barrilleaux and Grace, 2000; Denslow and Battaglia, 2002; Pattison, 2003). It was first introduced to the southeastern United States late in the 18th century to Savannah, Georgia (Bell, 1966) and Charleston, South Carolina (Michaux, 1803; Elliott, 1824; Schoepf, 1911; Hunt, 1947) and was later planted widely across the rest of the southeastern United States in the early 20th century (Jamieson and McKinney, 1938). The native source(s) of these introductions was not documented, but a variety of sources are possible given that it is widely distributed in its native range of central and eastern China and northern Vietnam (Lee, 1956; Jin and Huang, 1984; Zhang and Lin, 1994; Pattison and Mack, 2008) and that it is cultivated and naturalized in other parts of Asia. It is also becoming invasive in California, United States (Bower et al., 2009) and Australia (Hosking et al., 2003; Pattison and Mack, 2008).

Evidence for genetically determined phenotypic differences among populations of Chinese tallow tree comes from a 14-yr common garden experiment that showed that genotypes arising from Taiwan in the native range had higher tannin concentrations and less basal area than introduced genotypes in the southeastern USA (Siemann and Rogers, 2001). In addition, Georgia genotypes, which are derived from the earliest introductions in the southeastern United States, had higher allocation to defense and less to growth than did genotypes originating from more recent introductions in the early 1900s to the states of Texas and Louisiana. These phenotypic differences could have resulted from selection-driven evolution; native genotypes may allocate more to defense and less to growth because of selective pressure from high herbivore and pathogen loads (Siemann and Rogers, 2001). Low natural enemy loads in Texas and Louisiana may favor genotypes with high growth and low allocation to defense, whereas early introductions to Georgia may have lost their defenses initially when there was a demographic release from natural enemies, but then reacquired them as initially naïve herbivores came to use Chinese tallow tree over the 200 yr the species has been present there (Siemann and Rogers, 2001). However, the introduction history and nonselective evolutionary processes could concomitantly or alternatively contribute to these phenotypic differences in defense and growth.

We used microsatellite markers to compare the genetic diversity and composition of native, Chinese and introduced, southeastern United States populations of Chinese tallow tree to determine whether the introduction history or nonselective evolutionary processes could contribute to phenotypic differences observed in the long-term common garden study. We also examined one population in Australia to address whether introductions to different parts of the world may stem from different source populations. We addressed two questions regarding the invasion of Chinese tallow tree: (1) What do the patterns of genetic composition and genetic variation among native and introduced populations indicate about the introduction history and postintroduction nonselective evolutionary processes? (2) Specifically, do they indicate introductions of different source material to different parts of the southeastern United States (i.e., Georgia vs. Texas/Louisiana) or to different parts of the world (i.e., southeastern United States vs. Australia)?

MATERIALS AND METHODS

Study species—Chinese tallow tree was introduced to the United States and various other countries (Howes, 1949) primarily to make candles and soap from the sarcotesta, the white waxy layer surrounding the seeds (Jamieson and McKinney, 1938; Bell, 1966). A reproductive individual was collected in New Orleans, Louisiana in 1832, but Chinese tallow tree seems not to have been widely planted across the Gulf States until the early 20th century (Howes, 1949). At that time, the Foreign Plant Introduction Division of the Bureau of Plant Industry helped establish plantations across the southeastern United States to make soap (Jamieson and McKinney, 1938). Additionally, a chemist from Dow Chemical distributed 20000 trees to farmers across the Gulf of Mexico coast (Gray, 1950). It has also been propagated as an ornamental tree and as a source of nectar for honeybees (Bruce et al., 1997).

Chinese tallow tree is tetraploid in both its native and introduced ranges (Perry, 1943; Hans, 1973), but it is not known whether it is auto- or allotetraploid. Chinese tallow tree is a dichogamous, monoecious species, with separate male and female flowers that mature at different times, reducing the probability of self-fertilization and promoting cross-pollination. Pollination occurs via generalist insects (Duke, 1983; Bruce et al., 1997).

Southeastern USA and Australia collections—One to two leaves were collected from at least 10 trees in 51 populations across the southeastern United States (USA) (Fig. 1, Table 1) and 17 trees from one population in Australia. Populations in the USA were at least 8 km and a maximum of 2002 km apart. USA populations represent the entirety of the area in which Chinese tallow tree forms extensive, naturalized stands in the southeastern USA (shown in gray shading in Fig. 1), from its most northern population in Wilmington, North Carolina to its most southern population in the East (near Tampa, Florida) and its most western populations in east Texas (TX07). In addition, we sampled one small population outside this core range (SC06 in Clemson, South Carolina), which likely established from a few trees planted in the South Carolina Botani-cal Garden. Our distribution map is more restricted than that of Pattison and Mack (2008) because we show only areas where Chinese tallow tree is abundant.

In both the USA and Australia, samples were collected preferentially from naturally occurring trees, but some deliberately planted material may have been collected because it was often difficult to determine whether trees were planted or naturalized. Populations were located based on herbarium specimens, known locations based on personal communications, and new searches. Trees in the USA were sampled in a variety of different habitats including roadsides, pastures, forests, coastal prairies, and dunes. Trees in the Casino, New South Wales, Australia population (28.85°S, 153.05°E) were collected from an invaded wetland. Leaves were dried in silica gel for at least 48 h, ground to a fine powder with liquid nitrogen, and then stored at -80°C until DNA extraction.

China collections—One to two leaves from at least four individuals within each of 12 populations in China (Table 1) were collected and stored in a similar manner to those from Australia and the USA. Populations in China were at least 22 km and at most 1124 km apart (Fig. 1). Trees were sampled in a variety of different habitats including roadsides, parks, gardens, pastures, forests, and wetlands. Unfortunately, populations from Taiwan that were used in the common garden with Chinese and USA populations (Siemann and Rogers, 2001) were unable to be used because seeds were no longer available, DNA did not amplify from oven-dried leaves of individuals harvested from the 14-yr common garden, and our collections in the native range did not include Taiwan.

Microsatellite analysis—Total genomic DNA was extracted using DNeasy Plant Mini Kits (Qiagen, Valencia, California, USA). Each polymerase chain reaction (PCR) of 10 μ L contained approximately 5–10 ng of template DNA, 1× PCR buffer (NH₄-based reaction buffer without MgCl₂, Bioline, Randolph,



Fig. 1. Locations of the Chinese tallow tree populations sampled in its native range in China and part of its introduced range of the southeastern USA. Numbers correspond to the population codes found in Table 1. The gray shading in the inset map of China denotes the native range of Chinese tallow tree (Pattison and Mack, 2008) and in the southeastern USA map denotes the distribution of substantially sized naturalized populations. The range of small, naturalized populations is larger than depicted here and includes populations much farther inland, including ones such as Clemson, South Carolina (SC06). Different symbols placed next to states (USA) correspond to those used in Fig. 3. The cities to which Chinese tallow was originally introduced (Charleston, South Carolina and Savannah, Georgia) are indicated on the map.

Massachusetts, USA), 0.25 mmol/L of each dNTP (Promega, Madison, Wisconsin, USA), 0.6 μ mol/L of each primer, 2.0–3.0 mmol/L MgCl₂ (specific MgCl₂ concentrations for each primer pair are specified in DeWalt et al., 2006), and 0.25 U of BIOLASE *Taq* DNA polymerase (Bioline). PCR was performed and the products treated as previously described (DeWalt et al., 2006). Each individual was genotyped at six microsatellite loci described in DeWalt et al. (2006), representing CA, GA, or AAG repeats on an ABI 3100 Genetic Analyzer with a GeneScan 400HDRox size standard (Applied Biosystems, Foster City, California, USA). Up to four loci were multiloaded per lane. Fragment lengths were scored with the aid of the program Genotyper version 3.6 NT (Applied Biosystems). Thirty-four individuals, found across populations, were deleted from the data set because more than one locus failed to resolve after multiple attempts, indicating poor DNA quality. However, we retained the 143 individuals for which no alleles were scored at one locus. Null alleles were

likely present in these individuals. In particular, a null allele was likely present at D101, for which no alleles were scored for 95 individuals. Almost all populations had at least one individual with a null allele at D101, but almost 60% of GA04 had the putative null allele.

Data analyses—Allele frequency is difficult to estimate in polyploids because of complex segregation patterns. In addition, even co-dominant markers, such as microsatellites, cannot be used to assess allele dosage (number of copies of each allele) because the relative fluorescence units do not correspond to the initial number of copies of each allele before PCR. Exact genotypes can be assigned only when marker phenotypes show a single allele (and null alleles are assumed absent) or the number of alleles equal to the ploidy level (in the case of a tetraploid, four alleles). Only allele phenotypes can be determined when two or three alleles are present. For example, for a tetraploid with alleles A and

 TABLE 1. Sampling location, number of samples that successfully genotyped at more than five loci, and mean ± 1 SD for four measures of within-population genetic variation for Chinese tallow tree in native populations in China and introduced populations in Australia and the southeastern USA. The total number of samples in each of these three regions and regional averages for the measures of within-population variation are in boldface. Populations are listed roughly in decreasing latitude in China and decreasing latitude and increasing longitude in the southeastern USA.

Location	Ν	A _O	Minimum H_0	Maximum H ₀	$H_{\rm E}$	Location	N	A _O	Minimum H_0	Maximum H ₀	$H_{\rm E}$
China	129	2.27 ± 0.11	0.54 ± 0.04	0.60 ± 0.05	0.70 ± 0.03	FL09	10	2.26 ± 0.40	0.53 ± 0.14	0.58 ± 0.15	0.68
Jiangsu1	19	2.31 ± 0.39	0.55 ± 0.14	0.60 ± 0.14	0.68	FL10	10	2.27 ± 0.18	0.55 ± 0.07	0.61 ± 0.08	0.66
Jiangsu2	11	2.17 ± 0.24	0.50 ± 0.08	0.56 ± 0.09	0.67	AL01	20	2.19 ± 0.27	0.53 ± 0.10	0.60 ± 0.11	0.68
Anhui	5	2.27 ± 0.19	0.53 ± 0.06	0.59 ± 0.06	0.66	AL02	18	2.30 ± 0.35	0.55 ± 0.13	0.61 ± 0.14	0.69
Zhejiang1	6	2.28 ± 0.40	0.55 ± 0.15	0.63 ± 0.13	0.71	AL03	20	2.24 ± 0.40	0.53 ± 0.15	0.59 ± 0.15	0.69
Zhejiang2	6	2.56 ± 0.33	0.65 ± 0.08	0.73 ± 0.07	0.73	AL04	10	2.42 ± 0.43	0.58 ± 0.15	0.64 ± 0.15	0.71
Fujian	19	2.30 ± 0.29	0.58 ± 0.09	0.65 ± 0.09	0.72	MS01	31	2.25 ± 0.33	0.52 ± 0.13	0.58 ± 0.14	0.68
Jiangxi1	13	2.17 ± 0.45	0.52 ± 0.15	0.58 ± 0.15	0.67	MS02	20	2.23 ± 0.27	0.53 ± 0.09	0.60 ± 0.10	0.70
Jiangxi2	8	2.19 ± 0.35	0.55 ± 0.14	0.62 ± 0.13	0.73	MS03	9	2.04 ± 0.37	0.47 ± 0.13	0.56 ± 0.12	0.67
Jiangxi3	18	2.23 ± 0.29	0.50 ± 0.09	0.55 ± 0.10	0.72	LA01	20	2.20 ± 0.30	0.52 ± 0.11	0.60 ± 0.11	0.65
Guangdong	15	2.36 ± 0.42	0.57 ± 0.14	0.62 ± 0.13	0.74	LA02	39	2.34 ± 0.26	0.56 ± 0.09	0.62 ± 0.10	0.71
Guangxi1	5	2.24 ± 0.30	0.52 ± 0.10	0.57 ± 0.11	0.66	LA03	9	2.28 ± 0.30	0.53 ± 0.09	0.58 ± 0.08	0.70
Guangxi2	4	2.17 ± 0.43	0.49 ± 0.13	0.55 ± 0.09	0.68	LA04	26	2.24 ± 0.28	0.53 ± 0.10	0.60 ± 0.10	0.70
USA	878	2.21 ± 0.13	0.51 ± 0.05	0.58 ± 0.05	0.66 + 0.06	LA05	26	2.15 ± 0.28	0.50 ± 0.11	0.57 ± 0.11	0.67
NC01	38	2.21 ± 0.15 2 10 + 0 25	0.31 ± 0.03 0.48 ± 0.09	0.55 ± 0.05	0.65	LA06	10	2.04 ± 0.29	0.43 ± 0.12	0.46 ± 0.12	0.64
SC01	30	2.10 ± 0.23 2.26 ± 0.34	0.40 ± 0.00	0.55 ± 0.07 0.61 ± 0.11	0.72	TX01	25	2.16 ± 0.31	0.50 ± 0.12	0.57 ± 0.14	0.62
SC02	20	2.20 ± 0.34 2.58 ± 0.33	0.54 ± 0.12 0.65 ± 0.10	0.01 ± 0.01	0.72	TX02	14	2.18 ± 0.27	0.48 ± 0.10	0.52 ± 0.10	0.60
SC02	10	2.30 ± 0.33 2.35 ± 0.34	0.05 ± 0.10 0.59 ± 0.11	0.71 ± 0.09 0.68 ± 0.09	0.74	TX03	26	2.27 ± 0.24	0.54 ± 0.09	0.59 ± 0.09	0.70
SC03	18	2.33 ± 0.34 2 37 + 0 35	0.59 ± 0.11 0.56 ± 0.13	0.00 ± 0.00	0.74	TX04	9	1.96 ± 0.33	0.42 ± 0.13	0.48 ± 0.14	0.59
SC05	17	2.37 ± 0.33 2 31 + 0 21	0.50 ± 0.13 0.57 ± 0.08	0.61 ± 0.19	0.73	TX05	10	2.20 ± 0.30	0.51 ± 0.10	0.58 ± 0.09	0.68
SC06	28	2.01 ± 0.21 2.06 ± 0.25	0.57 ± 0.00 0.45 ± 0.10	0.05 ± 0.09	0.54	TX06	10	2.33 ± 0.31	0.57 ± 0.10	0.64 ± 0.10	0.69
GA01	23	2.00 ± 0.25 2.34 ± 0.36	0.45 ± 0.10 0.55 ± 0.13	0.50 ± 0.10 0.59 ± 0.12	0.64	TX07	10	2.30 ± 0.33	0.55 ± 0.12	0.60 ± 0.12	0.69
GA02	19	2.34 ± 0.30 2.23 ± 0.33	0.53 ± 0.13 0.52 ± 0.12	0.59 ± 0.12 0.59 ± 0.12	0.70	TX08	10	2.09 ± 0.28	0.46 ± 0.09	0.52 ± 0.09	0.59
GA03	30	2.23 ± 0.33 2.03 + 0.23	0.32 ± 0.12 0.47 ± 0.09	0.55 ± 0.12 0.55 ± 0.11	0.65	TX09	9	2.00 ± 0.20	0.44 ± 0.07	0.51 ± 0.08	0.64
GA04	17	2.05 ± 0.25 2 10 + 0 35	0.47 ± 0.09 0.47 ± 0.11	0.55 ± 0.11 0.51 ± 0.11	0.03	TX10	10	2.18 ± 0.17	0.51 ± 0.06	0.57 ± 0.06	0.67
FI 01	0	2.10 ± 0.33 2.11 ± 0.27	0.47 ± 0.11 0.47 ± 0.11	0.51 ± 0.11 0.53 ± 0.12	0.57	TX11	10	2.27 ± 0.29	0.53 ± 0.11	0.57 ± 0.12	0.68
FL02	22	2.11 ± 0.27 2.34 ± 0.42	0.17 ± 0.11 0.56 ± 0.14	0.53 ± 0.12 0.62 ± 0.14	0.68	TX12	9	2.13 ± 0.18	0.50 ± 0.06	0.58 ± 0.06	0.59
FL 03	28	2.34 ± 0.42 2.24 ± 0.29	0.50 ± 0.14 0.54 ± 0.10	0.02 ± 0.14 0.60 ± 0.10	0.66	TX13	10	2.00 ± 0.32	0.43 ± 0.11	0.49 ± 0.12	0.56
FL 04	21	1.98 ± 0.27	0.34 ± 0.10 0.43 ± 0.14	0.00 ± 0.10 0.49 ± 0.13	0.66	TX14	10	2.13 ± 0.30	0.48 ± 0.13	0.54 ± 0.14	0.61
FL05	20	2.25 ± 0.22	0.13 ± 0.11 0.52 ± 0.08	0.19 ± 0.13 0.58 ± 0.07	0.63	TX15	10	2.27 ± 0.38	0.54 ± 0.12	0.61 ± 0.12	0.66
FL 06	10	2.23 ± 0.22 2.07 ± 0.21	0.32 ± 0.00 0.47 ± 0.08	0.50 ± 0.07 0.54 ± 0.09	0.58	TX16	10	2.26 ± 0.18	0.52 ± 0.06	0.56 ± 0.06	0.62
FL07	10	2.07 ± 0.21 2.04 ± 0.20	0.46 ± 0.07	0.57 ± 0.09 0.52 ± 0.07	0.63	TX17	10	2.54 ± 0.17	0.63 ± 0.06	0.68 ± 0.08	0.67
FL08	19	2.04 ± 0.20 2.10 ± 0.26	0.47 ± 0.07	0.52 ± 0.07 0.53 ± 0.10	0.64	Australia	17	2.39 ± 0.34	0.57 ± 0.12	0.64 ± 0.11	0.63

B found at a marker locus, we could not determine whether there were two copies of A and two copies of B (AABB) or three copies of A and one copy of B (or vice versa; AAAB or ABBB). We were therefore unable to assign genotypes or estimate allele frequencies needed for classical analyses of genetic diversity and structure, including $F_{\rm ST}$.

To examine geographic patterns in genetic similarity, we calculated the pairwise genetic distance (D) between all pairs of populations using the genetic distance measure of Tomiuk and Loeschcke (1991) in the program POPDIST (Guldbrandtsen et al., 2000). This genetic distance measure is based on a maximum-likelihood based approach that uses a simple mutation model and groups genotypes into one of four classes based on the presence/absence of alleles in each population: (1) homozygotes observed in both populations (e.g., A in Pop1, A in Pop2), (2) heterozygotes that have at least one allele in common in both populations and differ for at least one (e.g., AB in Pop1, BC in Pop2), and (4) genotypes that have no common alleles in the populations (e.g., AB in Pop1, CD in Pop2). The different categories decrease or increase in frequency only by mutation.

The Tomiuk–Loeschcke genetic distances among pairs of populations were used to conduct nonmetric multidimensional scaling (NMDS) ordinations in the program SAS version 9.1 (SAS, Cary, North Carolina, USA) to view graphically the relationships among populations. We examined solutions with two and three axes and only used solutions that reduced the stress below 0.15. Statistical significance between groups identified in the NMDS ordination was evaluated with permutational multivariate analysis of variance (PERMANOVA) implemented with the adonis function of the vegan package of the program R version 2.8.1 (R Development Core Team, 2009).

We compared the population clustering pattern of native and introduced populations using the program STRUCTURE version 2.3 (Pritchard et al., 2000). This program takes a Bayesian approach to infer population structure by clustering similar multilocus genotypes (microsatellite genotypes in our study) into K clusters without using the geographical locations of the populations. We used the default parameters of the program to allow population admixture and correlated allele frequency across populations. Because Chinese tallow is a polyploid and there was ambiguity about the number of copies of each allele, we used the recessive alleles option and set the recessive allele code to the integer used for missing data, as recommended by the manual. We coded the data so that there were two copies for each allele when two alleles were detected (e.g., BBCC) and two copies of the largest allele (most number of repeats) when three alleles were detected (e.g., BCDD). Alternate coding had no appreciable effect on the results. We used a burn-in period of 50 000 iterations and collected data from an additional 100000 Markov chain Monte Carlo steps for five independent runs for K (number of clusters) set from 1 to 10. To identify the most likely number of clusters (K'), we calculated ΔK as the difference in the log likelihood of data between consecutive K values according to recommendations by Evanno et al. (2005) and designated ΔK_{max} as K'. An additional 15 simulations were run for K'. For each simulation, the membership of each individual in a cluster is estimated as a clustering coefficient that ranges from 0-1.0, with 1.0 indicating full membership in a cluster. We used the populationlevel output clustering coefficients of STRUCTURE from the 20 runs at K and averaged across runs using the Greedy algorithm of the program CLUMPP (Jakobsson and Rosenberg, 2007). Membership in clusters was visualized using the program DISTRUCT (Rosenberg, 2004).

Populations within the southeastern USA grouped together in the STRUC-TURE and NMDS analyses are hereafter referred to as groups. Genetic variation was then compared between native and introduced ranges as well as between groups within the southeastern USA. Using only the allele phenotypes, we directly calculated the mean number of alleles per locus, A_0 , and put minimum and maximum bounds on the observed heterozygosity of individuals, H_0 , for each population. The observed heterozygosity value of each locus is equal to one minus the probability that any two alleles drawn at random are identical by descent. The H_0 for phenotypes with one allele is equal to 0. For two alleles (e.g., A and B), the minimum H_0 is 0.50 assuming a genotype of AAAB, and the maximum is 0.66 assuming AABB. For three alleles, H_0 is 0.83, regardless of which allele has two copies; for four alleles, it equals 1 (Bever and Felber, 1992; Brown and Young, 2000). In addition, the program ATETRA 1.0 was used to calculate estimated heterozygosity (H_E) corrected for sample size using 10000 Monte Carlo simulations (Van Puyvelde et al., 2010). Finally, we used a rarefaction approach to compare estimated allelic richness (A_c) found in equal-sized subsamples from Australia, China, and the southeastern USA using the program ADZE (Szpiech et al., 2008). Kruskal–Wallis nonparametric tests were conducted to compare within-population genetic diversity measures (A_0 , minimum H_0 , maximum H_0 , H_E , A_r) between native and introduced populations and between southeastern USA regions.

We examined whether populations closer in space in the native and introduced range were more similar genetically by testing for isolation by distance (IBD) among China and USA populations separately. Geographic distances among pairs of populations were calculated from the latitude and longitude coordinates of the approximate center of each population using the program PASSaGE version 1.1 (Rosenberg, 2001). The significance of the relationship between pairwise geographical distance and genetic distance (*D*) between populations was calculated using a Mantel test with 3000 permutations implemented with the mantel function of the vegan package of R.

RESULTS

We identified 72 alleles across six microsatellite loci for the 1024 Chinese tallow individuals sampled across the 64 populations. Almost all individuals had different genotypes, but individuals sharing multilocus allele phenotypes were found in three populations; there were duplications (i.e., two individuals) of three phenotypes in TX12, three phenotypes in FL03, and one phenotype in Zhejiang2. The same multilocus allele phenotype was not found in more than one population. Nine alleles were found exclusively in the native range of China (3 for A10, 3 for B103, 1 for D101, 1 for D117, and 1 for B5). Of these, six were private alleles, found only in one population. Five alleles were found only in the USA, with three of them occurring in multiple populations and two being private alleles found only in Texas populations (one private allele in each of TX03 and TX04). Two alleles, one of D101 in AL02 and one of B103 in TX13, were found only in one USA population as well as in several populations of China. No alleles were found only in Australia, and all alleles found in Australia were found both in the USA and China.

Genetic relationships among native and introduced populations—Genetic distances calculated with the Tomiuk–Loeschcke algorithm ranged from 0.002 (between LA05 and TX03) to 0.422 (GA04 and Guangxi2). Populations within the USA tended to be genetically more similar to each other (mean = 0.076; range: 0.002-0.334) than those in China (mean = 0.12; range: 0.025-0.361). The mean genetic distance between Australian and USA (0.09) populations was lower than between Australian and China populations (0.12).

The NMDS ordination of all populations (Fig. 2) showed that China and USA populations differed in their allelic composition as seen by the separation of most of these populations along dimension 3 (3D solution, stress = 14.1). The Australian population had alleles found in both China and the USA, leading to an intermediate position along dimension 3. PERMANOVA analyses confirmed that native and introduced populations differed significantly in their allelic composition ($F_{1,62} = 14.1, P < 0.001$).

Based on the NMDS ordinations and STRUCTURE results (see below), we identified two groups within the southeastern USA: a "GA/SC group" that encompassed populations in South Carolina and Georgia, with the exception of GA04, and a "rest of the southeastern USA group" that encompassed all other populations. Populations from South Carolina and Georgia (with the exception of GA04) differed from the rest of the southeastern USA as seen by the separation along dimension 1 in Fig. 2 and along dimension 1 in the ordination of southeastern USA populations in Fig. 3 (3D solution, stress = 14.0). The most northern population sampled in the southeastern USA (Wilmington, North Carolina-NC01) was more closely related (and therefore closer along dimension 1 in Fig. 3) to some Florida, Texas, and Alabama populations than to South Carolina populations that were closer geographically. GA/SC populations differed significantly from the rest of the southeastern USA according to the PERMANOVA ($F_{1,49} = 35.1$, P < 0.001). Some Georgia and South Carolina populations had alleles for loci B5 and B103 that were not found outside this region within the USA; similarly, alleles at loci D11, B103, D101, and B5 were found in the rest of the southeastern USA but not in GA/ SC. All but one of these alleles (at the D101 locus) were found also in China.

Of the China populations sampled, Jiangsu and Zhejiang populations were the most similar in their allelic composition to the rest of the southeastern USA group as evidenced by their positions along dimensions 1 and 2 in Fig. 2 (also see STRUC-TURE results below). Jiangsu1 was found in the same position as many USA populations along all three dimensions. However, allelic composition of these four northern China populations was significantly different from the rest of the southeastern USA populations according to a PERMANOVA ($F_{1,44} = 5.21$, P = 0.04).

The Bayesian clustering analysis also showed differences among native and introduced populations and among southeastern USA populations and corroborated the NMDS and PER-MANOVA analyses. Based on ΔK_{max} , three clusters best explained the genetic structuring among populations. Most native, China populations as well as most populations within the GA/SC group had their greatest membership coefficients in the dark gray cluster, whereas none of the populations in the rest of the southeastern USA or Australia were assigned primarily to the dark gray cluster (Fig. 4). Instead, their membership was greatest in the white cluster and was greater in the light gray cluster than most China populations. The two Jiangsu populations in or near the city of Nanjing as well as the two Zhejiang populations differed from the rest of the Chinese populations by having membership coefficients similar to the Australian population and the rest of the southeastern USA group. The other Chinese populations, which were located more to the west or south, had membership coefficients similar to the GA/SC populations. These Chinese populations were most similar in their membership coefficients to USA populations in and around Savannah, Georgia (i.e., GA01 and GA02) and most South Carolina populations (all but SC01, which is located 85 km north of Charleston).

Southeastern USA populations and Chinese populations both showed significant patterns of isolation by distance (USA: r =0.26, P < 0.001; China: r = 0.26, P = 0.03), but geographic distance explained little (~7%) of the variation in genetic distance. Even when populations from the GA/SC group were omitted from the southeastern USA analysis, there was a significantly positive relationship between genetic and geographic distance



Fig. 2. Nonmetric multidimensional scaling ordination based on Tomiuk–Loeschcke genetic distances among Chinese tallow tree populations in the southeastern USA (circles and squares), Australia (star), and China (triangles). A three-dimensional solution was necessary to adequately reduce stress. Gray circles and open squares represent two seemingly genetically distinct groups within the southeastern USA: GA/SC and the rest of the southeastern USA, respectively. China populations that clustered with the "rest of the southeastern USA" populations and Georgia population GA04, which did not cluster with the GA/SC group, are labeled.

(r = 0.25, P = 0.008). A strong IBD pattern was found for GA/SC populations (r = 0.70, P = 0.004).

Genetic diversity of native and introduced populations—More alleles were found in China (67) than in the USA (63) despite sampling far fewer individuals in China (129) than in the USA (878). Thirty-one alleles were detected among the 17 individuals sampled in one Australia population. Fifty-four alleles were sampled in Georgia and South Carolina, and 60 were found in the rest of the southeastern USA. In a standardized sample size of 56, rarefaction computed 8.09 (\pm 1.15) alleles in China, 6.96 (\pm 0.87) in the southeastern USA, and 5.06 (\pm 0.86) in Australia.

Native and introduced populations of Chinese tallow tree did not differ significantly in any of the three measures of genetic diversity using allele phenotypes: A_0 (Kruskal–Wallis $\chi_1^2 = 1.43$, P = 0.23), minimum H_0 ($\chi_1^2 = 1.99$, P = 0.16), and maximum H_0 ($\chi_1^2 = 1.85$, P = 0.17). However, estimated heterozygosity (H_E) and allelic richness rarefied to a sample size of 16 [A_r (16)] differed significantly between native and introduced populations [H_E ($\chi_1^2 = 6.48$, P = 0.01) and A_r (16) ($\chi_1^2 = 13.13$, P < 0.001)], with China populations having significantly greater H_E and A_r (16) than USA or Australia populations (Table 1; Fig. 5). However, these two measures of genetic diversity did not differ between China and GA/SC populations (Fig. 5; data not shown for H_E). H_E and A_r (16) were significantly greater within populations of the GA/SC group than the rest of the southeastern USA [H_E ($\chi_1^2 = 4.52$, P = 0.04) and A_r (16) ($\chi_1^2 = 5.51$, P = 0.02)], and there was a trend for greater genetic diversity according to the other three measures [A_0 ($\chi_1^2 = 2.99$, P = 0.08), minimum H_0 ($\chi_1^2 = 3.62$, P = 0.06), and maximum H_0 ($\chi_1^2 = 3.71$, P = 0.05)].



Fig. 3. Nonmetric multidimensional scaling ordination based on Tomiuk–Loeschcke genetic distances among 51 Chinese tallow tree populations in the southeastern USA using a three-dimensional solution. Different symbols represent populations collected in different states and correspond to those in Fig. 1.



Fig. 4. Bayesian estimates of population structure based on microsatellite variation among native (China) and introduced (Australia and USA) populations of Chinese tallow tree. Each population is represented by a bar, and width of bar is determined by the number of individuals sampled within the population. Membership coefficients for each population for each of three clusters (K = 3) are depicted with different colors (dark gray, white, and light gray). The order of populations follows Table 1.

DISCUSSION

Molecular markers exhibiting neutral variation can help elucidate how introduction history and nonselective evolutionary processes contribute to biological invasions. For Chinese tallow tree, an aggressive invader of various habitats in the southeastern USA, microsatellite markers point to at least two separate introductions that produced the majority of the genetic structure of the resulting populations—one to Georgia and South Carolina and another to the rest of the southeastern USA and Australia. Comparison with native, Chinese samples suggests that these introductions came from different parts of the native range. Phenotypic differences present in the native range could therefore be reflected in the introduced range leading to observed genetic differences among introduced genotypes in herbivore tolerance and resistance, growth rates, and other traits.

Reconstructed introduction history—Present populations near the sites of the earliest introductions of Chinese tallow tree to the southeastern USA (Charleston, South Carolina and Savannah, Georgia) appear to differ in genetic composition as well as genetic diversity from populations resulting from introductions made approximately 120 yr later to the rest of the southeastern USA. The higher genetic variation [H_E and $A_r(16)$] found within Georgia and South Carolina populations compared to populations of the rest of the southeastern USA suggests that the early introductions were from a more diverse gene pool, entailed multiple introduction events (i.e., larger propagule number) or were founded by more individuals than the later ones (i.e., larger propagule pool). Although Chinese tallow tree naturalized quickly along the coast in Georgia and South Carolina (Michaux, 1803; Elliott, 1824) and present populations show isolation by distance, range expansion of these genotypes appears limited given that populations to the north and south of them (NC01, FL01, FL03) are genetically distinct from the coastal South Carolina and Georgia populations. Based on the difference in genetic composition between NC01 and South Carolina populations close to Charleston (SC02, SC03, SC04; Figs. 3, 4), the NC01 population must have been established from an introduction event involving different source material rather than from range expansion northward from Charleston. Even the slightly inland Jesup, Georgia (GA04) population differed in its pattern of membership with other Georgia populations and therefore likely established from a separate introduction event of different genotypes.

The one Australian population was genetically similar to the rest of the southeastern USA populations and therefore was probably established from the same or genetically similar native sources. We base this conclusion on the following results: no alleles were found in Australia that were not found in the southeastern USA; the NMDS ordination of all populations placed the Australian population closer to the points representing the southeastern USA populations outside of Georgia and South Carolina; and the Bayesian clustering analysis showed that the Australian population had membership coefficients in the three clusters that were more similar to populations in the southeastern USA that were outside of South Carolina and Georgia. We are unaware of when Chinese tallow was introduced to Australia; however, its genetic similarity to the rest of the southeastern USA indicates that it may have been introduced in the early 1900s to Australia at the same time it was being extensively planted in areas along the USA coast of the Gulf of Mexico or that it represents a secondary invasion from the southeastern USA.



Fig. 5. Allelic richness (A_i) with rarefaction to a sample size of 16 for Chinese tallow tree populations sampled in its native and introduced ranges. Bars are means ± 1 SE. Summaries by group (China, GA/SC, and the rest of the southeastern USA) are shown on the right. Significantly different groups (P < 0.05) are indicated by different letters.

Native sources of introduced populations—The genetic similarity of populations in Jiangsu and Zhejiang provinces of China to populations in the rest of the southeastern USA and Australia as indicated by the NMDS ordination (Fig. 2) and Bayesian clustering analysis (Fig. 4) suggests that this region of central, eastern China is a possible source for those introduced populations. The PERMANOVA analysis indicated that the genetic composition was still significantly different between this putative source region and the rest of the southeastern USA, which is expected given the high rate of mutation in microsatellites and lack of gene flow between native and invasive populations. Both native and introduced genotypes likely have diverged from the original introductions.

Unlike the rest of the southeastern USA populations, the source region of Georgia and South Carolina introductions is far less clear. Most sampled Chinese populations had Bayesian clustering patterns similar to these USA populations but were distinct in their allelic composition as visualized on the NMDS ordination. Thus, the source of the late 18th and early 19th century introductions to Charleston and Savannah cannot be narrowed down even to a region with our data set. Which, if any, of the native populations sampled were the source of GA/SC populations may be obscured by reshuffling and recombination of genetic material from the native range through intraspecific hybridization among multiple introductions. We think it unlikely, however, that substantial hybridization among multiple introductions from disparate areas of the native range occurred in GA/SC. When gene flow among multiple introductions leads to admixtures, genetic diversity generally is significantly higher in the introduced range compared to the native range (Novak and Mack, 1993, 2005; Maron et al., 2004; Bossdorf et al., 2005; Dlugosch and Parker, 2008), but we found similar diversity levels between GA/SC and native populations.

Introduction of different genotypes from the native range into different parts of the introduced range is not unique to Chinese tallow tree. This pattern has also been found for various plants including Bromus tectorum (Novak and Mack, 2001; Bartlett et al., 2002; Valliant et al., 2007), Butomus umbellatus (Kliber and Eckert, 2005), Centaurea diffusa (Marrs et al., 2008a), C. stoebe micranthos (Marrs et al., 2008b), Hypericum perforatum (Maron et al., 2004), and Schinus terebinthifolius (Williams et al., 2005). We found no evidence, however, of extensive gene flow among the separate introductions of Chinese tallow tree. Thus, intraspecific hybridization appears not to be a mechanism of invasion for Chinese tallow tree as has been found for other species such as Ambrosia artemisiifolia (Chun et al., 2010), Phalaris arundinacea (Lavergne and Molofsky, 2007), Spartina alterniflora (Blum et al., 2007), and Schinus terebinthifolius (Williams et al., 2005).

Role of introduction history on phenotypic traits between the native and introduced range-Publication of the evolution of increased competitive ability (EICA) hypothesis by Blossey and Nötzold in 1995 spurred an explosion of common garden experiments testing whether introduced genotypes grow faster but have lower herbivore resistance than native conspecifics (see references in Bossdorf et al., 2005). Few of these studies conducted extensive native range sampling to determine whether the native genotypes used were from source populations. Early experiments using Chinese tallow tree to test the EICA hypothesis also used native genotypes without regard to whether they were from source regions (e.g., Siemann and Rogers, 2001, 2003b; Rogers et al., 2003; Rogers and Siemann, 2005). However, one of our recent experiments likely made a more appropriate native-source-to-introduction comparison (Jiangsu vs. Texas) to study genetic differences in biomass allocation and leaf defoliation as well as the effect of genotype on

soil processes (Zou et al., 2006). In that study, results were consistent with all of the previous common garden experiments: introduced genotypes grew faster and attained greater biomass than native ones. In addition, recent studies on herbivore resistance and tolerance conducted with a broad set of native and introduced populations have confirmed earlier findings based on fewer populations (Huang et al., 2010; Wang et al., 2011).

We cannot pinpoint source populations in this study for either group in the southeastern USA, but we now know that future sampling to elucidate GA/SC origins should include more populations in the southern and western portions of its native range and possibly also Taiwan and Vietnam. Native range sampling was limited to mainland China because of budgetary and logistical constraints, but we are presently expanding our research in China to sample more localities. We will also include more areas in the northeast of China, particularly in Jiangsu and Zhejiang provinces, to determine whether Texas and other southeastern USA populations are more similar to populations in those areas. Despite uncertainty in which native regions were the source of USA and Australia introductions, our study shows that there were different sources for the two genotypes found presently within the southeastern USA (i.e., GA/SC vs. the rest of the southeastern USA).

Role of introduction history on phenotypic traits within the introduced range—The greater allocation to defense and lower allocation to growth of genotypes from Georgia than those from Texas and Louisiana found in the 14-yr common garden study (Siemann and Rogers, 2001) may mirror preexisting differences between native source populations. Greater tolerance of North Carolina seeds and seedlings to freezes than South Carolina genotypes may also reflect among-population differences in the native range (Park, 2009). The different origins of early introductions (South Carolina and Georgia) vs. later ones (the rest of the southeastern USA) may therefore have led to genotypes that have different abilities to colonize, establish, and proliferate. Consistent differences among China populations in germination success, growth rates, or herbivore resistance and tolerance in common garden studies would support this hypothesis. However, broad-stroke comparisons between northern and southern populations in China have not revealed differences (Zou et al., 2008b; Park, 2009), potentially because "northern China" populations were not homogenous in their phenotypic traits. Certainly, the Anhui Province population (Anhui1) we sampled in the northern portion of Chinese tallow tree's range does not appear genetically similar according to molecular markers to the other northern China populations (Fig. 4). Long-term common garden studies investigating variation in phenotypic traits among separate native populations are needed to test whether preexisting differences are present in the native range.

Our results for Chinese tallow tree support the findings for other plant invasions that introductions from different areas of the native range contribute to different phenotypic traits and invasiveness in the introduced range. For example, introductions of *Bromus tectorum* (cheatgrass) to eastern North America stem from a different source population than those in western North America, and these eastern genotypes appear to be more restricted in habitat distribution than the more invasive western genotypes (Bartlett et al., 2002). Similarly, *Rubus alceifolius* may be less invasive on Madagascar than other islands in the Indian Ocean because of the particular genotypes that were introduced there (Amsellem et al., 2000). Idiosyncratic features of different introduced lineages were also found to contribute to population variation in the aquatic plant *Butomus umbellatus* (Kliber and Eckert, 2005). This phenomenon may become apparent for other invasive species as more studies of neutral genetic differentiation are paired with studies of phenotypic genetic differentiation (e.g., Chun et al., 2009).

Different source populations do not tell the whole story for Chinese tallow tree, however, because environmental differences are also found within the southeastern USA that appear to affect Chinese tallow tree performance. Herbivore bioassays showed that Chinese tallow tree seedlings placed in Georgia sustained greater levels of herbivory than individuals of the same USA genotypes placed in Texas or Louisiana for 2 weeks (Siemann et al., 2006). Field experiments conducted in these same locations provided further evidence of a more benign environment in Texas/Louisiana than Georgia; seedlings grew faster in Texas and Louisiana grasslands than in Georgia grasslands over two growing seasons (Siemann et al., 2006). Thus, the selective regime in Georgia and Texas/Louisiana (and potentially in other areas of the southeastern USA) appears to differ and likely has also contributed to divergence in genetically determined patterns of growth and defense. In conclusion, within the USA, differences among genotypes of Chinese tallow tree in potentially adaptive traits may result from a combination of differences in source material from the native range (this study) as well as geographic differences in selective pressures (Siemann et al., 2006).

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