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PRIMER NOTE

Microsatellite markers for an invasive tetraploid tree, Chinese tallow (*Triadica sebifera*)

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Abstract

We present six polymorphic microsatellite loci for Chinese tallow tree (*Triadica sebifera*, Euphorbiaceae), which is native to East Asia and is an aggressive, invasive species in the southeastern USA, particularly along the Coastal Plain. Microsatellite loci were tested for polymorphism across a total of 79 individuals from populations across the native range of China and in introduced populations of the southeastern USA, California, Hawaii, and Australia. Across these samples, we found a high level of polymorphism, suggesting they will be useful for deducting the introduction history of this invasive species.

Keywords: exotic, introduction, polyploid, population genetics, *Sapium sebiferum*, weed

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Molecular markers can aid in reconstructing the introduction history of non-native invasive plants. Chinese tallow tree [*Triadica sebifera* (L.) Small, previously known as *Sapium sebiferum* Roxb.] is a tetraploid tree in the Euphorbiaceae native to China (Lee 1956) and northern Vietnam. It was first introduced to the southeastern USA in the late 18th century (Bell 1966) and was later widely planted across the Gulf States in the early 20th century (Jamieson & McKinney 1938). The geographical source(s) of the introduced genetic material is not known. Chinese tallow tree has become an aggressive invader of abandoned fields, forests, coastal prairies, pastures and waste areas primarily in the Coastal Plain in the southeastern USA (Bruce *et al*. 1997). We developed microsatellite primer pairs for polymerase chain reaction (PCR) amplification of six polymorphic loci to investigate the population genetics of this species and determine whether introductions to different areas of the introduced range stem from similar or different source populations in the native range.

Chinese tallow tree DNA was pooled from 20 extractions, using a DNeasy Plant Mini Kit (QIAGEN), from leaves of a single cultivated tree growing near Rice University in Houston, TX, USA. Microsatellite libraries were constructed, enriched and screened by Genetic Identification Services (GIS, Chatsworth, CA, http://www.genetic-id-services.com/) as described previously (Jones *et al*. 2002). Briefly, genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa*I, *Hae*III, *Bsr*B1, *Pvu*II, *Stu*I, *Sca*I, *Eco*RV). DNA fragments between 350 and 700 bp long were adapted and subjected to magnetic bead capture (CPG) using biotinylated-capture molecules. Four microsatellite-enriched libraries were produced from these DNA fragments, each enriched for a specific microsatellite di- or trinucleotide repeat: CA, GA, ATG or AAG. Enriched DNA was ligated into the pUC19 plasmid that had been cut with *Hin*dIII (New England Biolabs), and the recombinant plasmids were electroporated into *Escherichia coli* strain DH5α. Nine recombinant clones from each library were selected at random for sequencing on an ABI 377, using ABI PRISM *Taq* dye terminator cycle sequencing methodology. These yielded five microsatellites from each of the CA and GA libraries, four from the AAG library and two from the ATG library. Based on these enrichment results, sequences were obtained for 22 additional clones of the CA and GA libraries and 20 from the AAG library.

Twenty-six pairs of primers (4 CA, 12 GA, 1 ATG, and 9 AAG) were designed using DESIGNERPCR version 1.03 (Research Genetics), synthesized and tested on eight samples from the USA by running the PCR products on 3.5%

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Table 1 Primers, fluorescent label, amplification conditions and estimated measures of polymorphism for *Triadica sebifera* microsatellite loci. Annealing temperature was 58 °C for all primers. Number of alleles and maximum and minimum observed heterozygosities for each locus were calculated for 79 individuals collected in the USA, Australia and China

Locus	Repeat motif	Primer sequence (5′–3′)	Allele size range (bp)	$MgCl_2$ (mM)	No. of alleles	Min. H_O	Max. H_E	GenBank Accession no.
Ts-A10	$(CA)_{11}$	F: (6-FAM) CCAACAAGTTAGCATCACCT R: CAACAGAAGTTCCTCAATGTG	128–149	3.0	9	0.493	0.576	DQ307023
Ts-B5	$(CT)_{15}$	F: (6-FAM) CTCCAGCAGCTCTTCATCT R: CGAACCAAGAATTAGGAAAAC	186–226	2.5	18	0.728	0.775	DQ307024
Ts-B103	$(GA)_{19}$	F: (6-FAM) TTCAAATCAGCCGATAATAGAG R: GCTACCTGTCTCCGTAGGA	104–135	2.5	10	0.255	0.322	DQ307025
Ts-D11	$(AAG)_{10}$	F: (HEX) GCCTTAAAGACATGGGATTC R: CGATCCATTCTCTCTTGACA	145–166	2.0	8	0.659	0.726	DQ307026
Ts-D101	$(CTT)_9$	F: (HEX) TCGCTATTTCTGCCTATTTTC R: TTCCAAAGCCCCAAGATA	238–260	2.0	8	0.665	0.736	DQ307027
Ts-D117	$(CTT)_6$	F: (HEX) CTGATGGCAGTTCTTTGAGAT R: GCCTGTTGTGGAATAGTGG	233–276	2.5	12	0.733	0.785	DQ307028

Repeat motif is listed 5′ to 3′ with respect to the forward primer (F).

agarose gels. Of these loci, 10 that appeared polymorphic were amplified for an additional four USA and four China samples on an ABI 3100. Six primer pairs were selected that were polymorphic, reproducible and scorable.

We screened 79 individuals to characterize the microsatellite loci. One individual each was screened from 26 native populations across China and 53 introduced populations from across the southeastern USA, Hawaii, California, and Australia. Total genomic DNA was extracted using DNeasy Plant Mini Kits (QIAGEN) from leaves that had been dried in silica gel, ground in liquid nitrogen and kept at −80 °C. Each PCR (10 μL) contained approximately 5–10 ng of template DNA, 1× PCR buffer (NH_4-based reaction buffer without $MgCl_2$), 0.25 mM of each dNTP (Promega), 0.6 μM of each primer, 2.0–3.0 mM $MgCl_2$ (specific $MgCl_2$ concentrations are shown in Table 1) and 0.25 U of BIOLASE *Taq* DNA polymerase (Bioline). Forward primers were labelled with a fluorescent dye (Table 1). PCR was performed on a PTC-100 thermal cycler (MJ Research) with the following cycling parameters: 3 min denaturation at 94 °C; 33 cycles of 40 s at 94 °C, 40 s at 58 °C for annealing and 30 s at 72 °C; and finally 4 min at 72 °C for a final extension. PCR products were generally diluted by half, and then 1 μL of PCR product was precipitated using ethanol. Cleaned PCR products were then resuspended in Hi-Di formamide with GeneScan 400HD ROX size standard and denatured at 95 °C for 5 min. Fragments were analysed on an ABI PRISM 3100 Genetic Analyser and scored with the aid of GENOTYPER version 3.6 NT (Applied Biosystems). Up to four bands were found for each locus per individual, which is consistent with the tetraploidy of *T. sebifera*. We could not assign multilocus genotypes, determine allele frequencies or test for linkage disequilibrium, because we could not determine allele dosage when two or three bands were present. As a result, we only determined allele phenotypes and put minimum and maximum bounds on the observed heterozygosity (Table 1). The values for observed heterozygosity for each allele phenotype at each locus followed those of Bever & Felber (1992) and assume that *T. sebifera* is an autotetraploid, as the banding pattern suggests. The heterozygosity value was equal to one minus the probability that any two alleles drawn at random were identical by descent (H_O for phenotypes with one allele = 0, two alleles = 0.50 for minimum H_O and 0.66 for maximum H_O, three alleles = 0.83 and four alleles = 1). The primers also successfully amplified microsatellites for the congener *Triadica cochinchinensis* Lour. (formerly *Sapium discolor*), which appears diploid based on the banding patterns.

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