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PRIMER NOTE

CHARACTERIZATION OF MICROSATELLITE LOCI IN *TILIA PLATYPHYLLOS* (MALVACEAE) AND CROSS-AMPLIFICATION IN RELATED SPECIES[1]

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- *Premise of the study:* Microsatellite markers in the genus *Tilia* were developed to investigate the genetic variation in *T. platyphyllos* and its relationship with *T. cordata*.
- *Methods and Results:* Fifteen microsatellite markers were developed using a microsatellite enrichment protocol. Most loci show a high level of polymorphism in two *T. platyphyllos* populations from France. The number of alleles ranged from one to 15, with a mean of 8.96. The mean observed and expected heterozygosities were 0.71 and 0.70, respectively. Cross-amplification results indicated that 12 out of 15 loci amplified polymorphic loci in 23 species in the genus.
- *Conclusions:* These markers will be useful tools for investigating the phylogeography and hybridization of *Tilia* species.

Key words: hybridization; Malvaceae; microsatellite marker; population structure; *Tilia platyphyllos.*

Tilia L. (lime or basswood) is a genus of large trees with approximately 20–25 species in the family Malvaceae of the order Malvales (Angiosperm Phylogeny Group, 2002). These species are distributed in temperate regions of the northern hemisphere, throughout Europe, and in parts of Asia and the North American continent. *Tilia cordata* Mill. (small-leaved lime) and *T. platyphyllos* Scop. (large-leaved lime) are the two species that are widely distributed in Europe. They can hybridize, which results in the common lime, *T.* ×*europaea* (Pigott, 1969). Species relationships within the genus are unknown and are complicated by frequent hybridization. Although there are some morphological differences between *T. cordata* and *T. platyphyllos*, it is difficult to identify species in the absence of flowers and from characteristics of leaves at ground level; in particular, hybrids can vary in morphology (Pigott, 1991). In the United Kingdom, lime trees were a common species 5000 yr ago (Huntley and Birks, 1983), and trees alive today may be several hundred or even 1000 yr old.

There are no nuclear markers suitable for population genetics currently available within this genus. Here, we report on the development of the first set of microsatellite markers and their transferability to other species in the genus for future population genetic studies.

METHODS AND RESULTS

Genomic DNA was extracted from a fresh leaf bud of a mature *T. platyphyllos* tree at Chanstone Wood (52°0′52.36″N, 2°56′37.98″W) using a cetyltrimethylammonium bromide (CTAB) procedure (Morgan-Richards and Wolff, 1999). A microsatellite enrichment protocol was used, based on Edwards et al. (1996) and Squirrell and Wolff (2001). In short, 100 ng of purified DNA was digested with *Mbo*I (New England Biolabs, Beverly, Massachusetts, USA) and ligated to *Sau*LA and *Sau*LB linkers (Squirrell and Wolff, 2001). The ligated DNA was PCR amplified, and the product was hybridized at 47°C to nylon membranes with dot blots of $(GA)_{15}$ and $(CA)_{15}$ oligos. The eluted enriched DNA was PCR amplified and enriched for a second time, following the same method. The doubly enriched DNA was digested with *Mbo*I and ligated in pUC19 vector (QIAGEN, Hilden, Germany). The plasmids were transformed into competent *E. coli* cells (Bioline, London, United Kingdom). Recombinant colonies were selected by blue/white screening, and M13 PCR amplification was used to estimate the size of the inserts. Inserts with a size between 300 and 700 bp were sequenced using BigDye Terminator version 3.1 (Applied Biosystems, Foster City, California, USA). A total of 104 clones from the enriched library were sequenced on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems), and 96 clones contained microsatellite repeats with a minimum of five repeats. Inserts with 9–20 dinucleotide repeats were chosen for primer design. Thirty-one primer pairs were designed using Primer3 software version 0.4.0 using the default setting (Rozen and Skaletsky, 2000) but selecting primers longer than 21 nucleotides. The PCR amplification was performed in 10-μL multiplex reactions containing 5 ng of DNA, 1× reaction buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1–0.2 mM of each primer, and 0.5 U of *Taq* DNA polymerase (Bioline). Forward primers were labeled with FAM, HEX (Integrated DNA Technologies, Coralville, Iowa, USA), or NED (Applied Biosystems) (see Table 1 for multiplex sets, specific concentration of primers, and fluorescent label). The PCR reaction for all multiplex sets was as follows: an initial predenaturation step at 95°C for 5 min, followed by 15 cycles of 95°C for 15 s, 54°C for 15 s, and 72°C for 15 s, and 20 cycles of 89°C for 20 s, 52°C for 20 s, 72°C for 20 s, and a final extension step of 72°C for 30 min. Complete PCR reactions were diluted 1 : 10 with distilled water, and 1 μL of this dilution was mixed with 10 μL of Hi-Di Formamide (Applied Biosystems) and 0.1 μL of ROX-500 size standard before analysis on an ABI 3130xl Genetic Analyzer (Applied Biosystems). The data were analyzed by GeneMapper software (Applied Biosystems) and GenAlEx version 6 (Peakall and Smouse, 2006). Fifteen out of 31 primer pairs provided good patterns with the expected product size and were used for further characterization. The other 16 primer pairs failed to amplify targets, amplified nontarget sequences, or had nonspecific band patterns in preliminary tests and were discarded.

Polymorphism was studied in two populations of *T. platyphyllos* in France, one from Issole (Alpes de Haute Provence; 44°2′N, 6°49′E; $N = 20$) and the other from Gorges de la Carança (Pyrénées Orientales; 42°51′N, 2°22′E; $N = 20$). In addition, for testing transferability of microsatellite primers, leaves were collected from 23 species of this genus (52 individuals) from a living collection

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TABLE 1. Characterization of 15 microsatellite markers developed in the genus *Tilia*.

Locus	Primer sequences (5′–3′)	Repeat motif	Size (bp)[a]	Primer conc. (mM)	Fluorescent dye[b]	Multiplex sets	GenBank accession no.
Tc4	F: ATTTTAGAATGCCAACCTGCTAAG	$T_6(GT)_{12}$	224	0.2	HEX	B	JQ289157
	R: TATTGAAGTCCATTTCCAATTGTC						
Tc5	F: TTTTCATACATTTAGAGACTTTTAGCA	$(AG)_{12}$	150	0.2	FAM	D	JQ289158
	R: TGCATGATTTGTATGTTTAGGG						
Tc6	F: CCATATCTTCTGCCAGTTTTCC	$(AG)_{12}$	143	0.2	HEX	A	JQ289159
	R: GGACTAATTTCTTCCTTTTATTAGGC						
Tc7	F: TTTACTTTTGCCAGTTGTGAGG	$(GA)_{13}$	234	0.1	FAM	D	JQ289160
	R: CACCTAGAATGCCTCCTATTCG						
Tc8	F: CGAAGAAACTGTCAAAACAACG	$(GA)_{13}$	160	0.1	HEX	B	JQ289161
	R: AGCTGGGTTTTAGAGGATAGGG						
Tc11	F: AGCTATGAAAGAACTATCAAGAGAAAG	$(AG)_{13}$	146	0.1	NED	C	JQ289162
	R: CCCCAAGACATTGCAGTAGAAC						
Tc31	F: TTTGCAAAGACTACTCCAAGAATC	$(GA)_{12}$	205	0.2	FAM	B	JQ289164
	R: AAATCGATGGTCAAGAACTAAATC						
Tc915	F: ACATCGATTGTATTTCCCTTTAAC	$(CT)_{16}$	165	0.2	HEX	C	JQ289165
	R: GTTGTATTTTGCCCTTAACATTG						
Tc918	F: AACGGCTAATTACTCCTAGTTTCG	$(AC)_9(TC)_2$	240	0.2	HEX	A	JQ289166
	R: TGTTCAGCTCACTACTACCTTTCAC						
Tc920	F: AAATGTCTTCAGAGTGACTAGATGG	$(GA)_2(GT)_{15}(AG)_4$	232	0.1	FAM	A	JQ289167
	R: TGCCTCATTATTCTCCTAATTCTC						
Tc927	F: AGTCCTCCTGTCAAATGCTG	$(AG)_{10}$	157	0.1	FAM	C	JQ289168
	R: ATCACACTCGTTTATGACATCTTG						
Tc937	F: AGCCAACCAACTTTTACAATACAG	$(AG)_{13}$	162	0.1	NED	A	JQ289169
	R: AGATAAAAGCACATAAATCGATGG						
Tc943	F: ATTTCATCTTTCTCTAAAGCCTTG	$(CA)_{10}$	150	0.2	FAM	B	JQ289170
	R: GGGAAAGCCTGTGTTAGTTTC						
Tc951	F: TGTTATGACCTCACTTATAACCAAGT	$(CT)_{12}$	160	0.2	NED	D	JQ289171
	R: GGGTGAGCTGACAATATAGAAGAG						
Tc963	F: CTAACCCCATTCTCTTTAATTCTG	$(CT)_{11}$	238	0.2	HEX	C	JQ289172
	R: GCTTTCATTTCAGTTTTCCTCTAC						

[a] Size of the original fragment.
[b] Fluorescent label on the forward primer.

in Cartmel, Cumbria, United Kingdom (Pigott, 2002). No voucher specimens have been deposited because only leaves from ground level were available.

All 15 loci were highly polymorphic in the samples analyzed. One locus (Tc943) was monomorphic within the Issole population, but was polymorphic in the Gorges de la Carança population. In these two populations, the number of alleles of the 15 loci ranged from one to 15, with a mean of 8.96. The observed and expected heterozygosities varied from 0.25 to 1.00 (average: 0.71) and 0.18 to 0.90 (average: 0.70), respectively. Significant departures from Hardy–Weinberg equilibrium (HWE) were detected at Tc918 and Tc963 in the Issole population ($P < 0.05$). For the Gorges de la Carança population, Tc4 and Tc920 deviated from HWE ($P < 0.01$), which may indicate population substructure or the presence of null alleles for those loci (Table 2). However, we did not find homozygous nulls in these populations.

These 15 loci were also tested for their amplification in 23 species in the genus *Tilia*. Most of the loci (12 out of 15) were transferable and polymorphic in most of the related species (Appendix 1). Tc915 failed to amplify in two species (*T. americana* and *T. caroliniana*), and Tc920 failed to amplify in three species (*T.* ×*euchlora*, *T. dasystyla*, and *T. endochrysea*). Tc918 was successfully amplified in only five

TABLE 2. Results of 15 microsatelite markers in two populations from France (Issole and Gorges de la Carança).

	Issole ($N = 20$)				Gorges de la Carança ($N = 20$)			
Locus	A	H_o	H_e	HWE[a]	A	H_o	H_e	HWE[a]
Tc4	8	0.85	0.82	ns	11	0.70	0.85	**
Tc5	15	1.00	0.86	ns	10	0.90	0.82	ns
Tc6	8	0.70	0.82	ns	7	0.75	0.76	ns
Tc7	9	0.80	0.76	ns	7	0.85	0.79	ns
Tc8	6	0.90	0.77	ns	8	0.80	0.80	ns
Tc11	9	0.95	0.81	ns	8	0.65	0.73	ns
Tc31	9	0.80	0.80	ns	9	0.90	0.80	ns
Tc915	12	0.90	0.88	ns	12	0.75	0.87	ns
Tc918	2	0.10	0.18	*	2	0.25	0.22	ns
Tc920	9	0.65	0.59	ns	5	0.30	0.54	**
Tc927	14	0.85	0.90	ns	11	0.75	0.82	ns
Tc937	10	0.85	0.81	ns	9	0.95	0.80	ns
Tc943	1	0.00	0.00	Monomorphic	2	0.30	0.26	ns
Tc951	8	0.60	0.64	ns	6	0.75	0.71	ns
Tc963	14	0.85	0.87	*	15	0.90	0.88	ns

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; HWE = Hardy–Weinberg equilibrium; N = sample size.
[a] ns = nonsignificant departure from HWE; *$P < 0.05$; **$P < 0.01$.

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species (*T.* ×*euchlora*, *T.* ×*europaea*, *T. dasystyla*, *T. paucicostata*, and *T. platyphyllos*). Because Tc918 amplifies in *T. platyphyllos* but not in *T. cordata*, this locus may be useful for the identification of the two species and their hybrid.

CONCLUSIONS

These 15 highly polymorphic microsatellite markers will be useful tools for the study of population structure in *T. platyphyllos* and increase our understanding of their phylogeography and of the hybridization between *Tilia* species. Furthermore, the success of cross-amplification in related species will assist the future study of genetic diversity across the genus.

LITERATURE CITED

Angiosperm Phylogeny Group. 2002. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APGII. *Botanical Journal of the Linnean Society* 141: 399–436.

Edwards, K. J., J. H. A. Barker, A. Daly, C. Jones, and A. Karp. 1996. Microsatellite libraries enriched for several microsatellite sequences in plants. *BioTechniques* 20: 758–760.

Huntley, B., and H. J. B. Birks. 1983. An atlas of past and present pollen maps for Europe: 0–13,000 B.P. Cambridge University Press, Cambridge, United Kingdom.

Morgan-Richards, M., and K. Wolff. 1999. Genetic structure and differentiation of *Plantago major* reveals a pair of sympatric sister species. *Molecular Ecology* 8: 1027–1036.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Pigott, C. D. 1969. The status of *Tilia cordata* and *T. platyphyllos* on the Derbyshire limestone. *Journal of Ecology* 57: 491–504.

Pigott, C. D. 1991. Biological flora of the British Isles. *Journal of Ecology* 79: 1147–1207.

Pigott, C. D. 2002. A review of chromosome numbers in the genus *Tilia* (Tiliaceae). *Edinburgh Journal of Botany* 59: 239–246.

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

Squirrell, J., and K. Wolff. 2001. Isolation of polymorphic microsatellite loci in *Plantago major* and *P. intermedia*. *Molecular Ecology Notes* 1: 179–181.

Appendix 1. Amplification of 15 microsatellite loci across 23 species in the genus *Tilia*.

Species[a]	Tc4	Tc5	Tc6	Tc7	Tc8	Tc11	Tc31	Tc915	Tc918	Tc920	Tc927	Tc937	Tc943	Tc951	Tc963
T ×*euchlora* (1)	++	++	+	++	++	++	++	++	+	–	++	++	++	++	++
T ×*europaea* (4)	++	++	++	++	++	++	+	++	+	++	++	++	++	++	++
T. americana L. (2)	++	++	++	++	+	++	++	–	–	++	++	++	++	++	++
T. amurensis Rupr. (4)	++	++	++	++	+	++	++	++	–	++	++	++	++	++	++
T. callidonta Hung T. Chang (1)	+	+	++	++	+	++	++	++	–	++	++	++	++	+	++
T. caroliniana Mill. (5)	++	++	++	++	++	++	++	–	–	+	++	++	++	++	++
T. chingiana Hu & Cheng (1)	++	++	++	++	+	+	++	++	–	++	+	+	++	++	++
T. concinna Pigott (1)	++	++	+	++	+	++	++	++	–	++	++	++	++	++	++
T. cordata Mill. (6)	++	++	++	++	+	++	++	++	–	++	+	++	++	++	++
T. dasystyla Steven (2)	++	++	++	++	++	++	++	++	++	–	++	++	++	++	++
T. endochrysea Hand.-Mazz. (1)	++	++	++	++	+	++	++	++	–	–	+	++	+	+	+
T. henryana Szyszył. (1)	++	++	++	++	+	++	++	++	–	++	++	++	++	++	++
T. japonica (Miq.) Simonk. (2)	++	++	++	++	+	++	++	++	–	++	++	++	++	++	++
T. kiusiana Makino & Shiras. (1)	++	++	++	++	+	+	++	++	–	+	++	++	++	+	+
T. mandshurica Rupr. & Maxim. (1)	+	++	++	++	+	++	++	+	–	++	++	++	+	+	++
T. maximowicziana Shiras. (2)	++	++	++	++	+	++	++	++	–	++	++	++	+	++	++
T. mongolica Maxim. (1)	++	++	++	++	++	++	++	++	–	++	++	++	++	++	++
T. nobilis Rehder & E. H. Wilson (1)	+	+	++	++	+	+	++	++	–	+	++	++	++	++	++
T. oliveri Szyszył. (1)	++	++	++	++	+	+	+	++	–	+	++	+	++	+	++
T. paucicostata Maxim. (1)	++	++	+	++	++	++	++	++	+	++	++	++	++	++	++
T. platyphyllos Scop. (7)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
T. tomentosa Moench (4)	++	++	++	++	+	++	++	+	–	++	++	++	++	+	++
T. tuan Szyszył. (2)	++	++	++	++	+	++	++	++	–	++	++	++	++	++	++

Note: – = failed amplification; + = successful amplification with one allele; ++ = successful amplification with more than one allele.
[a]Numbers in parentheses show number of samples tested.

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