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PRIMER NOTE

Identification and characterization of microsatellite loci in the common fig (*Ficus carica* L.) and representative species of the genus *Ficus*

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Abstract

We developed microsatellites in fig (*Ficus carica* L.). A TC and TG-enriched genomic library was screened, and after sequencing, primers were designed for 20 microsatellites. Eight primer pairs produced amplification products that were both interpretable and polymorphic in 14 fig cultivars and two French wild-growing populations of *F. carica* (n_1 = 9 and n_2 = 10). Number of alleles per locus ranged from three to six. Except for one microsatellite locus, the observed heterozygosity was higher than the expected value. The *F. carica* microsatellites gave amplification products in 17 other *Ficus* species in 86% of the cases.

Keywords: *Ficus*, fig cultivars, microsatellites

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The fig–pollinator mutualism is a model system for the study of coevolution (Anstett *et al*. 1997). The use of molecular markers is becoming essential to understand the evolutionary biology of this obligate relationship. For example, genotyping pollen carried by the associated wasps using microsatellite markers would enable one to determine on which tree those wasps were born and hence to determine patterns of wasp movement among trees. We chose to identify microsatellite loci in *Ficus carica* because it is a Mediterranean crop plant presenting cultivar identification problems and for which we plan to analyse the domestication process.

In this note, we report the development of eight microsatellite primers from *F. carica* and their polymorphism in fig cultivars and two French wild-growing populations. We also report results of amplification on species of *Ficus* covering the whole phylogeny of the genus (Weiblen 2000) and showing the potential usefulness of these microsatellite loci within the genus.

Genomic DNA from the French cultivar 'Violette de Solliès' of *F. carica* was digested with *Rsa*I. DNA fragments were ligated to synthetic adapters, denatured and hybridized to biotinylated $(TC)_{15}$ and $(TG)_{15}$ oligonucleotides. Oligo-fragment hybrids were selectively separated from the remaining DNA using streptavid-coated paramagnetic beads (Streptavidin MagneSphere Paramagnetic Particles, Promega). The DNA fragments containing simple sequence repeats (SSRs) were eluted as single strands from beads and the second strand was regenerated and amplified by polymerase chain reaction (PCR), using an adapter-specific primer (Rafalski *et al*. 1996). PCR fragments were ligated into pGEM-T Easy plasmid (Promega) following the manufacturer's instructions and cloned in *Escherichia coli* XL1-Blue cells (Stratagene). Inserts of a set of 564 colonies were amplified by PCR with an adapter-specific primer, run on a 2% agarose gel and transferred onto a charged Nylon membrane (Hybond N+, Amersham-Pharmacia) using alkaline transfer. Membranes were hybridized with a mix of [γ^{32}P]-dATP end-labelled $(TC)_{10}$ and $(TG)_{10}$ probes. Selected clones were sequenced using dye-primer chemistry and analysed on an ABI377 semi-automated sequencer. Primers flanking microsatellite repeats were designed for 20 loci, and eight primer pairs produced amplification products that were both interpretable and polymorphic in fig cultivars and wild-growing populations of *F. carica*.

For PCR, DNA was extracted from 100 mg of frozen material (leaves) according to the Dneasy Plant Mini Kit (Qiagen) with the following modification: 1% of Polyvinylpyrrolidone (PVP 40 000) was added to buffer AP1.

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Table 1 *Ficus carica* microsatellite loci motifs, primer sequences, size of cloned alleles and annealing temperature (T_a). Number of alleles observed (N_a), observed heterozygosity (H_O) and expected heterozygosity (H_E) are shown for cultivars (C) and two wild-growing populations (Grotte de la Clamouse: GC, Pont d'Hérault: PH) and for the total sample (T)

							N_a				H_E/H_O			
Locus	Accession no.	Repeat motif	Primer sequence (5′–3′)	Size*	$MgCl_2$†	T_a	C	GC	PH	T	C	GC	PH	T
MFC1	AF333696	$[CT]_{13}$	F: ACTAGACTGAAAAAACATTGC R: TGAGATTGAAAGGAAACGAG	192	2	55	6	3	5	6	0.689/0.643	0.611/0.889	0.735/0.8	0.691/0.758
MFC2	AF333697	$[AC]_{18}[AT]_7$	F: GCTTCCGATGCTGCTCTTA R: TCGGAGACTTTTGTTCAAT	172	2	55	4	5	5	5	0.602/0.714	0.772/0.667	0.595/0.8	0.742/0.727
MFC3	AF333698	$[AC]_{15}TC[AC]_8[AT]_7$	F: GATATTTTCATGTTTAGTTTG R: GAGGATAGACCAACAACAAC	136	2	55	5	4	5	6	0.671/0.714	0.698/1	0.73/1	0.716/0.879
MFC4	AF333699	$[AT]_4[AC]_{11}$	F: CCAAACTTTTAGATACAACTT R: TTTCTCAACATATTAACAGG	218	2	55	4	4	4	4	0.707/1	0.636/0.889	0.715/0.9	0.698/0.939
MFC5	AF333700	$[GA]_{13}$	F: ACCAATCCAAATAATAATCC R: ACACGCTTACTAGAATTACC	140	3	50	4	3	3	4	0.61/1	0.494/0.667	0.535/0.9	0.535/0.879
MFC6	AF333701	$[TAA]_3,[GT]_8$	F: AGGCTACTTCAGTGCTACA R: GCCATAAGTAATAAAAACC	313	2.5	50	4	4	5	6	0.737/0.929	0.735/0.778	0.76/0.9	0.777/0.879
MFC7	AF333702	$[AG]_{11}$	F: CACAATCAAAATAGTTACCG R: AGCGAAGACAGTTACAAAGC	150	1.5	50	4	3	3	4	0.52/0.429	0.426/0.556	0.525/0.6	0.504/0.515
MFC8	AF333703	$[CA]_9TA[CA]_{14}[TA]_6$	F: GTGGCGTCGTCTCTAATAAT R: TATTCTATGCTGTCTTATGTCA	179	2	50	3	2	2	3	0.528/0.714	0.105/0.111	0.18/0.2	0.374/0.394

*Size of cloned allele. †Concentration of $MgCl_2$ (mM).

Table 2 Ability of *Ficus carica* microsatellite primers to generate PCR products in 17 species representing 14 out of 15 *Ficus* sections. An individual of each species was tested. The section is indicated for each species which are listed in order of increasing phylogenetic distance. The bold bars separate groups of sections of increasing phylogenetic distance

Species	Section	MFC1	MFC2	MFC3	MFC4	MFC5	MFC6	MFC7	MFC8
F. deltoidea	*Ficus*	+	+	+	+	+	+	+	+
F. pumila	*Rhizocladus*	+	+	+	+	+	+	+	+
F. punctata	*Kalosyce*	–	–	–	+	–	+	–	+
F. parietalis	*Sycidium*	+	–	+	+	+	+	+	+
F. condensa	*Sycocarpus*	+	+	+	+	+	+	+	+
F. mauritania	*Sycomorus*	+	–	–	–	+	+	–	+
F. callosa	*Oreosycea*	+	+	+	+	+	+	+	+
F. prolixa	*Urostigma*	+	+	–	+	+	+	+	+
F. macrophylla	*Malvanthera*	+	–	–	+	+	+	–	+
F. schumacheri	*Americana*	+	+	+	+	+	+	+	+
F. lutea	*Galoglychia*	+	+	+	+	–	+	+	+
F. glumosa	*Galoglychia*	+	+	+	+	+	+	+	+
F. xylophylla	*Conosycea*	+	+	+	+	+	+	+	+
F. consociata	*Conosycea*	+	+	+	–	–	+	+	+
F. elastica	*Stilpnophyllum*	+	+	+	+	+	+	+	+
F. insipida	*Pharmacosycea*	+	–	–	+	+	+	+	+
F. glabrata	*Pharmacosycea*	+	+	+	+	+	+	+	+
No. of species with PCR products		16	12	12	15	14	17	14	17

Amplification reactions were performed in a final volume of 25 µL in the presence of 50 ng of template DNA, 10 pmoles of each primer, 0.2 mM of each dNTP, and 1 unit *Taq* polymerase (Appligene-Oncor). The buffer used was the one supplied by the manufacturer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% TritonX100, 0.02% gelatin) with the final concentration of $MgCl_2$ ranging between 1.5 and 3 mM according to primer pair. The PCR was carried out using a PTC 100 thermocycler (MJ Research). After 4 min at 94°, 35 cycles were performed with 30 s at 94 °C, 45 s at 50–55 °C, 1 min at 72 °C and a final extension step of 4 min at 72 °C. Amplification products were mixed with an equal volume of Loading Sequence Buffer (98% formamide, 10 mM EDTA pH 8, 1% xylene cyanol, 1% bromophenol blue). The samples were denatured by incubation at 95° for 5 min and placed on ice. Four µL of each sample were loaded onto 6% denaturing polyacrylamide gels (7.5 M urea, 6% acrylamide, 1× TBE). The gels were run in 1× TBE at 70 W. Microsatellites were visualized by silver staining with a commercial kit (Promega).

The eight selected loci were tested on 14 cultivars from the fig germplasm in the 'Conservatoire Botanique National Méditerranéen de Porquerolles' and two wild-growing populations from Southern France, Hérault: Grotte de la Clamouse (n = 9) and Pont d'Hérault (n = 10).

DNA from 17 species representing 14 out of the 15 sections of genus *Ficus* were used to test transferability of the microsatellite primers. Amplification and visualization conditions were identical to those used in *F. carica*.

For five microsatellite loci, all alleles observed were found in fig cultivars (Table 1). Except for the microsatellite locus MFC2, the observed heterozygosity was higher than the expected values suggesting the absence of null alleles (Table 1). In other *Ficus* species, the *F. carica* microsatellites gave an amplification product in 86% of the cases (Table 2). This rate was higher than we expected in view of the supposedly 'old age' of diversification of the genus. However, it is comparable to transportability rates of microsatellites within other plant genera.

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