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PRIMER NOTE

Characterization of eight new microsatellite loci in the invading maritime pine bast scale *Matsucoccus feytaudi* (Hemiptera: Coccoidea: Margarodidae)

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Abstract

The bast scale *Matsucoccus feytaudi* is a specific pest of maritime pine that is endemic in the Iberian Peninsula and southwestern France, and invasive in southeastern France, Italy and Corsica where it causes heavy damages. We developed eight microsatellite polymorphic markers using an enrichment protocol in order to study the invasion pathways and infer the genetic diversity of the populations. All loci could be successfully amplified with no evidence of null alleles. Cross-species amplifications failed to amplify the congeneric *Matsucoccus josephi*.

Keywords: maritime pine, *Matsucoccus*, microsatellites, scale insect

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The bast scale *Matsucoccus feytaudi* Duc. (Hemiptera: Coccoidea: Margarodidae) is a specific pest of maritime pine (*Pinus pinaster* Ait.). It is distributed in the western part of the Mediterranean basin. In the Iberian Peninsula and southwestern France, the insect is endemic and its impact on the host tree is negligible. On the contrary, the scale is supposed to have been introduced in southeastern France where it caused severe outbreaks responsible for the decline of 120 000 ha of maritime pine in the 1960s (Schvester 1967; Schvester & Fabre 2001). It reached Italy in the late 1970s (Covassi & Binazzi 1992) and is still expanding southwards there. Moreover, *M. feytaudi* was detected in Corsica in 1994 (Jactel *et al*. 1996) where its range is continuously growing, causing heavy tree mortality (Jactel *et al*. 1998, 2006). The phylogeography of *M. feytaudi* was studied using mitochondrial markers (Burban *et al*. 1999) and showed that the populations are highly structured in space. However, the invasive populations were all monomorphic for the chosen marker. To study the expansion of the species and to infer the geographic origin of the introduced populations, we needed to develop polymorphic nuclear markers.

A microsatellite-enriched library was constructed following the procedure of Edwards *et al*. (1996) modified according to Butcher *et al*. (2000) using dinucleotide motifs (GA and CA). Enriched polymerase chain reaction (PCR) products were cloned using the TOPO TA cloning kit (Invitrogen) following the manufacturer's instructions. Approximately 400 recombinant clones were screened for microsatellites using digoxigenin (DIG)-labelled oligonucleotide probes. Sixty-nine positive clones were revealed and sequenced with the universal M13 primer (5'GTAAAACGACGGCCAGT3') using the dye primer cycle sequencing core kit (PE Applied Biosystems) on alkaline-denaturated plasmids. Sequencing was carried out using a 4000 L automatic DNA sequencer (Li-Cor) using 6% Long Ranger gels (TEBU).

Sixteen microsatellite loci were found on different clones and PCR primers were designed in the simple sequence repeat (SSR) flanking regions using the OLIGO software. We were able to define PCR conditions for eight polymorphic loci. Technical details and GenBank Accession nos for each marker are given in Table 1. The loci were amplified on 32 individuals collected in Sintra, Portugal and 32 individuals collected in Pinetto, Corsica, France to test the polymorphism and determine if null alleles occurred. The amplifications were carried out using a GeneAmp PCR System 2700 (Applied Biosystems) and Invitrogen *Taq* polymerase. For all loci but Mat61, the total reaction volume was 10 μL, containing 10 ng of genomic DNA, 1× *Taq* buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl], 0.5 mM

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Table 1 Characteristics of the eight microsatellite loci. The fluorescent-labelled primers are indicated by H (HEX), F (FAM) or N (NED) at the 5′ end. N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; # amp. ind., number of amplified individuals (32 individuals tested per population). H_E and H_O were not computed in cases when one allele was found per population

Locus name	Primer sequences (5′–3′)	Motif	NA	Size range	Sintra H_O/H_E (# amp. ind.)	Pinetto H_O/H_E (# amp. ind.)	GenBank Accession
Mat56M	**N**-ACATCCACAGGCGTGGTG TTTTGGCTTCAAAGGTGGC	$(CA)_{11}$	2	142–144	–/– (32)	0.56/0.51 (32)	DQ460042
Mat61	**H**-GGTTGCGAGTGGCGATGCT TGAGGTGCAAGTGGGGACACA	$(GT)_3AT(GT)_8$	2	152–160	0.19/0.25 (31)	–/– (32)	DQ460036
Mat123	**F**-CAGAAATCAAGGTGCGAAAATC CGAAAGATCGACTTTGACCGAT	$(TG)_{12}$	6	142–156	0.56/0.67 (31)	–/– (31)	DQ460038
Mat211B	**F**-CTCCGTTCCTGCTGTCTCATG ACACACTCAAAGCATTCACATCAAC	$(CA)_{27}$	15	133–195	0.72/0.79 (31)	0.59/0.61 (32)	DQ460037
Mat212	**F**-CCCTAACGATATACGGCAACA CGATGGAATGAAAAACTTACGA	$(GA)_{12}CG(CA)_5$	3	140–146	0.12/0.15 (32)	0.44/0.51 (31)	DQ460039
Mat234	**N**-CTGTTAAACGGGGACCTTGA GAATGTTTGCAGATGTTTACCATTT	$(GT)_6GATT$ $(GT)_7TT(GT)_{10}$	8	150–170	0.75/0.79 (32)	0.41/0.42 (32)	DQ460040
Mat182	**N**-GGGCGCTGCACACCTAAT GGCGAAAAATTCTGCGATAAAA	$(GT)_9GAA(G)_8$	6	154–166	0.50/0.76 (31)	0.19/0.26 (31)	DQ51910
Mat252	**H**-ATCGCAGAAATCAAGGTG CGACTTTGACCGATATTATGTA	$(GT)_{11}$	6	138–152	0.56/0.65 (32)	–/– (32)	DQ460041

dNTP mix, 1.5 mM $MgCl_2$, 0.4 μM of nonlabelled primer 1, 0.4 μM of fluorescent-labelled primer 2, 0.1 mg/mL of bovine serum albumin (BSA) and 0.5 U of *Taq*. For locus Mat61, $MgCl_2$ concentration was raised to 2 mM and BSA to 0.25 mg/mL. The cycling conditions were 3 min at 94 °C followed by 26–30 cycles of [30 s at 94 °C, 30 s at 50 °C (except for locus Mat61 at 55 °C) and 30 s at 72 °C] and a final elongation step of 5 min at 72 °C. The PCR products were diluted 10 times and run on a MegaBACE1000 (Amersham) automatic sequencer using ET400-R size standard to size alleles. Raw data were analysed using MegaBACE fragment profiler version 1.2 (Amersham).

Most of the tested individuals were successfully amplified and only seven null alleles were found (out of 512 amplifications, see Table 1). The number of alleles per locus ranged from two to 15. Exact tests of Hardy–Weinberg equilibrium were performed for each locus and population with ARLEQUIN 3.0 (Excoffier *et al*. 2005). In most cases, the observed heterozygosities were not significantly different from the expected heterozygosities except for Mat182 and Mat61 in Sintra, and Mat182 and Mat212 in Pinetto (Table 1). Linkage disequilibrium between each pair of loci was not significant in Sintra except for the pair Mat123–Mat252 ($P < 0.001$). In Pinetto, three pairs of loci were significantly linked (Mat182-Mat234; Mat182-Mat212 and Mat56M-Mat212), which could be due to the recent origin of this population in the expansion range of the species.

We investigated cross-species amplification for 16 *Matsucoccus josephi* individuals sampled in Israel with the same PCR conditions as for *M. feytaudi*. No amplification was obtained for any individual.

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