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PRIMER NOTE

Characterization of five microsatellite loci in the pine shoot beetle *Tomicus piniperda* (Coleoptera: Scolytidae)

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Abstract

***Tomicus piniperda* is a bark beetle that causes damage to various pine species across a wide geographical range. We developed five microsatellite polymorphic markers using an enrichment protocol. All loci could be successfully amplified with no evidence of null alleles and will be useful for population genetic studies. Cross-species amplifications show that at least some of the markers could be useful in four other *Tomicus* species.**

Keywords: microsatellites, Scolytidae, *Tomicus*

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The bark beetle *Tomicus piniperda* (Coleoptera: Scolytidae) is one of the main pests of pine forests in Europe. The damage it causes is mostly due to its shoot-feeding behaviour, which weakens the tree and reduces its growth. *T. piniperda* is widely distributed from Europe to Japan (Balachowsky 1949), and has been introduced in the USA (Alosi Carter *et al*. 1996). It attacks the majority of the *Pinus* species within this range. Little is known about the genetic structure of this species or about its phylogeography. Understanding the factors responsible for the genetic structuring and the relationships between ecologically distinct populations may be important in building pest management programmes and decisions regarding quarantine laws. For these reasons, we developed five microsatellite loci that were tested on various *Tomicus* species. Microsatellite markers have been reported from numerous insects including Coleoptera (Liewlaksaneeyanawin *et al*. 2001; Rasplus *et al*. 2001; Brouat *et al*. 2002; Dhuyvetter *et al*. 2002), but no Scolytidae microsatellites have been published to date despite the economic importance of this family.

Microsatellites were isolated following a biotin-enrichment protocol modified from Kijas *et al*. (1994). Genomic DNA was extracted from 20 individuals using the DNeasy Tissue Kit (Qiagen). Total DNA was digested with *RSA*I and the 300–900-bp fragments were isolated on a 1.5% agarose gel using the Qiaquick Gel Extraction kit (Qiagen). Oligonucleotide adaptators (RSA21: 5′-CTCTTGCTTACGCGTGGACTA-3′ and RSA25: 5′-TAGTCCACGCGTAAGCAAGAGCACA-3′) were subsequently ligated with T4 DNA ligase (Promega). Biotin-labelled oligonucleotides corresponding to microsatellite motifs $(CT)_{10}$, $(GT)_{10}$ and $(GAA)_8$ were then hybridized to the modified DNA, and enrichment was completed using Streptavidine Magnesphere Paramagnetic Particles (Promega). The resulting enriched DNA was amplified using RSA21 primer, with 25 cycles of 40 s at 94 °C, 1 min at 60 °C and 2 min at 72 °C and a final elongation step of 10 min at 72 °C. The polymerase chain reaction (PCR) product was cloned into the pGEM plasmid vector (pGEM-T easy vector, Promega) using *Escherichia coli* strain JM109 (Promega). Recombinant clones were screened by PCR. Forty-eight positive clones were purified using the Qiaprep Spin Miniprep Kit (Qiagen). Sequencing of the mini-preparation was performed using the Big-Dye Terminator sequencing kit and carried out with a ABI 3100 automatic sequencer (Applied Biosystems).

Of the 48 clones, 17 microsatellite loci were found. We were able to define PCR conditions for five loci. Technical details and Genbank Accession nos for each marker are given in Table 1. The loci were amplified on 30 individuals collected in Orléans forest (France) to test polymorphism and determine whether null alleles occurred. Amplifications were carried out using a MJ Research PTC100 thermal cycler and the Red Taq package (Sigma). The total reaction volume was 10 μL, containing 10 ng of genomic DNA, 1 μL of 10× buffer, 1 μL of 10 mM dNTP mix, 0.6 μL of 25 mM $MgCl_2$, 0.4 μL of nonlabelled primer 1 (10 μM), 0.36 μL of nonlabelled primer 2 (10 μM), 0.04 μL of fluorescent-labelled

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Table 1 Characteristics of the five microsatellite loci. Allelic diversity and heterozygosities were calculated on 30 individuals from Orléans (France)

Locus	Primer sequences (5′ → 3′)	Annealing temp. (°C)	Motif	No. alleles	Size (bp)	H_O	H_E	P	GenBank Accession no.
TP-CT2-8F	TGGCTGCTCTTGCTAACGC* CCACCTATCTATAACGACTTCGCC	55	$(CT)_{10}$	11	113–136	0.900	0.885	0.51	AY165173
TP-CT2-5F	TGACGACCGTGGGCTACG* AACCGTTTCGACGCCAAAG	57	$(CA)_7TACGAACACGA(AC)_{12}$	30	248–320	0.965	0.966	0.77	AY165174
TP-CT1-4F	GGAGCACACGACGCAACT* CCGCATCACAAAGAAGAACTG	53	$(CT)_{10}$	6	162–175	0.600	0.691	0.60	AY165175
TP-CT2-5H	ACCACGACGGAACGACC* CTTAGGTTCTCCAGATTTGCG	54	$(TG)_8$	3	115–119	0.333	0.315	1	AY165176
TP-CT1-8B	AGATGTCGCCTGGTTCGG* TCCACACACAACATTTAATGCG	54	$(AC)_8$	4	198–206	0.367	0.532	0.17	AY165177

*Fluorescent-labelled primers.

Table 2 Cross-species amplifications of the five loci. Results are shown when more than 50% of the individuals were successfully amplified

Species	*T. destruens*		*T. minor*		*T. brevipilosus*		*Tomicus nsp*	
Locus	*TP-CT2-5F*	*TP-CT2-5H*	*TP-CT2-5H*	*TP-CT2-5F*	*TP-CT2-8F*	*TP-CT2-5H*	*TP-CT2-8F*	*TP-CT2-5H*
Tested individuals	19	19	5	8	8	8	8	8
Amplified individuals	11	19	5	5	8	8	6	6
Number of alleles	7	4	3	6	1	3	9	2

primer 2 (10 μM), 0.2 μL of BSA (1 mg/mL) and 0.4 U of *Taq* DNA polymerase. The cycling conditions were 3 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at the specific annealing temperature (Table 1) and 30 s at 72 °C. The amplified products were detected on an ABI 3100 automatic sequencer and their sizes were estimated using GENESCAN software (Applied Biosystems).

All the tested individuals were successfully amplified and no null alleles were found. The number of alleles per locus ranged from 3 to 30. For all loci, the observed heterozygosity was not significantly different from the expected heterozygosity (Table 1), as tested using ARLEQUIN software (Schneider *et al*. 1997). Linkage desequilibrium between each pair of loci was not significant (*P*-values of 0.071–0.994) except for *TP-CT2-8F* and *TP-CT2-5F* ($P = 0.045$).

We investigated cross-species amplification for *T. destruens* (southeast France), *T. minor* (St André les Alpes, France), *T. brevipilosus* (Yunnan Province, China) and *Tomicus nsp* (Yunnan Province, China) using the same PCR conditions as for *T. piniperda*. The most interesting results are summarized in Table 2. We obtained some promising amplifications, especially for the locus *TP-CT2-5H*.

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