Molecular Ecology Notes (2004) **4**, 213–214 doi: 10.1111/j.1471-8286.2004.00620.x

PRIMER NOTE

Characterization of five microsatellite loci in the Pine Processionary Moth *Thaumetopoea pityocampa* (Lepidoptera Notodontidae Thaumetopoeinae)

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Abstract

Five microsatellite markers were developed for the lepidopteran species *Thaumetopoea pityocampa* using an enrichment protocol. All loci could be amplified with no evidence of null alleles and will be useful for population genetic studies. The number of alleles ranged from three to 12 for a population of 30 individuals. Observed heterozygosities ranged from 0.53 to 0.80. No significant heterozygote deficiency was detected. Four markers might be of interest for *Th. wilkinsoni*.

Keywords: Lepidoptera, microsatellites, *Thaumetopoea*, *Thaumetopoea pityocampa*, *Thaumetopoea wilkinsoni*

Received 9 December 2003; revision received 16 January 2004; accepted 16 January 2004

The winter pine processionary moth *Thaumetopoea pityocampa* is a serious and increasing economic and public-health concern in Southern Europe and North Africa. Its defoliating caterpillars cause potentially severe damage to various pine species. They have urticating hairs and may cause strong allergic reactions. In recent years, this Mediterranean insect has shown range expansion to upper latitudes and elevations (e.g. Démolin *et al*. 1996; Benigni & Battisti 1999). Genetic markers could be very helpful to understand these colonization processes and to define integrated management strategies. Despite the importance of this forest and urban pest species, there is to date only one study on its population genetic structure (Salvato *et al*. 2002) that indicates a strong geographical structure among eight Italian and one Spanish populations using AFLP and SSCP markers. Thanks to their codominant properties, the microsatellites reported in the present paper open broad possibilities for further studies, including genetic structure and dynamics through space and time. Moreover, microsatellite markers tend to be rare in the Lepidoptera (e.g. Ji *et al*. 2003) and no Notodontidae microsatellites have been published so far.

Microsatellite loci were isolated following a biotin-enrichment protocol modified from Kijas & Fowler (1994). Genomic DNA was extracted from 60 heads of L3 larvae using the DNeasy Tissue Kit (Qiagen). Total DNA was digested with *RSA*I and the 300–900 bp-long fragments were isolated on a 1.5% agarose gel using the Qiaquick Gel Extraction kit (Qiagen). Oligonucleotide adaptators (RSA21: 5′CTCTTGCTTACGCGTGGACTA3′ and RSA25: 5′TAGTCCACGCGTAAGCAAGAGCACA3′) were subsequently ligated with T4 DNA ligase (Promega) and a Polymerase Chain Reaction (PCR) was performed using RSA21 as primer. Biotin-labelled oligonucleotides corresponding to microsatellite motifs $(CT)_{10}$, $(GT)_{10}$ and $(GAA)_8$ were then hybridized to the modified DNA, and the enrichment was completed using the Streptavidine Magnesphere Paramagnetic Particles (Promega). The resulting enriched DNA was amplified using RSA21 primer, with an initial step of 1 min at 95 °C, 27 cycles of 40 s at 94 °C, 1 min at 60 °C and 2 min at 72 °C and a final step of 10 min at 72 °C. The PCR product was cloned into the plasmid pGEM-T easy vector (Promega) using the *Escherichia coli* strain JM109 (Promega). Recombinant clones were screened by PCR using vector primers plus the repeat-specific primer $(CT)_{10}$ $(GT)_{10}$ or $(GAA)_8$. Fifty-five 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 55 clones, 33 different inserts containing microsatellites were found. We could design primers and define PCR conditions for five polymorphic loci. Technical details and gene bank accession numbers are given in Table 1. The loci were amplified on 30 individuals collected in Orléans

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Table 1 Characteristics of five polymorphic microsatellite loci in *Thaumetopoea pityocampa*. Allelic diversity and heterozygosities were calculated on 30 individuals from Orléans forest (France) collected in October 2002

Locus name GeneBank Accession	Repeat	Primer sequences (5→3′)	T_a	[$MgCl_2$] (mM)	No. of alleles	Size range (bp)	H_O	H_E
MS-*Thpit*1-CT AY520916	$(GA)_{11}$	GCAGTTGAACACGTGGTTAGCG* GGTTAACGCACTCCTTCTTTCC	53 °C	1.2	5	159–173	0.800	0.800
MS-*Thpit*2-CT AY520913	$(GA)_{19}$	CCTCGTTCGCAATAACTTTGAC† CAAAATCGGGATAGTATTAGGTCG	53 °C	1.2	12	137–167	0.633	0.818
MS-*Thpit*3-CT AY520914	$(CT)_{16}$	GGCCCTGAATCCTGATTGG‡ ATCACGTTTTGGTGTTTGACGT	54 °C	1.0	6	231–253	0.567	0.728
MS-*Thpit*4-CT AY520915	$(GA)_{11}$	CGCGAAAGCAATATAATTGTGC‡ TGACTGGGCTGAATGCAGG	54 °C	1.0	7	159–189	0.600	0.733
MS-*Thpit*5-CT AY520912	$(GA)_{11}$	CAATGACGTGCTCGTAGAGCCA* TCGTTGGCAAGTGTTTTGGAGA	56 °C	1.2	3	193–207	0.533	0.444

Primer end-labelled with *6-FAM, †HEX, ‡NED; T_a: annealing temperature; [$MgCl_2$]: $MgCl_2$ concentration; H_O: observed heterozygosity; H_E: expected heterozygosity.

forest (France), where the species has been present for 10 years. The 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, 0.4 U of *Taq* DNA polymerase (Sigma), 1 × buffer (100 mM tris HCl, 500 mM KCl and 0.1% gelatin), 20 mg/L of BSA, 250 μM of each dNTP, 0.4 μM of each primer. $MgCl_2$ concentration is given in Table 1. 20% of the forward primer was 5′ end-labelled with a fluorescent dye, either 6-FAM (Sigma), HEX (Sigma) or NED (Applied Biosystems). The cycling conditions were 3 min at 95 °C followed by 30 cycles of 50 s at 95 °C, 1 min at annealing temperature (Table 1) and 30 s at 72 °C. The amplified products were detected on a ABI-3100 automatic sequencer and their sizes were estimated using the GENESCAN software (Applied Biosystems).

All the tested individuals were successfully amplified with no evidence of null alleles. For all loci, the observed heterozygosities were not significantly different from the expected heterozygosities (*P*-values of 0.07–0.41) and linkage desequilibrium between each pair of loci was not significant (*P*-values of 0.23–0.97), as tested using ARLEQUIN software (Schneider *et al*. 1997). The number of alleles per locus ranged from three to 12 (Table 1).

These markers were tested on three congeneric species of major importance, *viz*. its Middle Eastern sibling *Th. wilkinsoni* and two summer species occurring, respectively, in Middle East and in Europe, the pistachio processionary moth *Th. solitaria* and the oak processionary moth *Th. processionae*. Cross-species amplifications were investigated on samples from Israel (eight individuals), Lebannon (six individuals) and France (two individuals), respectively, using the same PCR conditions as for *Th. pityocampa*. Amplifications were obtained only for *Th. wilkinsoni*, for all loci but MS-*Thpit*2-CT. All tested individuals were successfully amplified. Two alleles were revealed for MS-*Thpit*1-CT, MS-*Thpit*3-CT, MS-*Thpit*4-CT and one single allele was found for MS-*Thpit*5-CT.

Acknowledgements

The authors are grateful to A. Battisti (Università di Padova, Italy), Z. Mendel (ARO, Israel), J. Poirot (DSF-Nancy, France), B. Frérot (INRA-Versailles, France), F. Goussard and P. Pineau (INRA-Orléans) for the samples of *Thaumetopoea*. This work was supported by a grant from the European Union (Promoth project).

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