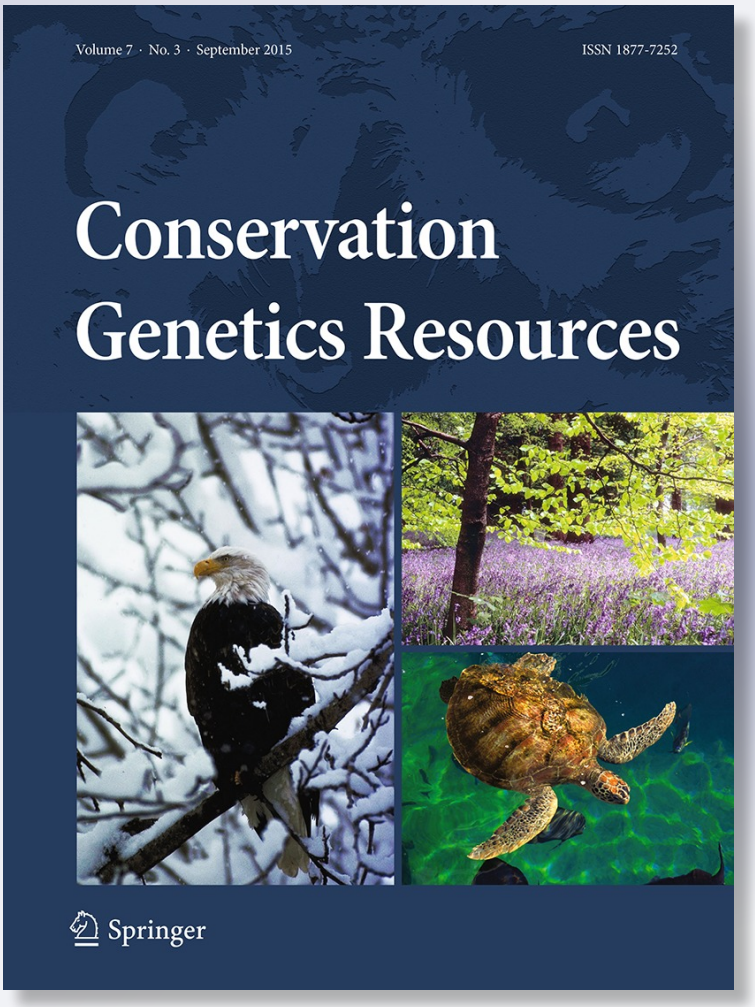
Isolation, characterization and PCR multiplexing of 17 microsatellite loci in the pine processionary moth Thaumetopoea pityocampa *(Lepidoptera, Notodontidae)*

L. Sauné, F. Abella & C. Kerdelhué

Conservation Genetics Resources

ISSN 1877-7252
Volume 7
Number 3

Conservation Genet Resour (2015)
7:755-757
DOI 10.1007/s12686-015-0453-3



Springer

Conservation Genet Resour (2015) 7:755–757
DOI 10.1007/s12686-015-0453-3

MICROSATELLITE LETTERS

Isolation, characterization and PCR multiplexing of 17 microsatellite loci in the pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera, Notodontidae)

L. Sauné · F. Abella · C. Kerdelhué

Received: 10 February 2015 / Accepted: 16 February 2015 / Published online: 22 February 2015

Abstract Seventeen polymorphic microsatellite markers were developed for the pine processionary moth, *Thaumetopoea pityocampa* and organized in three multiplex. The number of alleles ranged from 1 to 18 and observed heterozygosities from 0.068 to 0.892. Tests of cross-amplifications are also reported, and show that these loci can be used in divergent clades of the same species, and most of them for the sister species *T. wilkinsoni*. These markers will be useful to develop fine-scale population genetics study and adapt pest management strategies for this insect, which was proved to threaten relict pine populations in the Mediterranean Basin.

Keywords Microsatellites · Genetic diversity · *Thaumetopoea pityocampa*

The pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae) is one of the main pests in Mediterranean pine and cedar forests. It can cause heavy defoliations that weaken the host tree, which then becomes prone to secondary pest infestation or draught damage. It was proved to threaten relict populations of *Pinus sylvestris* in southern Spain (Hódar et al. 2003). To investigate fine-scale population structure and correctly design management strategies, polymorphic markers are needed to complement the already existing loci. We here report the development of 17 new microsatellites.

We used draft genomic sequences available for this species (Kerdelhué, unpublished data) to design PCR primers using QDD (Meglezc et al. 2010) with the following stringent criteria: (1) target microsatellites had at least seven repetitions, (2) length of PCR products between 90 and 300 bp, (3) flanking regions did not contain either any homopolymer stretch of more than four bases or any di-hexa motifs of more than two repetitions, (4) annealing temperatures optimized to 55 °C and (5) microsatellites were not compound or interrupted. We finally selected 48 sequences for which primers were designed.

Individuals used for amplification tests were collected from five localities located in France (Mont-Ventoux, Southern France and the island of Corsica) and in neighbouring European countries (Sierra Nevada, Spain; Venostra, Italy; Leiria, Portugal). The DNA of two individuals per locality was extracted using the DNeasy Blood and Tissue kit (Qiagen®). For each primer pair, PCR amplifications were performed in a total volume of 10 µL using the Multiplex PCR Kit (Qiagen®). Thermocycling was performed on a Mastercycler® gradient (Eppendorf) with the following protocol: 94 °C for 15 min, followed by 35 cycles (94 °C for 30 s, 55 °C for 90 s, 72 °C for 30 s), and 72 °C for 30 min. Out of the 48 primer pairs, 23 displayed clear PCR products on agarose gel electrophoresis, i.e. one or two clear bands of the expected size. The other 25 primer pairs either did not amplify in some of the 10 individuals or produced multiple bands or smears. The selected loci were further amplified using forward primers labelled with the fluorescent dyes 6-FAM, PET, NED or VIC (Applied Biosystems). The PCR products were visualized using an ABI 3500XL Genetic Analyzer (Applied Biosystems). Allele sizes were scored against an internal GeneScan-500 LIZ® Size Standard (Applied Biosystems) and genotypes obtained using GeneMapper® 5.1 (Applied Biosystems). Among the 23 screened markers, 17 showed unambiguous genotype patterns. They were then

L. Sauné (✉) · F. Abella · C. Kerdelhué
UMR1062 CBGP (INRA, CIRAD, IRD, Montpellier Supagro), INRA, 755 avenue du campus Agropolis, CS 30016, 34988 Montferrier-sur-lez cedex, France
e-mail: laure.saune@supagro.inra.fr

Table 1 Microsatellite data and polymorphism characterization of the populations from Varges and Fundao, Portugal

Locus	Motif	Primer sequence (5′–3′)	Scaffold number	Set	Dye	Expected size (bp)	Na	Null allele frequency	*Ho*	*He*	Na	Null allele frequency	*Ho*	*He*
Ppit09	$(ATCC)_7$	F:TTATTGACGATTCACTGACG R:ACCATAATCTCCATGTTTGG	scf_1131814_304779	1	6-FAM	247	5	0.000	0.714	0.702	5	0.000	0.800	0.684
Ppit16	$(ATC)_8$	F:ATTAACGGAATCACTCGAAA R:GGGACGATATAGACTGGGTA	scf_1146282_290221	3	VIC	102	4	0.140	0.357	0.564	4	0.142	0.367	0.561
Ppit20	$(ACT)_9$	F:CCAGTTGGGTACAATTTCAA R:ACAGATTAAGCTTGCAGGAG	scf_1118793_118958	1	VIC	191	5	0.000	0.464	0.419	3	0.067	0.500	0.615
Ppit24	$(ATC)_8$	F:CAACAGACCCTGAGTTCAAT R:TGTCGTGGATATTGTGAGAA	scf_1129246_284397	1	NED	258	2	0.181	0.214	0.486	2	0.044	0.433	0.508
Ppit30	$(AG)_{10}$	F:AGGTCTGTTTCATTCTCAGC R:GGATGATCCTAGGTCTAGAGAAA	scf_1106604_7884	2	PET	96	6	0.000	0.714	0.683	7	0.059	0.633	0.672
Ppit31	$(AG)_{13}$	F:CATGTTGCTGCTTTCTACAT R:TTTCCAGGCTGTTGTTATTC	scf_1119121_50344	1	VIC	101	8	0.034	0.679	0.740	8	0.000	0.667	0.671
Ppit33	$(AG)_{10}$	F:TAAATTCGGACCTACTTCGT R:TTTGTTTACGTATGTGTCGG	scf_1145908_27939	1	NED	101	7	0.066	0.536	0.654	4	0.157	0.267	0.514
Ppit34	$(AG)_{11}$	F:GGTTCAGCATTCCAATTAAA R:TTTGTTACATTTCCCGTTCT	scf_182056_1193	1	6-FAM	105	11	0.094	0.679	0.849	10	0.000	0.867	0.846
Ppit35	$(AG)_{14}$	F:TTGAGATGTGAACCTTGGTA R:TCGGGATAATTCTAGGTCG	scf_1136567_89093	3	6-FAM	106	12	0.013	0.821	0.792	8	0.085	0.667	0.815
Ppit37	$(AC)_{11}$	F:AGGCTCCCGTTATTACTGAT R:TTAAATCACAGACAGAGCGA	scf_1099096_20807	1	PET	198	3	0.208	0.143	0.408	2	0.000	0.233	0.210
Ppit38	$(AC)_{13}$	F:CGTCAACACTGGAACATAAA R:TTGAACAACATAATACGGCTT	scf_1139294_162794	3	VIC	187	5	0.000	0.857	0.751	6	0.048	0.633	0.747
Ppit39	$(AG)_{12}$	F:GGTGACCGTTGTTCTTTAGT R:GCCGATTACAATCTACTGCT	scf_1139882_168290	2	VIC	193	18	0.000	0.893	0.898	15	0.008	0.833	0.876
Ppit41	$(AC)_{12}$	F:ACAGTATTTGTGGACGACAC R:GAATTAAATCATTTGTTCCGA	scf_141399_363	2	6-FAM	190	5	0.273	0.179	0.643	5	0.365	0.069	0.692
Ppit43	$(AG)_{10}$	F:AATATTCGACGCTTAACCTTT R:CCACTTCTCTTTGTCCCTTT	scf_1133323_162357	1	VIC	264	4	0.004	0.571	0.556	3	0.052	0.367	0.412
Ppit46	$(AG)_{12}$	F:ATCATGCACACGTAGTCAAA R:GTGTCGTTCATAATGTCTGGT	scf_1148450_230898	2	NED	270	10	0.224	0.393	0.797	13	0.000	0.867	0.836
Ppit47	$(AC)_{12}$	F:CACAATGAGACAACACAAAGA R:TAGATCCCTCGATTTACACG	scf_1152280_208560	2	6-FAM	261	4	0.000	0.571	0.573	3	0.080	0.367	0.501
Ppit48	$(AG)_{10}$	F:GGTTGGTTAGTACTCGTTGC R:AACGAATAATTTCAGGAGCA	scf_1152398_78471	3	PET	263	3	0.000	0.607	0.595	4	0.036	0.400	0.439

Results in *italics* indicate significant deviation from Hardy–Weinberg equilibrium

Na number of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity

Table 2 Cross–species amplification results for *Thaumetopoea spp.*: number of successful amplifications/number of alleles in the taxon

Species	country	N	Ppit09	Ppit16	Ppit20	Ppit24	Ppit30	Ppit31	Ppit33	Ppit34	Ppit35	Ppit37	Ppit38	Ppit39	Ppit41	Ppit43	Ppit46	Ppit47	Ppit48
T. pityocampa	Corsica (France)	2	2/1	2/2	2/1	2/1	2/1	0	2/2	2/2	2/3	2/1	2/1	2/4	2/1	2/1	2/1	2/1	1/1
T. pityocampa	Morocco	2	2/1	2/3	1/2	2/1	2/2	2/3	2/4	2/2	2/2	2/2	2/3	2/3	1/1	2/1	2/2	2/2	2/3
T. pityocampa ENA	Tunisia	2	2/1	2/2	2/1	2/1	2/2	2/2	1/2	2/3	2/4	2/2	2/3	2/4	0	2/1	2/2	2/1	2/4
T. pityocampa ENA	Libya	2	2/2	2/2	2/2	2/1	2/3	2/1	2/2	1/2	2/2	2/2	0	2/2	0	2/1	2/2	2/3	2/2
T. wilkinsoni	Turkey	2	2/1	2/1	2/1	2/1	2/4	0	0	2/1	2/4	2/1	0	2/3	0	2/1	2/1	1/1	2/3
T. wilkinsoni	Israel	2	2/1	2/1	2/1	2/1	2/2	0	1/1	2/1	2/2	2/1	0	2/1	0	2/1	2/1	0	2/2
T. wilkinsoni	Cyprus	2	2/1	2/1	2/2	2/1	2/3	0	2/3	2/2	2/3	2/1	0	2/2	0	2/1	2/1	2/3	2/2
T. wilkinsoni	Crete	2	2/1	2/3	2/3	1/1	2/2	2/1	1/1	2/2	2/2	2/1	1/1	2/3	0	1/1	2/1	2/3	2/2
T. bonjeani	Algeria	2	0	0	2/1	0	2/1	2/1	0	0	2/2	0	2/2	2/1	0	2/1	0	0	2/1
T. libanotica	Lebannon	2	0	0	2/2	0	0	0	1/1	2/2	2/1	0	2/1	0	0	2/2	2/1	0	2/1
T. pinivora	France	2	0	0	2/1	0	2/1	2/1	1/1	1/2	2/1	0	2/3	1/1	0	1/1	0	0	2/1

N number of individuals tested

successfully organized into three PCR multiplex kits using the amplification conditions described above (Table 1). Two primer pairs previously described, namely Thpit07 and Thpit10 were added in multiplex 3 (A'Hara et al. 2012). The microsatellites were successfully used to genotype 2 populations sampled in Portugal (Varges and Fundao).

Deviations from Hardy–Weinberg equilibrium (HWE), expected and observed heterozygosities and linkage disequilibrium (LD) were calculated using ARLEQUIN 3.11 (Excoffier et al. 2005). The existence of null alleles was tested using FreeNA (Chapuis and Estoup 2007).

The number of alleles ranged from 1 to 18 and the expected heterozygosity from 0.068 to 0.892 (Table 1). Significant departures from HWE (heterozygote deficiency) were detected for five loci in Varges (Ppit16, Ppit24, Ppit37, Ppit41, Ppit46) and for four loci in Fundao (Ppit16, Ppit33, Ppit35, Ppit41). No primer pair was in significant LD in both populations.

Cross amplification was also tested in divergent populations of *T. pityocampa* including the Eastern North African (ENA) clade, in four lineages of the sister species *T. wilkinsoni* and in three congeneric species. Amplifications were mostly successful in all clades of *T. pityocampa* and *T. wilkinsoni*, and 8 to 10 loci could be useful for the other tested species (Table 2).

Acknowledgments This work was supported by the ANR-10-JCJC-1705-01 GenoPheno. Data used in this work were (partly) produced through molecular genetic analysis technical facilities of the Labex "Centre Méditerranéen de l'Environnement et de la Biodiversité". We gratefully thank Jérôme Rousselet and Manuela Branco for collecting insects samples.

References

A'Hara SW, Amouroux P, Argo EE et al (2012) Permanent genetic resources added to molecular ecology resources database 1 August 2011–30 September 2011. Mol Ecol Res 12(1):185–189

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24(3):621–631

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolut Bioinform Online 1:47–50

Hódar JA, Castro J, Zamora R (2003) Pine processionary caterpillar *Thaumetopoea pityocampa* as a new threat for relict Mediterranean Scots pine forests under climatic warming. Biol Cons 110(1):123–129

Meglecz E, Costedoat C, Dubut V, Gilles A, Malausa T, Pech N, Martin J-F (2010) QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics 26:403–404