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TECHNICAL NOTE

First microsatellite panel for the Wood Tiger Moth (*Parasemia plantaginis*)

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Abstract Ten polymorphic microsatellite loci were isolated and characterized for the Wood Tiger Moth (*Parasemia plantaginis*), a nocturnal moth of the Arctiidae family distributed throughout the globe at mid to northerly latitudes. Characterization of 25 *P. plantaginis* individuals form central Austria showed moderate to high allelic diversity ranging from 2 to 14 alleles per locus. One locus showed significant departures from Hardy-Weinberg equilibrium presumably due to null alleles. No evidence of linkage disequilibrium was found for any locus pair. Polymorphism is also reported in four related Arctiidae species. This set of markers can be useful for the evaluation of genetic composition, conservation, population genetics and might prove useful in other phylogenetically close species of the Arctiidae family.

Keywords *Parasemia plantaginis* · Microsatellite · Aposematism · Lepidoptera

Colour polymorphism is one manifestation of intra-specific variability that species commonly display across their distribution range. Such variability may be generated and maintained by ecological and evolutionary processes such as local adaptations, disruptive selection, geographic isolation, and sexual selection (Gray and McKinnon 2007). In aposematic species, colouration plays a central role in the individual's fitness since it is mainly used as a warning signal that advertises unpalatability (Ruxton et al. 2004). The theoretical expectation is that selection should favour traits that positively affect fitness, and therefore, traits are expected to reach fixation preventing the evolution of polymorphism. In aposematic species, polymorphism is puzzling since avoidance learning of predators is faster when warning signals are invariable (Rowland et al. 2007). As a result, maintenance of colour polymorphisms within species is a topic that has invoked much attention (Przeczek et al. 2008; Wang and Shaffer 2008). Lepidopterans (butterflies and moths) provide an ideal system for research on the evolution of colour polymorphisms because of their high variability in coloration between and within species. The aposematic wood tiger moth (*Parasemia plantaginis*) belongs to the Arctiidae family occurring throughout the globe at mid to northerly latitudes and displays a high variability in coloration throughout its range. Although *P. plantaginis* is not globally endangered, populations from central Europe and the United Kingdom have experienced severe declines and are now considered threatened. Here we developed a panel of microsatellite markers to study the proximate causes of colour polymorphism and population declines in *P. plantaginis.* Further, we test its applicability in related taxa.

Microsatellite markers were identified through the development of an enriched genomic library as described in Galarza et al. (2009). DNA was extracted from 5 living larvae from Finland, Canada and Austria using a DNeasy Blood + Tissue extraction kit (Qiagen) by a Robot (Kingfisher) and concentrated via ethanol precipitation. Simultaneous restriction-ligation of genomic DNA was carried out using *Rsa*I and *Mse*I restriction enzymes and double stranded linker-adapted primers according to Hamilton et al. (1999). Ligated DNA was enriched by magnetic bead selection with a biotin-labeled probe mixture consisting of $(GA)_{12}$ and $(CATA)_8$ at 1 μM each.

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Enriched DNA was eluted in 100 µl dH_2O from the bead probes and concentrated by ethanol precipitation. Both enrichments were amplified by PCR, pooled and purified using QIAquick PCR purification kit (Qiagen). Approximately 60 ng/µl of purified DNA was cloned using CloneJET^{TM} PCR Cloning kit (Fermentas). A total of 490 positive clones were screened and checked for inserts using ABI PRISM BigDye Terminator Cycle kit (Applied Biosystems) and resolved on an ABI 3130xl Genetic Analyser (Applied Biosystems). Primer pairs for 14 potential usable microsatellite loci were designed using Primer3 software (Rozen and Skaletsky 2000). Polymorphism was tested by PCR reactions performed in 20 µl total volume, which include ~50 ng of DNA, 2 mM of $MgCl_2$, 0.5 µM of reverse primer, 0.45 forward primer, 0.05 µM labeled forward primer, 200 µM dNTP's, 1× DreamTaq Buffer (Fermentas) and 0.75 units DreamTaq DNA polymerase (Fermentas). Reaction conditions were as follows: an initial denaturation step of 5 min at 95°C, 30 cycles consisting of 30 s at 95°C, 30 s at 55°C annealing temperature, 30 s at 72°C followed by 10 min at 72°C.

Microsatellite variability was assessed in 25 individuals from a single sampling site in central Austria. Individuals were genotyped by assessing allele size on an ABI 3130xl Genetic Analyser (Applied Biosystems) using forward primers labeled with 6-FAM, NED, PET and VIC (Applied Biosystems). Allele scoring was carried out using GENEMAPPER V.4.0 software (Applied Biosystems). Expected and observed values for heterozygosity were determined using ARLEQUIN V.3.5 (Excoffier and Lischer 2010). The number of alleles per locus, allele size range as well as deviations from Hardy-Weinberg expectations and linkage disequilibrium between pairs of loci were estimated using FSTAT V.2.9 (Goudet 1995) and GENEPOP V.4.0 (Rousset 2008) software. Ten loci were polymorphic, the total number of alleles per locus and heterozygosities estimates are listed in Table 1. We found no evidence of linkage disequilibrium between locus pairs. One locus (Ppla313) showed significant deviations from Hardy-Weinberg expectations after correction for multiple test (Rice 1989). This could be due to the presence of null alleles or the inclusion of individuals from cryptic subpopulations in the analysis.

Polymorphism was examined in five individuals from other Arctiidae namely *Arctia caja, Diacrisia sannio, Rhyparia purpurata* and *Spilosoma urticae* using the same conditions detailed for *P. plantaginis*. Three loci amplified in *A. caja, D. sannio* and *R. purpurata* species with the number of alleles ranging from 1 to 6 depending on the locus and the species (Table 2). No amplification was

Table 1 Characterization of 10 *P. plantaginis* microsatellite loci ($N = 25$)

Accession no.	Locus	Repeat motif	Primer sequence (5′ → 3′)	Allele no.	Allele size (bp)	H_O	H_E	F_{IS}
HM997001	Ppla107	$(GAT)_5$	F: AGCGTGGTATGGTTGCCTAA R: ATCGTCCACTTGCGTAACGA	3	116–125	0.357	0.320	−0.121
HM997002	Ppla109	$(CT)_{20}$	F: GCTTTTGCATTCACTTGGTG R: GCCGTTACATATTGCGATCC	10	172–214	0.854	0.904	0.117
HM997003	Ppla279	$(CA)_6CG(CA)_{13}$	F: CACGGTGTTGTCAAACCAAT R: TAGGCGGTTGGAAGGTGTTA	14	121–158	0.928	0.947	0.021
HM997004	Ppla313	$(GA)_{38}$	F: TCACCATCTAATTCCAAAACGA R: TGAAAAATAATCTTTATTGCTCTGTG	11	223–253	0.585	0.883	0.385*
HM997005	Ppla317	$(GA)_{10}$	F: ATAATGACGCCAACGCCAAT R: GGGCACTCTTTCAGAGCCTA	13	181–217	0.885	0.917	0.105
HM997006	Ppla323	$(CA)_{12}$	F: GCCAGACGGGTTGGTATAGA R: CCAGACCAGGGATTCAAAAA	2	236–238	0.442	0.507	0.213
HM997007	Ppla363	$(TAA)_6$	F: AATCCGTCACCACACCTCTC R: ACAATCTCGTGGGCAAAATC	4	345–356	0.514	0.600	0.152
HM997008	Ppla382	$(CAT)_6$	F: TGTTGCTCGATGACCGTAAC R: AGAACCGATAACCGTTGGTG	2	355–357	0.493	0.518	0.107
HM997009	Ppla414	$(CT)_{10}$	F: CAGCTTGAGCTCGAGTCGTTA R: GTAAAGTTCCCCGCGACATA	10	266–284	0.897	0.916	0.034
HM997010	Ppla439	$(GA)_{11}$	F: GTGGTGAGCTTACGTAATC R: ACACCGGCTTTCTGGGAG	7	178–198	0.685	0.722	0.113

H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient

* $P < 0.005$

Table 2 Cross-species amplification of 10 microsatellite loci from *P. plantaginis* in five individuals of *Arctia caja, Diacrisia sannio, Rhyparia purpurata* and *Spilosoma urticae*

Locus	*Arctia caja*		*Diacrisia sannio*		*Rhyparia purpurata*		*Spilosoma urticae*	
	Allele no.	Range	Allele no.	Range	Allele no.	Range	Allele no.	Range
Plan107	NA		NA		NA		NA	
Plan109	NA		NA		NA		NA	
Plan279	NA		NA		NA		NA	
Plan313	NA		NA		NA		NA	
Plan317	NA		NA		NA		NA	
Plan323	6	214–244	4	262–284	2	188–198	NA	
Plan363	NA		NA		NA		NA	
Plan414	1	284	2	304-340	1	333	NA	
Plan439	1	190	NA		2	211–217	NA	
Plan382	NA		NA		NA		NA	

No. of alleles and allele range (bp)

NA no amplification

obtained in *S. urticae,* which is consistent with the taxonomical relationship between the species. This set of markers can be useful for the evaluation of genetic composition, conservation, population genetic studies, and might prove useful in other phylogenetically close species of the Arctiidae family.

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References

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Galarza JA, Boulay R, Cerda X, Doums C, Federici P, Magalon H, Monnin T, Rico C (2009) Development of single sequence repeat markers for the ant *Aphaenogaster senilis* and cross-species amplification in *A-iberica*, *A. gibbosa*, *A-subterranea* and *Messor maroccanus*. Conserv Genet 10:519–521

Goudet J (1995) F_STAT (2.9.3) a program for IBM compatible PCs to calculate Weir and Cockerman's (1984) estimators of F-statistics. J Hered 86:485–486

Gray SM, McKinnon JS (2007) Linking color polymorphism maintenance and speciation. Trends Ecol Evol 22:71–79

Hamilton MB, Pincus EL, Di Fiore A, Flesher RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27:500–507

Przeczek K, Mueller C, Vamosi SM (2008) The evolution of aposematism is accompanied by increased diversification. Integr Zool 3:149–156

Rice W (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103–106

Rowland HM, Ihalainen E, Lindstrom L, Mappes J, Speed MP (2007) Co-mimics have a mutualistic relationship despite unequal defences. Nature 448:64–67

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Ruxton GD, Sherratt TN, Speed MP (2004) Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry. Oxford University Press, Oxford

Wang IJ, Shaffer HB (2008) Rapid color evolution in an aposematic species: a phylogenetic analysis of color variation in the strikingly polymorphic strawberry poison-dart frog. Evolution 62:2742–2759