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Isolation and characterization of microsatellite loci in the Alpine leaf beetle, *Oreina elongata*

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Abstract

For a study of local adaptations in the Alpine leaf beetle, *Oreina elongata*, we developed six microsatellite loci and screened them in 305 individuals from 13 populations. All markers were polymorphic with three to 15 alleles per locus. Average observed and expected heterozygosity values were 0.14 and 0.62, respectively. Four markers showed heterozygote deficiency and deviated significantly from Hardy–Weinberg expectations, indicating the presence of null alleles.

Keywords: Chrysomelidae, Coleoptera, microsatellites, *Oreina elongata*

The balance between gene flow and spatial variation in selection pressure has often been considered to be the critical determinant of the degree of adaptation of separated populations to their local environment (Slatkin 1973, 1987; Peterson & Denno 1998). The study of both mechanisms is essential to understand local adaptation but both approaches have rarely been combined. We required a set of microsatellite markers to fulfil the genetic side of our study.

Oreina elongata is an Alpine leaf beetle that can be found in patchy populations throughout the Alps and the Apennines at altitudes between 1500 and 2200 m above sea level. It feeds exclusively on two very dissimilar types of host plants, which provide the insect with either mechanical or chemical protection. This specialist beetle has never been seen flying and because its isolated populations can be found on one or both types of host plant, it is very likely to exhibit local adaptations. Indeed, it has been shown that populations differ in some life history traits related to host plant use (Ballabeni *et al*. 2003; Gotthard *et al*. 2004) and morphological studies have described five subspecies that differ in their geographical range and host plants (Daccordi & Ruffo 1976, 1986).

During the summer 2001, we collected individuals from 13 populations of *O. elongata* throughout the Alps and the Appenines. The individuals were starved overnight to avoid contamination by plant material contained in the gut, preserved in pure ethanol and stored at –80 °C as quickly as possible. DNA was extracted using the PUREGENE Kit (Gentra Systems) from the head, thorax and legs only, to ensure that no plant material contained in the gut could contaminate the samples.

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An enriched DNA library was made by ECOGENICS GmbH (Zurich, Switzerland) from size-selected genomic DNA ligated into TSPAD-linker (Tenzer *et al*. 1999) and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GA)_{13}$ oligonucleotide repeats (Gautschi *et al*. 2000a, b). Out of the 1536 recombinant colonies screened, 519 gave a positive signal after hybridization. Plasmids from 104 positive clones were sequenced as described in Gautschi *et al*. (2000a) and primers were designed for 14 microsatellite inserts. Of these, six were shown to be polymorphic (Table 1).

To assay variation among 305 individuals, polymerase chain reactions (PCR) were performed in a 5-μL volume containing about 1.5 ng of template DNA, 1.25 μM of each primer and 2.5 μL of HotStar*Taq* master mix (QIAGEN). The latter contains 400 μM dNTP each, 0.5 unit of HotStar-*Taq* DNA polymerase (QIAGEN) and 2× PCR buffer (QIAGEN) consisting of Tris-Cl, KCl $(NH_4)_2SO_4$ with a final concentration of 1.5 mM $MgCl_2$. The forward primers were labelled with an IR Dye at the 5′ end, using 800 IRD for *TGG6*, *CAB1*, *CAO7* and *CAA5* primers and 700 IRD for *CAB6* and *CAA3* so that they could be run together on gels. Amplification proceeded on a TGradient thermocycler (Biometra) using the following hotstart protocol: a first step of 15 min prolonged denaturation at 95 °C, followed by 25–30 cycles, each consisting of 30 s denaturation at 95 °C, 30 s annealing at the locus-specific annealing temperature (Table 1) and

Table 1 Characterization of six microsatellite loci in *Oreina elongata* based on 305 individuals originating from 13 populations

Locus	GenBank Accession no.	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	N_A	Size range (bp)	H_O	H_E	HW
Oel1	AY380123	$(GT)_{12}$	F: CCGAAGCTTCCACTTGAGAC	58	15	142–176	0.241	0.619	0.000
CAA3			R: CAGGGTACTTTGTCCCGAAC				(0.183)	(0.209)	
Oel2	AY380124	$(GT)_{15}$	F: AGGCAACAACGATGTCTCG	60	14	115–141	0.307	0.555	0.000
CAA5			R: GGTGCTGGCTGTAGGTTCAC				(0.217)	(0.151)	
Oel3	AY380129	$(CA)_8CC(CA)_2$	F: ATCCGGTGTGAAAGACTTCG	58	9	176–196	0.081	0.176	0.000
CAB1		$CGCCAA(CA)_{11}$	R: CATGCTCCTCTGAAGGAACC				(0.099)	(0.238)	
Oel4	AY380125	$(GT)_{13}$	F: GCAACAGTTCATGGCAGAATC	58	9	96–114	0.269	0.436	0.000
CAB6			R: CCATTGCGAGGTCTGTCC				(0.277)	(0.339)	
Oel5	AY380131	$(CA)_{12}$	F: AGATGCCGGTGACACAATG	62	10	106–126	0.62	0.584	0.770
CAO7			R: TTGGTCATTAGGGCTCCATC				(0.276)	(0.228)	
Oel6	AY380130	$(ACC)_6$	F: CACTCGATGCTGATGCAGAC	58	3	162–168	0.1	0.097	0.668
TGG6			R: ACGCTCGAAAAGATCACCTG				(0.175)	(0.168)	

T_a, annealing temperature of the primer pair; N_A number of alleles; H_O, observed heterozygosity (standard deviation); H_E, expected heterozygosity (standard deviation); HW, *P* value for the exact test of departure from Hardy–Weinberg equilibrium.

30 s extension at 72 °C. The last cycle was followed by an extra 8 min at 72 °C to complete extension. PCR products were mixed with a stop solution [95% (v/v) formamide, 400 μL EDTA 0.5 M and 5 mg bromophenol blue] and denatured at 95 °C for 1 min, before electrophoresis on a 6% denaturing polyacrylamide gel Sequagel *XR* (National Diagnostics) running on a sequencing system (IR2 DNA Analyser, LI-COR). Isolated bands were scored according to their size, using SAGA IR^2 software, version 2.1.2 (LI-COR). We used an infrared dyed sizing standard ranging from 50 to 350 base pairs (IRDye, LI-COR). All six microsatellite loci were variable and consistently scorable in *Oreina elongata*. The number of alleles per locus ranged from three to 15 for the six microsatellite loci (Table 1).

For each population and locus, we calculated the mean and expected heterozygosities and tested for linkage disequilibrium using ARLEQUIN (Schneider *et al*. 2000). We assessed the degree of departure from Hardy–Weinberg equilibrium using exact tests given by GENEPOP (Raymond & Rousset 1995). *CAA3*, *CAA5*, *CAB1* and *CAB6* showed heterozygote deficiencies and deviated significantly from HWE, probably because of the presence of null alleles. There was no evidence for linkage disequilibrium among the six microsatellite loci.

These loci will now provide a means to test to what extent Alpine populations of *Oreina elongata* really are isolated from one another and therefore to what extent we expect them to evolve independently.

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