Published in Molecular Ecology Notes 3, 12-14, 2003
which should be used for any reference to this work

PRIMER NOTE

Isolation and characterization of polymorphic microsatellite loci in *Acanthoscelides obvelatus* Bridwell (Coleoptera: Bruchidae)

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Abstract

Five microsatellite loci were isolated from the bruchid *Acanthoscelides obvelatus* Bridwell (Coleoptera: Bruchidae). Each locus was polymorphic, with the number of alleles ranging from two to 15. We found high levels of within-population variation at most loci, with heterozygosity ranging from 0.182 to 0.900. Cross-species amplification of these loci was tested in two other species of the genus *Acanthoscelides*, *A. obtectus* Say and *A. argillaceus* Sharp.

Keywords: *Acanthoscelides obvelatus*, Bruchidae, Coleoptera, microsatellite, pest species, *Phaseolus*

Almost all *Acanthoscelides* species (and more generally Bruchidae species) are predators of legume seeds (Johnson 1989). *Acanthoscelides obvelatus* Bridwell is specialized on seeds of the common bean (*Phaseolus vulgaris* L.). Nevertheless, the species is able to feed on other species of the genus *Phaseolus* such as *P. coccineus* L. and *P. polyanthus* Greenman, and also on other genera of legumes, such as *Vigna* (Delgado-Salinas *et al*. 1988). Its distribution is limited to Mexico, Central America and northern South America. *Acanthocelides obvelatus* represents a major problem in the management of bean stocks, and is considered a major pest of field crops and storage sites. This species belongs to the supraspecific taxonomic group of *A. obtectus* Say (which also includes *A. obtectus* Say and *A. argillaceus* Sharp). A previous study using allozyme markers investigated genetic diversity in *A. obtectus* and *A. obvelatus* (Gonzalez-Rodriguez *et al*. 2000). Sampling involved 14 populations throughout Mexico, which represents the native range of these species, and results revealed differences between taxa, but not at the population level. We isolated microsatellite loci in *A. obvelatus* in order to assess population genetic structure, and to test the role of ecological and human factors on the evolutionary forces acting on the different populations of *A. obvelatus*.

Total genomic DNA was extracted from a pool of 29 individuals using DNeasy™ kit (QIAGEN). Microsatellite-enriched libraries were built following Billotte *et al*. (1999): DNA was digested with *Rsa*I (Eurogentec) and DNA fragments ranging between 500 and 1000 bp were selected after migration on agarose gel and isolated using an extraction kit (Promega). The partial genomic library was then constructed by ligating the DNA fragments into a pGEM-T plasmid. Epicurian-coli XL1-Blue MRF' supercompetent cells (Stratagene) were used to transform the DNA fragments. One hundred and eighty white transformant clones were transferred on Hybon-N^+ nylon membranes (Amersham), and hybridized using inosin/biotin-labelled microsatellite oligoprobe $(CT)_8$ and $(GT)_8$. For 29 of these clones, which gave a satisfactory positive signal, the inserted DNA fragment was sequenced. Eighteen primer pairs were designed using OLIGO Version 3.3 (Rychlik & Rhoads 1989), among which five gave satisfactory amplification patterns [i.e. polymerase chain reaction (PCR) product of the predicted size, and supernumerary bands of low intensity].

PCR amplifications were performed in a final volume of 10 μL, which contained 1.25 μL of extracted DNA, 0.32–0.4 μL of 25 mM $MgCl_2$ (Table 1), 1 μL of 2.5 mM dNTPs, 1 μL of buffer 10× [750 mM Tris–HCl pH 8.8; 200 mM $(NH_4)_2SO_4$; 0.1% (v/v) Tween 20], 1.33 units of *Taq* DNA polymerase (Eurogentec Red Goldstar™), 0.25 μL of 0.01 μM reverse primer labelled with [γ^{33}P]-dATP, and 0.75 μL of 0.01 μM unlabelled forward primer. PCRs were performed on a PTC-100™ thermocycler using the following cycling conditions: initial denaturation at 94 °C (1 min); six (for locus *AcobD04*) or seven (for loci *AcobC09*, *AcobC10*,

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Table 1 Primer sequences, PCR conditions and polymorphism statistics for five microsatellite loci in two populations of *Acanthoscelides obvelatus* Bridwell. Repeat motif is listed 5′ to 3′ with respect to the forward primer (F). 'Size' refers to the length of the cloned allele. T_a: lower annealing temperature. [$MgCl_2$]: concentration of $MgCl_2$ for PCR reactions. *n* is the number of genes analysed. N_a: number of allele size variants observed. H_O: observed proportion of heterozygous individuals. H_E: expected heterozygosity (i.e. gene diversity; Nei 1987)

Locus	GenBank Accession no.	Primer sequences (5′ to 3′)	Repeat motif in library	Size (bp)	T_a (°C) [$MgCl_2$] (mM)	Size range (bp)	Valle de Bravo			Tzintzuntzan		
							n	N_a	H_O/H_E	*n*	N_a	H_O/H_E
AcobC09	AF527375	F: TAGGAAAGGGTGAGAGATGCG R: ATTGGAGACGAGAAAAAGAGGG	$(AG)_{10}GG(AG)_{13}ACGA(AG)_3$	246	59 [0.8]	216–266	20	12	0.800/0.937	20	10	0.900/0.900
AcobC10	AF527376	F: CACCACCCTACCACCTC R: TTCTTATAGGCGATGATGTG	$GC(GT)_8(GC)_2(CA)_5(GTGC)_2GT$	130	53 [1.2]	124–132	20	2	0.200/0.189	20	4	0.400/0.432
AcobD04	AF527377	F: CAGAAACAATTTGCACGAC R: CGGCTGAGACTATGAATCTG	$(AC)_2GC(AC)_8$	103	52 [1.0]	97–105	20	7	0.455/0.532	22	8	0.182/0.333
AcobD06	AF527378	F: ATTTACTGTCCTCGTTGTCG R: ATGCTTATCCGTTCTACTGC	$(TG)_9C(TG)_2$	350	59 [0.8]	316–362	22	11	0.500/0.842	20	9	0.818/0.792
AcobE04	AF527379	F: ACAGTGATGTTAAATAAAAA R: GGTTAGAGATAGGTTGAGTA	$(AT)_5(AC)_2C(CA)_4AAT(AC)_3(AT)_3(AC)_2$ $ATAC(AT)_3AC(AT)_3(AC)_8CCGC(AC)_9$	341	53 [1.0]	265–369	22	15	0.818/0.957	20	11	0.800/0.926

AcobD06 and *AcobE04*) 'touchdown' cycles: 92 °C (25 s), 1 °C drop per cycle to a final annealing temperature of T_a (35 s) (Table 1), 72 °C (30 s); 24 (for locus *AcobD04*) or 23 (for loci *AcobC09*, *AcobC10*, *AcobD06* and *AcobE04*) cycles of: 92 °C (25 s), T_a (35 s), 72 °C (30 s); final elongation at 72 °C (10 min). PCR products were separated by electrophoresis on a 5% denaturing polyacrylamide gel containing urea and 1× TBE buffer. Results were displayed by autoradiography, using water as the negative control.

The DNA of 20 individuals, sampled in two Mexican populations (10 individuals per population), Valle de Bravo (Mexico State) and Tzintzuntzan (Michoacan State), which are 159 km apart, was extracted using DNeasy™ kit (QIAGEN) and genotyped for the five loci. All loci were polymorphic in both populations. The observed number of alleles per population ranged between 2 and 15, and heterozygosities were between 0.182 and 0.900 (Table 1). No significant deviation from Hardy–Weinberg equilibrium and linkage equilibrium between loci was observed in either population when exact tests (GENEPOP Version 3.3 package; Raymond & Rousset 1995) and a correction for multiple tests (Dunn-Sydák method for sequential Bonferroni procedure) were performed.

Cross-species amplifications were tested on individuals of the other species of the *A. obtectus* group (*A. obtectus* and *A. argillaceus*). PCR conditions were identical to those used for *A. obvelatus*. *Aconthocelides argillaceus* feeds on seeds of *P. lunatus* L. (Johnson 1989) and its distribution overlaps broadly with that of *A. obvelatus*, whereas *A. obtectus*, also native to Mexico, is now worldwide in distribution. Despite their taxonomic proximity, *A. argillaceus* amplified successfully for only two of the five microsatellite loci (*AcobD04* and *AcobD06*), whereas *A. obtectus* amplified for three (*AcobD04*, *AcobD06* and *AcobE04*). All cross-amplified loci showed polymorphism (*AcobD04*: two alleles, ranging from 220 to 222 for *A. argillaceus* and from 184 to 186 for *A. obtectus*; AcobD06: three alleles ranging from 344 to 350 for *A. argillaceus* and two alleles ranging from 346 to 350 for *A. obtectus*; *AcobE04*: four alleles ranging from 265 to 321 for *A. obtectus*). Heterozygosities were not calculated as most individuals were collected from different populations.

Acknowledgements

The authors thank P. Jarne, G. Debout, A. Dalecky, D. McKey, P. Sourrouille and C. Debain for their helpful assistance and J.-Y. Rasplus for the identification of *Acanthoscelides* species. This work was financially supported by the Swiss National Science Foundation (project N°3100.064821.01).

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