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PRIMER NOTE

Isolation and characterization of polymorphic microsatellite loci in *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)

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Abstract

Six microsatellite loci were isolated from the bruchid *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Each locus was polymorphic, with the number of alleles ranging from 3 to 18. We found high levels of within-population variation at most loci, with heterozygosities ranging from 0 to 0.75. Cross-species amplification of these loci was tested in two other species of the genus *Acanthoscelides*, *A. obvelatus* Bridwell and *A. argillaceus* Sharp.

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

Acanthoscelides obtectus Say is a bruchid species of neotropical origin, whose larvae feed on beans of the *Phaseolus vulgaris* L. group. Since the domestication and diffusion of beans, this species has become cosmopolitan, through human-mediated migration. The consequence of its worldwide status is that *A. obtectus* is now a major problem in the management of bean stocks, and is considered a major pest of field crops and storage sites. In the last 30 years, the species has also been recorded on new host plants, such as *Pisum*, *Lens* and *Vigna (*e.g. Jarry & Bonet 1982). Therefore, there is a strong need to understand the population genetics of this species and to examine the roles of ecological and human factors in the genetics and demography of its populations. However, no useful markers have yet been developed, with the exception of three cross-amplifiying microsatellite loci developed for its sister species, *A. obvelatus* (Alvarez *et al*. 2003). Here we present sequences and preliminary data for six new microsatellite loci in *A. obtectus*.

Total genomic DNA was extracted from a pool of 20 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 (Promega). Epicurian-coli XL1-Blue MRF supercompetent cells (Stratagene) were used for the transformation of the DNA fragments. One hundred white transformant clones were transferred on to Hybond-N+ nylon membranes (Amersham), and hybridized using inosin/biotin-labelled microsatellite oligoprobes $(CT)_8$ and $(GT)_8$. For 14 of these clones, which gave a satisfactory positive signal, the inserted DNA fragment was sequenced using Applied Biosystems BigDye™ protocol, and further analysis using an automated ABI 310 genetic analyser. Eleven primer pairs were designed using OLIGO 3.3 software (Rychlik & Rhoads 1989), of which, six gave satisfactory amplification patterns (i.e. polymerase chain reaction product of the predicted size and supernumerary bands of low intensity).

Polymerase chain reaction (PCR) amplifications were performed following the standard protocol of the Qiagen Multiplex PCR kit, in a final volume of 10 μL, which contained ~5 ng of extracted DNA, 5 μL of 2× Multiplex PCR Master Mix (Qiagen; 1× at final concentration), 1 μL of 5× Q-Solution (Qiagen; 0.5× at final concentration) and 0.2 μM of each multiplexed primer. Primers were multiplexed as follows: *AcobtC12*, *AcobtE07* and *AcobtF01* (multiplex #1); *AcobtE01*, *AcobtF09* and *AcobtG08* (multiplex #2). Reverse primers were labelled with fluorochromes HEX, 6-FAM and NED (Applied Biosystems), as follows: *AcobtC12*, *AcobtE07* and *AcobtF01* (HEX); *AcobtF09* and *AcobtG08* (6-FAM); *AcobtE01* (NED). PCRs were performed on a PTC-100™ thermocycler (MJ Research) using the following

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Table 1 Primer sequences, PCR conditions and polymorphism statistics for six microsatellite loci in two populations of *Acanthoscelides obtectus* Say

Locus	GenBank Accession no.	Primer sequences (5′–3′)	Repeat motif in library	Size (bp)	Size range (bp)	Coeneo			Xochitlan		
						n	N_a	H_O/H_E	*n*	N_a	H_O/H_E
AcobtC12	AY681082	F: GATCCTCTGATGCTACATTTGGTC R: GAGCACGAGCACACGCA	$(TG)_{25}$	311	262–358	34	15	0.294/0.945	34	8	0.118/0.816
AcobtE01	AY681083	F: ATTCACTTAACCACAATACG R: GCTCCTTGAACCTTCTAC	$(AC)_8(AT)_5AC(AT)_3$ $(AC)_2(AC)_3AT$	151	149–161	30	6	0.133/0.623	22	3	0.000/0.329
AcobtE07	AY681084	F: ACACAGTCATGATGACAGC R: AAGTAGAAAATGACGACGAC	$(AG)_{14}$	126	106–140	48	11	0.792/0.764	46	13	0.826/0.865
AcobtF01	AY681085	F: CATAAGGATATTGATTTCGTC R: TGTTCACAATTTCACAGC	$(GT)_8$	236	232–236	46	2	0.087/0.085	42	3	0.238/0.296
AcobtF09	AY681086	F: AGCAGACGACAAGCAGCACAC R: CGAGCCGCATACGCATTG	$(CA)_{11}$	169	158–170	40	7	0.750/0.763	48	4	0.542/0.556
AcobtG08	AY681087	F: GGTGGAGGGACCGCACAC R: CCTTCGGAAATCGTGGATACCC	$(GT)_{14}$	379	366–372	46	4	0.435/0.697	44	3	0.500/0.468

Repeat motif is listed 5′ to 3′ with respect to the forward primer (F). 'Size' refers to the length of the cloned allele. *n* is the number of genes analysed (i.e. two genes per individual). 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).

cycling conditions: initial denaturation at 95 °C (15 min); 30 cycles of: 94 °C (30 s), T_a (1 min 30 s), 72 °C (1 min); final elongation at 72 °C (10 min). The annealing temperature (T_a) was 57 °C for loci *AcobtC12*, *AcobtE07* and *AcobtF01* and 60 °C for loci *AcobtE01*, *AcobtF09* and *AcobtG08*. Fragments were separated on an ABI 310 genetic analyser (Applied Biosystems) with internal size standard ROX500 (Applied Biosystems).

The DNA of 48 individuals, sampled in two Mexican populations (24 individuals per population), Coeneo (Michoacan State) and Xochitlan (Puebla State), which are 412 km apart, was extracted using DNeasy™ kit (Qiagen) and genotyped for the six loci. All loci were polymorphic in both populations. The observed number of alleles per population ranged between 2 and 15, and heterozygosities per population and per locus were between 0 and 0.750 (see Table 1). No genotypic linkage disequilibrium between loci was observed in either population when exact tests (GENEPOP 3.3; Raymond & Rousset 1995) and a correction for multiple tests (Dunn–Sydák method for sequential Bonferroni procedure) were performed. No significant deviation from Hardy–Weinberg equilibrium was observed for loci *AcobtE07*, *AcobtF01*, *AcobtF09* and *AcobtG08* when exact tests (GENEPOP 3.3; Raymond & Rousset 1995) were performed. However, a significant heterozygote deficiency, that would probably be the consequence of null alleles, was found for loci *AcobtC12* and *AcobtE01*. We were unable to design new primer pairs at locus *C12*, because the flanking region in which the forward primer was designed was only 18 nucleotides long, and allowed only the definition of one unique forward primer. Concerning locus *E01*, we designed two other primer pairs, which unfortunately did not allow any amplification, because the flanking region where the forward primer was designed (despite the fact that it was 49 nucleotides long) was composed of several small repeated motifs fewer than 10 nucleotides long that resembled the major motif itself. Other forward primers did not anneal, probably because of interindividual variability in this flanking region.

Cross-species amplifications were tested on individuals of the other species of the *A. obtectus* group (*A. obvelatus* and *A. argillaceus*). PCR conditions were identical to those used for *A. obtectus*. *A. argillaceus* feeds on seeds of *Phaseolus lunatus* L., whereas *A. obvelatus* — the sister species of *A. obtectus* — also develops on seeds of beans from the *P. vulgaris* L. group (Johnson 1989; Alvarez *et al.* in press). The distributions of both species are restricted to Mesoamerica. Whereas *A. obvelatus* amplified none of the six loci, *A. argillaceus* successfully amplified one of the six microsatellite loci (*AcobtF01*: three alleles ranging from 230 to 236). Heterozygosities were not calculated, as most individuals were collected from different populations.

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References

Alvarez N, Aebi A, Risterucci A-M, Hossaert-McKey M, Benrey B (2003) Isolation and characterization of polymorphic microsatellite loci in *Acanthoscelides obvelatus* Bridwell (Coleoptera: Bruchidae). *Molecular Ecology Notes*, **3**, 12–14.

Alvarez N, Hossaert-McKey M, Rasplus J-Y *et al.* (2004) Sibling species of bean bruchids: morphological and phylogenetic studies among *Acanthoscelides obtectus* Say and *A. obvelatus* Bridwell. *Journal of Zoological Systematics and Evolutionary Research*, in press.

Billotte N, Lagoda PJL, Risterucci A-M, Baurens FC (1999) Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. *Fruits*, **54**, 277–288.

Jarry M, Bonet A (1982) La bruche du haricot, *Acanthoscelides obtectus* Say (Coleoptera, Bruchidae), est-elle un danger pour le cowpea, *Vigna unguiculata* (L.) Walp.? *Agronomie*, **2**, 963–968.

Johnson CD (1989) Adaptive radiation of *Acanthoscelides* in seeds: examples of legume–bruchid interactions. In: *Advances in Legume Biology* (eds Stirton CH, Zarucchi JL), pp. 747–779. Monographs in Systematic Botany from the Missouri Botanical Garden 29. Missouri Botanical Garden, St. Louis, MO.

Nei M (1987) *Molecular Evolutionary Genetics*, 2nd edn. Columbia University Press, New York.

Raymond M, Rousset F (1995) GENEPOP Version 1.2.: population genetics software for exact tests and oecumenicism. *Journal of Heredity*, **86**, 248–249.

Rychlik W, Rhoads RE (1989) A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and *in vitro* amplification of DNA. *Nucleic Acids Research*, **17**, 8543–8551.