

the  $H_E$  observed in other plant species (range  $H_E = 0.42$ – $0.79$ , e.g. Innan *et al.* 1997; White & Powel 1997), but it is considerably higher than the  $H_E = 0.038$  observed for allozymes in *P. formosum* (Van der Velde & Bijlsma 2000). Assuming the microsatellite loci to assort independently, the number of possible multilocus genotypes for the seven most polymorphic loci already exceeds four million, the expected frequency of the most common multilocus genotype being less than 0.002. Thus, the amount of variation revealed by these microsatellite loci in *Polytrichum formosum* is clearly large enough to perform clonal structure and paternity analysis in this species.

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- S.B.S., University of Wales, Bangor, Gwynedd, LL57 2UW, UK
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- Received* 11 March 2000; *revision accepted* 25 May 2000
- Correspondence: A. Stenson. Fax: number: 00 44 1248 37 16 44; E-mail:  
bss239@bangor.ac.uk

## Highly polymorphic microsatellite loci from the Dominican anole (*Anolis oculatus*) and their amplification in other *bimaculatus* series anoles

A. STENSON, A. MALHOTRA and  
R. S. THORPE

Microsatellite markers are widely employed in a wide range of population, evolutionary and/or ecological studies (Jarne & Lagoda 1996). Although exceptional cases of amplification of homologous loci in taxa separated by many millions of years of independent evolution have been reported (e.g. Rico *et al.* 1996), it is generally necessary to identify loci for specific species and/or species groups. Herein we report the identification of six highly polymorphic microsatellite loci from the Dominican anole (*Anolis oculatus*) and their amplification in other *bimaculatus* series anoles.

Two genomic libraries were established following the method of Rassmann *et al.* (1991) and the resulting colonies screened with a range of  $^{32}\text{P}$ -end-labelled oligonucleotide probes, including  $(\text{CA})_n$ ,  $(\text{CT})_n$ ,  $(\text{ACC})_n$  and  $(\text{GATA})_n$ . A total of 73 cloned inserts were fully or partially sequenced and primers were designed, using OLIGO 5.0 (National Biosciences, INC. Plymouth, MN, USA), from 20 of the sequences. Of these, four failed to produce any detectable product, four produced multiple products and six produced monomorphic products. The remaining six pairs of primers produced unambiguous and reproducible polymorphic bands.

Tissue samples for all taxa consisted of autotomised tail tips, which were immediately placed into 80% ethanol and the animals released at the point of capture. Polymerase chain reactions (PCR) were carried out in 25  $\mu\text{L}$  volumes containing 50–100 ng genomic DNA, 1 $\times$  reaction buffer (50 mM KCl, 20 mM Tris-HCl pH 8.4), each dNTP at 120  $\mu\text{M}$ , each primer at 400 nM, DMSO at 0.6% and 1 U *Taq* DNA polymerase (BRL). The concentration of magnesium chloride varied between loci (Table 1). Following an initial denaturation step of 3 min at 94  $^{\circ}\text{C}$ , the reactions underwent 35 thermal cycles (94  $^{\circ}\text{C}$  for 30 s, 30 s at the annealing temperature (Table 1) and 30 s at 72  $^{\circ}\text{C}$ ) and a final extension period of 5 min at 72  $^{\circ}\text{C}$ . Products were separated on 6% denaturing polyacrylamide gels and transferred to nylon membrane by Southern blotting. After hybridization at 45  $^{\circ}\text{C}$  in 6 $\times$  SSC, 0.1% SDS, 1% BSA (fraction V) with  $^{32}\text{P}$ - or  $^{33}\text{P}$ -end-labelled oligonucleotide probes corresponding to the repeat motif, products were visualized by autoradiography.

All six loci have been used to screen populations of *A. oculatus* and have proved to be highly polymorphic with between 13 and 25 alleles. GENEPOP version 3.1 (Raymond & Rousset 1995) was used to calculate expected heterozygosities ( $H_E$ ) and to test for significant departures from Hardy–Weinberg equilibrium. Expected heterozygosities were high, with average values across all populations of >70% for all loci. After corrections for multiple simultaneous tests, patterns of heterozygote deficiencies indicated the possible presence of null alleles at three of the loci (Ao10;13, AoSA18 & AoBA36). However, these appear to be at relatively low frequency and widely distributed between populations. Non-independence of loci was tested using the EM algorithm of ARLEQUIN version 1.1 (Schneider *et al.* 1997) and a small number of

**Table 1** Polymorphic microsatellite loci from *Anolis oculatus*. GenBank accession nos, primer sequences, the size and repeat region of the cloned allele and the annealing temperature and magnesium chloride concentration used in PCR reactions are given, as are the number of observed alleles and the average expected and observed heterozygosities across all populations. Sample sizes were 900 individuals from 39 populations for loci AoGT2, AoGT9, Ao7;73 and Ao10;13 and 180 individuals from eight populations for loci AoSA18 and AoBA36

Locus	Accession no.	Primers (5' to 3')	Cloned allele size (bp)	Repeat region of cloned allele	Annealing temperature (°C)	[MgCl <sub>2</sub> ] (mM)	Number of alleles	Mean $H_E$	Mean $H_E$
AoGT2	AF241567	GAAGTAACTTTTTGATTCTAGGTT TTACTCAGCACACCTTCCTG	100	(GT) <sub>10</sub>	50	3.0	17	0.8377	0.8208
AoGT9	AF241568	CATCTGTGGCTCATGGCTTT CTTTCTCCCACCTGGACATT	190	(TG) <sub>5</sub> (TA) <sub>4</sub> (TG) <sub>12</sub>	50	3.0	24	0.7601	0.7441
Ao7;73	AF241569	AGTCCAGAGAGCGGTATTTT AAAAAAGAGGTATAAAGACATA	155	(CT) <sub>8</sub>	45	3.0	18	0.8318	0.7538
Ao10;13	AF241570	AAGTCAAATCATGCTAAATA ACCCCTAAAGTAAAAATAATA	140	(CT) <sub>10</sub> AT(AC) <sub>7</sub>	45	2.0	25	0.8115	0.7884
AoSA18	AF241571	AGCTCGAATTGCCACCAATA TCCGACTCTTTGCACAGTTG	70	(CA) <sub>10</sub>	55	3.0	15	0.7081	0.6475
AoBA36	AF241572	TGTTATGCCATGCCTGATGT GCAACAGGACATAACCACCGT	121	(GT) <sub>10</sub>	55	3.5	13	0.8125	0.7824

**Table 2** Heterospecific amplification of microsatellite loci. Results of amplification are indicated by: P[n] = Polymorphic, with *n* alleles; M = Monomorphic; – = No detectable product

Locus	<i>A. marmoratus</i>	<i>A. leachi</i>	<i>A. gingivinus</i>	<i>A. bimaculatus</i>	<i>A. sabanus</i>	<i>A. nubilus</i>	<i>A. lividus</i>	<i>A. ferreus</i>	<i>A. wattsi</i>
AoGT2	P [18]	P [7]	P [7]	P [12]	P [3]	P [2]	M	P [2]	P [8]
AoGT9	P [28]	–	–	–	P [9]	M	P [7]	P [8]	–
Ao7;73	P [12]	P [2]	–	–	P [3]	M	P [6]	P [7]	P [6]
Ao10;13	P [23]	P [11]	P [4]	P [5]	P [10]	M	P [12]	P [4]	P [13]
AoSA18	P [9]	P [12]	P [12]	P [5]	P [5]	M	P [5]	P [3]	P [14]
AoBA36	P [11]	P [4]	M	P [2]	P [3]	M	P [2]	P [8]	P [6]

departures from expectation were detected. However, these were randomly distributed between locus pairs and populations and global tests across all populations, based upon tests implemented in GENEPOP, detected no significant departures from linkage expectations.

The amplification of these loci in other taxa was investigated in all recognized species of *bimaculatus* series anoles. Sample sizes for all taxa were >30, except for *A. nubilus* for which only three tissue samples were available. One locus (AoGT9) failed to produce detectable products in four species, while locus Ao7;73 also failed to produce a product in two of these species (Table 2). There was a marked tendency for fewer alleles being detected in non-source species, although locus AoGT9 displayed more alleles in *A. marmoratus* than in *A. oculatus*.

All observed bands were consistent with changes in the number of repeat units. A limited amount of sequence data was obtained from heterospecific amplification products and confirmed that the primers for all loci had amplified homologous loci in all taxa.

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### Isolation and characterization of microsatellite loci in marsupial gliders (*Petaurus norfolcensis*, *P. breviceps* and *P. gracilis*)

A. L. MILLIS