

Table 2 Cross-species amplifications data for *Cebus apella* and *Lagothrix lagotricha* microsatellites. Neotropical primates. Successful amplification is indicated by the observed genotype (in bp) of the single sample per species that was analysed

Locus	PEPC3	PEPC8	PEPC40	PEPC59	PEPL4
<i>Alouatta seniculus</i>	265	171/189	+	256	–
<i>Aotus trivirgatus</i>	268	150	+	232/256	–
<i>Ateles</i> sp.	251/262	176/189	+	320	+
<i>Brachyteles arachnoides</i>	263/281	180	+	253/257	+
<i>Callicebus molloch</i>	258/275	176	+	+	–
<i>Callimico goeldii</i>	266	+	+	+	–
<i>Callithrix jacchus</i>	259	172/174	+	221/232	–
<i>Chiropotes satanas</i>	244	208	+	+	+
<i>Lagothrix lagotricha</i>	211/222	277	+	288/290	296/298
<i>Leontopithecus rosalia</i>	264/304	209	+	226/236	–
<i>Pithecia pithecia</i>	266/307	+	+	233	–
<i>Saguinus geoffroyi</i>	264/327	146	+	281	–
<i>Saimiri sciureus</i>	327	+	+	277	–

– indicates no amplification.

The primers reported here give evidence of the high polymorphism found in microsatellites compared to other molecular markers. Given the high polymorphism in these primers they offer a powerful tool for studies on pedigree and population structure of neotropical primates.

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Polymorphic microsatellite DNA markers for the marine gastropod *Littorina subrotundata*

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Coastal habitats of the northern temperate zone are home to over 19 species of *Littorina* (Reid 1996). Due to its widespread abundance and phenotypic diversity, members of this

genus have been the model system of choice for numerous ecological and evolutionary studies (Reid *et al.* 1996 and references therein). Although mitochondrial DNA has been useful in the study of population structure in *Littorina*, it is clear from recent data (Kyle & Boulding 1998) that faster-evolving markers are needed for fine-scale metapopulation analysis. To this end, we have isolated 12 polymorphic microsatellite loci for *Littorina subrotundata* (Carpenter 1864), a direct-developing species from the North Pacific. We report preliminary data evaluating their potential utility as high-resolution genetic markers for this model taxon. The general methods for the isolation of simple sequence loci outlined in Rassmann *et al.* (1991) were followed, with the following alterations.

Total genomic DNA from the visceral mass of *L. subrotundata* was isolated via organic extraction with methylene chloride/isoamyl alcohol and subsequent ethanol precipitation (Claxton *et al.* 1997). DNA from five individuals was combined, digested with the restriction enzymes *Hae*III and *Rsa*I and size selected using 1% agarose gel electrophoresis. DNA fragments of 300–1000 bp in length were ligated into the *Eco*RV site of the pZErO plasmid vector (Invitrogen) which was then transformed into Top F' *Escherichia coli* cells (Invitrogen) via electroporation using standard methods (*E. coli* Pulser, Bio-Rad).

Approximately 32 000 colonies were screened with [γ^{33} P]-ATP-labelled AAT₁₀, AAAT₆ (hybridization $T = 52$ °C), AAG₈, AAC₉ and GATA₇ (hybridization $T = 55$ °C) oligonucleotide probes according to the methods of Shaw (1997; P. W. Shaw, personal communication). DNA sequences of inserts from clones positive in two successive hybridizations were obtained using the manufacturer's suggested cycle sequencing protocol for the ABI model 377 Version 2.1.1. using M13 sequencing primers and labelled ddNTPs.

Of the 50 sequences obtained, 48 contained unique microsatellites composed of at least one trinucleotide repeat array. PCR (polymerase chain reaction) primers for 10 of these loci were designed using Primer V0.5 (Lincoln & Daly 1991) and Gene Runner V3.0 (Hastings software) programs and are reported in Table 1. Two dinucleotide loci, VAM-F1 and

Table 1 Characterization of *Littorina subrotundata* microsatellites

Locus*	Repeat motif	Primer sequence (5'–3')	T_a (°C) used in PCR	Product length (bp)†	Number of alleles‡	Product size range (bp)	H_o	H_e §
Lsub4	(ATA) ₂₈	CCCCATTTGATAACAGGAACC GCTTTGAACCTTCGGATAAGGC	62	153	18 (27)	143–210	0.96	0.92
Lsub6	(TCAA) ₆ CA(AAT) ₂₇	GCCTTTAACAACAGGTCTTGG CTCTGGCACCTATAAACACGC	60	230	17 (27)	203–251	0.48	0.92
Lsub8	(ATA) ₂₉	CCAGTGACCAGATCATAGCG GGAATTGTAAAGTGCTTGGAGC	62	234	16 (27)	186–258	0.78	0.90
Lsub9	(ATA) ₂₅	TGTGACTGTGCTACAAGTCC TGACAAATTGTGATATGTTGATGC	62	231	15 (24)	195–237	1.00	0.92
Lsub13	(TTA) ₇ C(TAT) ₃₀	TCCCAGTGCCGTGTATCC ACAGGAAGCGACTATGGTGC	64	240	19 (27)	189–240	0.89	0.93
Lsub14	(ATT) ₁₉	GGCTTGGTCAGTGGATTATTG CGCTTTGAACCTTTTGATAAGGC	64	133	14 (26)	97–172	0.73	0.85
Lsub16	(CTT) ₁₁	TTTGCTGCTCTGAAAATTC GGAGTCTAAGCCCATGAACG	60	140	8 (23)	137–200	0.30	0.76
Lsub32	(CAA) ₁₃ TTG(AAC) ₅ TG(AAC) ₁₀	ATCACATCGCACACGCTTAC ACGGTGTGTCATCATCAACG	62	229	10 (27)	205–247	0.81	0.84
Lsub62	(AAC) ₁₄	CGCTTTCCCGTTATACCAAC CACCGTAAAACCTTGTGAGC	64	240	12 (27)	231–246	0.93	0.84
Lsub63	(CAA) ₅ AA(CAA) ₂ CA (CAA) ₇ CAT(CAA) ₁₃	GCCAATTGTATAACGGACTGTG TAAGTGCGGTATGGTCAAC	58	142	16 (27)	130–184	0.78	0.91
VAM-F1	(TC) ₇ N ₁₀ (TC) ₂₇	TTGGTAGTTAGGGCTAGAAAG ACCTGCAACCAGAATACACAG	60	212	16 (25)	196–262	0.80	0.88
VAM-B3	(CT) ₃₀ (CA) ₇	CTTGTATCTCTCGTATCCGGG AGAACCAGCAAGCGTAAAGC	62	173	22 (28)	143–213	0.75	0.93

*Genbank Accession numbers: AF167443–AF167454.

†Based on the sequence of the cloned fragment.

‡Number in parentheses indicates the number of individuals sampled at that locus.

§Using the formula $H_e = 1 - \sum P_i^2$ where P_i is the frequency of the i th allele.

VAM-B3 (Table 1), had been isolated at an earlier date, employing an enrichment method (Naish & Skibinski 1998).

Allelic variation was assayed in 27 individuals collected from Prasiola Point, Vancouver Island, British Columbia, Canada (48° 50'N, 125° 08'W). PCR amplifications were carried out in a final volume of 20 µL containing 10 ng of genomic DNA, 0.4 µM of each primer, 50 µM of each dNTP, 1.5 mM of MgCl₂, 0.5–1.0 units of *Taq* polymerase and 1× PCR buffer containing 20 mM Tris-HCl (pH 8.4) and 50 mM KCl (Gibco-BRL). Cycling parameters were: 5 min at 95 °C followed by 28 cycles of 30 s at 95 °C and 40 s + 1 s per cycle at the optimal annealing temperature (Table 1) using a PTC-100 thermocycler (MJ Research). PCR products were electrophoresed in 5% nondenaturing polyacrylamide gels and visualized by silver staining (Naish & Skibinski 1998). Sizes of PCR products were determined by comparison to a 3-bp allelic size standard made from the PCR products of different individual snails and calibrated with the original clone.

For VAM-F1 and VAM-B3 (Table 1), the PCR conditions were modified as follows: a final volume of 5 µL was used, the dNTP concentration was 40 µM, the forward primer was end-labelled with [³²P]-ATP and used at a concentration of 0.02 µM and the reaction included 0.1% Tween-20. Cycling parameters were: 5 min at 95 °C followed by 30–40 cycles of 30 s at 95 °C, 30 s at the optimal annealing temperature (Table 1) and 30 s at 72 °C. PCR products were resolved on 6% polyacrylamide gels under denaturing conditions and visualized by autoradiography. Allele sizes were determined by comparison to an M13mp18 sequencing size standard (Amersham).

Table 1 outlines the allelic variation, product size range and heterozygosity observed at each of the 12 loci. All loci were moderately to highly polymorphic with a population of 23–27 individuals showing between 8 and 22 alleles per locus and an observed heterozygosity in the range of 0.30–1.00. These preliminary results indicate the potential utility of microsatellite DNA markers in future studies of intra-specific variation, local gene flow and population substructure in *Littorina subrotundata*.

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New microsatellite markers for assessment of paternity in the squid *Loligo forbesi* (Mollusca: Cephalopoda)

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The availability of microsatellite DNA markers for the veined squid, *Loligo forbesi*, has allowed detailed analysis of population structuring and preliminary investigation into mating strategy and male–male competition in this northeastern Atlantic species (Shaw & Boyle 1997; Shaw 1997; Shaw *et al.* 1999). Mating involves the transfer of sperm packets (spermatophores) from male to female, after which eggs are laid in encapsulated strings (Boletzky 1997). Competition for mates between males has been observed in loliginid squid, where dominant males guard females against other males and smaller, 'sneaker' males (Hanlon 1996). Microsatellite markers have been used to demonstrate that multiple mating in *Loligo forbesi* can result in multiple paternity of the offspring of a single female, i.e. within an egg string (Shaw & Boyle 1997). To facilitate these studies, additional microsatellite loci have been isolated from *L. forbesi*.

A size-selected partial genomic library (Rassmann *et al.* 1991) was constructed using genomic DNA from ethanol-preserved mantle of *Loligo forbesi* extracted using a proteinase K, phenol–chloroform procedure (Jones *et al.* 1997). DNA from eight individuals was pooled to a total of 10 µg and digested with *AluI* and *RsaI* (Promega). DNA fragments of 300–600 bp were selected by electrophoresis and ligated into the PCRscript vector (Stratagene) following the manufacturer's instructions for cloning blunt-ended polymerase chain reaction (PCR) products. The ligation was used to transform strain XL-1 blue competent cells (Stratagene). Colonies positive for recombinant plasmids