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PRIMER NOTE

Microsatellite DNA markers developed for the Australian bass (*Macquaria novemaculeata*) and their cross-amplification in estuary perch (*Macquaria colonorum*)

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Abstract

The Australian bass is a catadromous species found in drainages of southeastern Australia. As an economically important resource that is declining in number, the Australian bass is currently extensively stocked in New South Wales and Victoria to meet the requirements of fisheries programs. We have developed six microsatellite markers that amplify in both Australian bass and the congeneric estuary perch. These markers are useful for investigating population genetic structure and for identifying hybrids between these two species.

Keywords: conservation genetics, freshwater fish, hybridization; microsatellites

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The catadromous Australian bass (*Macquaria novemaculeata*) is found in coastal rivers from southern Queensland to tributaries of Gisppsland lakes in Victoria (Harris & Rowland 1996). As with many other southeastern Australian freshwater fish species, Australian bass populations are declining ultimately as a result of anthropogenic effects such as riverflow regulation and overfishing (Harris 1984). In addition, this species is known to hybridize with the morphologically similar estuary perch (*Macquaria colondrum*) (Jerry *et al*. 1999). Management plans for Australian bass include breeding programs and reintroductions into historical ranges. For these plans to be effective, it is important to understand the patterns of population genetic structure and identify the hybrids between Australian bass and estuary perch. Here we describe the isolation and development of six microsatellite markers and their amplification in both the Australian bass and the estuary perch. These DNA markers are suitable for both population level analysis and identifying hybrids between these two *Macquaria* species.

We isolated microsatellites using a modification of an enrichment technique (Fisher & Bachmann 1998). Genomic DNA from the Australian bass was digested with *Rsa*I and *Hae*III, and fragments were ligated to two oligo adaptors (Edwards *et al*. 1996). Biotinylated oligo probes (dGA10 and dCA10) were hybridized to the digested DNA and separated using streptavidin magnetic particles (Promega). Polymerase chain reactions (PCRs) were performed on the microsatellite-enriched eluate using one of the oligo adaptors as a primer. The product from the first PCR was used as template to repeat the enrichment process. The enriched library was gel purified, ligated into pCR 2.1-TOPO vector (Invitrogen), and transformed into TOP10 cells (Invitrogen). Inserts were amplified from the clones using M13 primers, gel purified, and sequenced on an ABI 377 automated DNA sequencer (PE Applied Biosystems) using dye terminator chemistry. We sequenced 31 putative positive clones and used PRIMER 3 (Rozen & Skaletsky 1997) to develop microsatellite primers.

Microsatellite loci were amplified by PCR using a 10-μL radio-labelled reaction containing *c*. 50–100 ng of DNA, 4 μg of bovine serum albumin, 12 pmol of each primer, 0.5 units of *Taq* DNA polymerase (Promega), 200 μM of dCTP, dGTP, and dTTP, 20 μM of dATP, 2.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1% Triton X-100 and 0.05 μL [α^{33}P]-dATP at 1000 Ci/mmol overlaid with mineral oil. The PCRs were performed in a MJ Research thermocycler using cycling conditions designed by Beheregaray & Sunnucks (2000). The conditions consisted of 94 °C for 3 min, followed by a 'touchdown' (32 cycles at 94 °C/20 s, annealing/45 s and 72 °C/60 s), and a final step at 72 °C for 4 min. The annealing temperature of the touchdown PCRs decreased by two degrees per cycle until stabilizing at the fifth cycle (from 63 to 55 °C). The PCR products were separated by 6% polyacrylamide gel electrophoresis and visualized

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Table 1 Characteristics of six microsatellite loci isolated from the Australian bass, *Macquaria novemaculeata*. Number of alleles (N_a), observed (H_O) and expected (H_E) heterozygosities are based on a sample of 55 Australian bass and 19 estuary perch

Locus	Primer sequences (5′–3′)	Repeat structure	N_a Bass	N_a Perch	Allele size (bp) Bass	Allele size (bp) Perch	H_O/H_E	GenBank Accession no.
AB001	ACGCTGGTGTTAGGTGTGC	$CA_{(15)}$	5		232–238		0.51/0.54	AY819798
	CGAGCTATGATTGGGTCAGC		1		224		NA	
AB006	GGTACACTTGTCCACATTTGAGC	$GA_{(21)}$	3		188–192		0.87/0.62*	AY819799
	CAGGTGAGGGGAAAAGAGG		1		180		NA	
AB009	CAGCAAGACATAGCAGCTTCC	$GA_{(7)}$	2		275–285		0.46/0.43	AY819800
	GGTACACTTACCAACTCTCTGTTTC		3		271–275		0.21/0.24	
AB097	AAAACAGCCAAAAAGTTGACC	$CA_{(9)}$	2		104–112		0.26/0.28	AY819801
	AATGAGGGTAAACAGCACAGG		1		104		NA	
AB107	TGTTTAGGGAGCAATTGTGG	$CA_{(8)}$	1		290		NA	AY819802
	TGATTAGTAGAGATGAGAGGACTGG		1		300		NA	
AB114	TTTAAAATGCCCAACACTGA	$GA_{(9)}$	4		115–133		0.51/0.44	
	CATCGTCCATGGCTCACC		2		131–141		0.32/0.32	AY819803

*Out of Hardy–Weinberg equilibrium $P < 0.05$.

by autoradiography. The software GENEPOP version 3.3 (Raymond & Rousset 1995) was used to estimate expected (H_E) and observed (H_O) heterozygosities and to test for linkage disequilibrium (LD).

Six loci were successfully optimized to work in both species and were screened for variation in a sample of 55 Australian bass and 19 estuary perch. Loci revealed moderate to low genetic variation: number of alleles per locus ranged between one (a different fixed allele in each species) and five, and expected heterozygosities in nonfixed loci varied between 0.21 and 0.62 (Table 1). Most loci were at Hardy–Weinberg equilibrium, except for AB006 which showed significant excess of homozygotes in the bass. This excess may be due to the combination of individuals from different river populations in the analysis. No evidence for LD was detected in locus pair/population comparisons.

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