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PERMANENT GENETIC RESOURCES NOTE

Cross-species characterisation of polymorphic microsatellite loci in the giant otter (*Pteronura brasiliensis*)

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Abstract

Nineteen microsatellite loci developed for the Eurasian otter (*Lutra lutra*) and 15 loci developed for the North American river otter (*Lontra canadensis*) were tested for ease of amplification and degree of polymorphism on a set of 20 giant otter (*Pteronura brasiliensis*) faecal samples from the Bolivian Amazon basin. Nineteen loci amplified consistently well, with polymorphisms ranging from two to nine alleles and observed heterozygosity ranging from 0.15 to 0.85.

Keywords: *Lontra*, *Lutra*, microsatellite, polymorphism, *Pteronura brasiliensis*

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The giant otter, *Pteronura brasiliensis*, suffered a population collapse in the last century as a result of over-harvesting for the pelt trade. This hunting pressure extirpated the species from much of its eastern range in Brazil, and from Argentina and Uruguay (Carter & Rosas 1997). With the implementation of a continent-wide hunting ban in 1979, the population has been recovering; however, survival is now increasingly threatened by habitat degradation (Groenendijk *et al*. 2005).

In order to select a series of microsatellite loci for use in population genetic studies of the giant otter, we screened 19 loci derived from the Eurasian otter, *Lutra lutra* (Dallas & Piertney 1998; Dallas *et al*. 2000; Huang *et al*. 2005) and 15 loci from the North American river otter, *Lontra canadensis* (Beheler *et al*. 2004; Beheler *et al*. 2005) on a sample of 20 giant otters. The samples were faecal in origin, collected in 2007 from the Rio San Martin, located in the Beni Department of northern Bolivia. The samples constitute individuals from 12 social groups distributed over 280 km.

Faecal samples were preserved in ethanol and DNA was extracted using a QIAGEN QIAamp DNA Stool Mini Kit following the manufacturer's protocol. Amplification took place in 7 μL polymerase chain reactions (PCR) using 1.2 μL template DNA, 0.6 μM of primer solution (forward primers of each primer pair were fluorescently labelled using either FAM or HEX dye), BSA, autoclaved Milli-Q water and 4 μL QIAGEN multiplex PCR kit (containing master mix, HotStar *Taq*, $MgCl_2$, dNTPs and PCR buffer). PCR was performed using an ABI GeneAmp PCR System 9700 involving a 94 °C denaturing step for 15 min followed by 35 cycles of denaturing at 94 °C, annealing at 58 °C for 1 min 30 s with extension at 72 °C for 1 min and a final extension period at 60 °C for 30 min. Alleles were scored using GeneMapper (version 3.7). The comparative method approach was used as recommended by Hansen *et al*. (2008), with each sample independently genotyped at least twice for a heterozygote and three times for a homozygote. If ambiguities arose, further repeats would occur until a consensus was reached. Heterozygosity was calculated and Hardy–Weinberg equilibrium tested for using GenAlEx (Smouse & Peakall 1999). Linkage disequilibrium was tested for using GenePop (Raymond & Rousset 1995), with an alpha of 0.001 following Bonferroni correction. Micro-Checker (Van Oosterhoust *et al*. 2004) was used to estimate the probability of null allele occurrence.

In total, 32 of the 34 loci amplified; seven were monomorphic and in the remaining loci polymorphism varied from two to eight alleles per locus. Overall observed heterozygosity was lower than expected, although three loci had heterozygosities higher than expected by greater than 0.04 (Table 1). No significant departures from Hardy–Weinberg or linkage disequilibrium between loci were observed, although evidence of null alleles was suggested in loci Lut604, Lut615, 04OT04, Rio16, Rio20 ($P < 0.05$).

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Table 1 Number and range of alleles found in the giant otter, with expected (H_E) and observed (H_O) heterozygosities

Locus	Species of origin	Source	Sample size	PCR product (bp)	No. of alleles	H_E	H_O
Lut435	*Lutra lutra*	Dallas and Piertney 1998	19	113–125	5	0.634	0.579
Lut453	*Lutra lutra*	Dallas and Piertney 1998	20	113–125	5	0.733	0.75
Lut457	*Lutra lutra*	Dallas and Piertney 1998	20	172	M		
Lut604	*Lutra lutra*	Dallas and Piertney 1998	19	116–126	3	0.666	0.368
Lut615	*Lutra lutra*	Dallas and Piertney 1998	14	226–234	3	0.579	0.286
Lut701	*Lutra lutra*	Dallas and Piertney 1998	13	170–202	8	0.846	0.846
Lut715	*Lutra lutra*	Dallas and Piertney 1998	20	153–157	2	0.469	0.45
Lut717	*Lutra lutra*	Dallas and Piertney 1998	20	137	M		
Lut733	*Lutra lutra*	Dallas and Piertney 1998	20	150–170	5	0.59	0.5
Lut782	*Lutra lutra*	Dallas and Piertney 1998	20	158–174	3	0.585	0.6
Lut818	*Lutra lutra*	Dallas and Piertney 1998	20	135	M		
Lut832	*Lutra lutra*	Dallas and Piertney 1998	20	178–190	4	0.543	0.55
Lut833	*Lutra lutra*	Dallas and Piertney 1998	19	158–164	M		
Lut914	*Lutra lutra*	Dallas *et al*. 2000	20	129–133	2	0.145	0.158
04OT04	*Lutra lutra*	Huang *et al*. 2005	15	156–164	7	0.571	0.267
04OT05	*Lutra lutra*	Huang *et al*. 2005	20	148	M		
04OT07	*Lutra lutra*	Huang *et al*. 2005	19	120–124	2	0.301	0.263
04OT17	*Lutra lutra*	Huang *et al*. 2005	20	153–177	4	0.559	0.6
04OT19	*Lutra lutra*	Huang *et al*. 2005	19	212–228	5	0.474	0.526
RIO01	*Lontra canadensis*	Beheler *et al*. 2004	20	231	M		
RIO02	*Lontra canadensis*	Beheler *et al*. 2004	20	182–188	6	0.491	0.35
RIO03	*Lontra canadensis*	Beheler *et al*. 2004	18	176–190	6	0.673	0.833
RIO06	*Lontra canadensis*	Beheler *et al*. 2004	15	230–250	5	0.582	0.6
RIO07	*Lontra canadensis*	Beheler *et al*. 2004	17	160–168	6	0.721	0.941
RIO11	*Lontra canadensis*	Beheler *et al*. 2005	20	140	M		
RIO12	*Lontra canadensis*	Beheler *et al*. 2005	20	203–211	4	0.581	0.6
RIO13	*Lontra canadensis*	Beheler *et al*. 2005	10	246–264	4	0.65	0.6
RIO14	*Lontra canadensis*	Beheler *et al*. 2005	20	*n*	0		
RIO15	*Lontra canadensis*	Beheler *et al*. 2005	16	244–260	2	0.482	0.563
RIO16	*Lontra canadensis*	Beheler *et al*. 2005	20	262–268	4	0.591	0.35
RIO17	*Lontra canadensis*	Beheler *et al*. 2005	19	153–181	5	0.645	0.632
RIO18	*Lontra canadensis*	Beheler *et al*. 2005	20	*n*	0		
RIO19	*Lontra canadensis*	Beheler *et al*. 2005	14	266–270	3	0.538	0.5
RIO20	*Lontra canadensis*	Beheler *et al*. 2005	18	233–237	2	0.278	0

M, monomorphic; *n*, no amplification product.

The degree of amplification success varied depending on the locus. Whereas Lut615 (62%) and Rio13 (23%) amplified poorly, others amplified extremely well, with 87.9% in 04OT17 and 90.4% in Lut733, for example.

Following this study, 15 loci were chosen for suitability in population studies and divided into the following multiplexes. M1: Lut453, 04OT17, Rio12; M2: Lut435, Lut733, Rio16; M3: Lut604, Lut782, Rio15; and results indicate a 60% amplification success rate among giant otter faecal samples and suggest that using noninvasive methods of DNA collection these loci are sufficient to reveal patterns of relatedness and levels of gene flow occurring between populations.

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