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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, MgCl₂, 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 Oosterhout *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 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 19 | 113–125 | 5 | 0.634 | 0.579 |
| Lut453 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 20 | 113–125 | 5 | 0.733 | 0.75 |
| Lut457 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 20 | 172 | M | | |
| Lut604 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 19 | 116–126 | 3 | 0.666 | 0.368 |
| Lut615 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 14 | 226–234 | 3 | 0.579 | 0.286 |
| Lut701 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 13 | 170–202 | 8 | 0.846 | 0.846 |
| Lut715 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 20 | 153–157 | 2 | 0.469 | 0.45 |
| Lut717 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 20 | 137 | M | | |
| Lut733 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 20 | 150–170 | 5 | 0.59 | 0.5 |
| Lut782 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 20 | 158–174 | 3 | 0.585 | 0.6 |
| Lut818 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 20 | 135 | M | | |
| Lut832 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 20 | 178–190 | 4 | 0.543 | 0.55 |
| Lut833 | <i>Lutra lutra</i> | Dallas and Piertney 1998 | 19 | 158–164 | M | | |
| Lut914 | <i>Lutra lutra</i> | Dallas <i>et al.</i> 2000 | 20 | 129–133 | 2 | 0.145 | 0.158 |
| 04OT04 | <i>Lutra lutra</i> | Huang <i>et al.</i> 2005 | 15 | 156–164 | 7 | 0.571 | 0.267 |
| 04OT05 | <i>Lutra lutra</i> | Huang <i>et al.</i> 2005 | 20 | 148 | M | | |
| 04OT07 | <i>Lutra lutra</i> | Huang <i>et al.</i> 2005 | 19 | 120–124 | 2 | 0.301 | 0.263 |
| 04OT17 | <i>Lutra lutra</i> | Huang <i>et al.</i> 2005 | 20 | 153–177 | 4 | 0.559 | 0.6 |
| 04OT19 | <i>Lutra lutra</i> | Huang <i>et al.</i> 2005 | 19 | 212–228 | 5 | 0.474 | 0.526 |
| RIO01 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2004 | 20 | 231 | M | | |
| RIO02 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2004 | 20 | 182–188 | 6 | 0.491 | 0.35 |
| RIO03 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2004 | 18 | 176–190 | 6 | 0.673 | 0.833 |
| RIO06 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2004 | 15 | 230–250 | 5 | 0.582 | 0.6 |
| RIO07 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2004 | 17 | 160–168 | 6 | 0.721 | 0.941 |
| RIO11 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 20 | 140 | M | | |
| RIO12 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 20 | 203–211 | 4 | 0.581 | 0.6 |
| RIO13 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 10 | 246–264 | 4 | 0.65 | 0.6 |
| RIO14 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 20 | <i>n</i> | 0 | | |
| RIO15 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 16 | 244–260 | 2 | 0.482 | 0.563 |
| RIO16 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 20 | 262–268 | 4 | 0.591 | 0.35 |
| RIO17 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 19 | 153–181 | 5 | 0.645 | 0.632 |
| RIO18 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 20 | <i>n</i> | 0 | | |
| RIO19 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 2005 | 14 | 266–270 | 3 | 0.538 | 0.5 |
| RIO20 | <i>Lontra canadensis</i> | Beheler <i>et al.</i> 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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References

- Beheler AS, Fike JA, Dharmarajan G, Rhodes OE, Serfass TS (2005) Ten new polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and their utility in related mustelids. *Molecular Ecology Notes*, **5**, 602–604.
- Beheler AS, Fike JA, Murfitt LM, Rhodes OE, Serfass TS (2004) Development of polymorphic microsatellite loci for North American river otters (*Lontra canadensis*) and amplification in related mustelids. *Molecular Ecology Notes*, **4**, 56–58.
- Carter SK, Rosas FCW (1997) Biology and conservation of the giant otter *Pteronura brasiliensis*. *Mammal Review*, **27** (1), 1–26.
- Dallas JF, Piertney SB (1998) Microsatellite primers for the Eurasian otter. *Molecular Ecology*, **7**, 1247–1263.

- Dallas JF, Carss DN, Marshall F, Koepfli K-P, Kruuk H, Pieltney SB, Bacon PJ (2000) Sex identification of the Eurasian otter *Lutra lutra* by PCR typing of spraints. *Conservation Genetics*, **1**, 181–183.
- Groenendijk J, Hajek F, Duplaix N *et al.* (2005) Surveying and monitoring distribution and population trends of the giant otter (*Pteronura brasiliensis*). Guidelines for a standardisation of survey methods as recommended by the giant otter section of the IUCN/SSC Otter Specialist Network. *Habitat*, **16**, 1–100.
- Hansen H, Ben-David M, McDonald DB (2008) Effects of genotyping protocols on success and errors in identifying individual river otters (*Lontra canadensis*) from their faeces. *Molecular Ecology Resources*, **8**, 282–289.
- Huang CC, Hsu YC, Lee LL, Li SH (2005) Isolation and characterization of tetramicrosatellite DNA markers in the Eurasian otter (*Lutra lutra*). *Molecular Ecology Notes*, **5**, 314–316.
- Raymond M, Rousset F (1995) GenePop (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.