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PRIMER NOTE

Characterization of microsatellite loci in *Tursiops aduncus*

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Abstract

We describe the cloning and characterization of five highly polymorphic microsatellite loci cloned from aduncus dolphins (*Tursiops aduncus*) from Western Australia. Five polymorphic microsatellite loci were isolated and tested on up to 350 animals, showing 7–23 alleles and expected heterozygosity values from 0.68 to 0.89. We also tested the loci on striped dolphins and franciscana dolphins, where we also found high levels of polymorphism (9–16 alleles in 102 striped, and 4–5 alleles in 13 franciscana dolphins). Considering that the cetacean genome is highly conserved, the characterized markers are likely to be useful in a number of cetacean population studies.

Keywords: CA repeats, cetaceans, GT repeats, microsatellites, odontoceti, *Tursiops aduncus*

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Behavioural findings in dolphins suggest a very complex social structure (Wells *et al*. 1987; Connor *et al*. 1992a,b, 1999). It is vital for genetic studies of behaviour to be able to unambiguously resolve kinship, which requires a large number of highly polymorphic loci. Although a wide range of cetacean-specific microsatellite markers are already available for both toothed (e.g. Schloetterer *et al*. 1991; Valsecchi & Amos 1996; Shinohara *et al*. 1997) and baleen whales (e.g. Valsecchi & Amos 1996; Palsboll *et al*. 1997; Waldick *et al*. 1999), recent studies suggest that a larger number of loci than previously thought are needed for unambiguously resolving genetic relationships (Slate *et al*. 2000). Furthermore, endangered species, which (some more than others) might require a molecular assessment of their status for conservation purposes, have often low genetic variability. This also requires more loci to be screened. Here we present five highly polymorphic microsatellite loci cloned from Tursiops aduncus (Wang *et al*. 1999), which we employed among other loci in a large-scale population analysis for investigating paternity and genetic relatedness among dolphins in Shark Bay, Western Australia (Krützen *et al*. in preparation).

Samples from *Tursiops aduncus* were obtained in three consecutive field seasons using a modified air gun and biopsy tips specially designed for the use in small cetaceans (Krützen, unpublished data). Samples were stored in a saturated NaCl/20% dimethyl sulfoxide (DMSO) solution (Amos & Hoelzel 1991) at –20 °C. Genomic DNA was extracted from the tissue following standard phenol–chloroform procedures (Davis *et al*. 1986). A genomic library was isolated from the DNA extracted from the liver of a juvenile female dolphin. The library was screened for microsatellites following the method described by Rassmann *et al*. (1991). DNA was digested with *Hae*III, *Bsu*RI, *Hpa*I, and *Eco*RV (all Promega) and size selected. Then 400–600 base pair (bp) fragments were ligated into a pUC18 vector (Pharmacia). After ligation and transformation, the library was screened for CA-repeats using a radioactively labelled CA_{250}-polymer (Pharmacia). Plasmid DNA from putative positive colonies was prepared and sequenced with the dye-terminator kit according to the manufacturer's instructions (Perkin Elmer) on a GeneAmp® PCR System 9600 (Perkin Elmer). Sequencing fragments were detected on an ABI 377 Automated Sequencer (Perkin-Elmer). Primers were designed using the program PRIMER 0.5 for Macintosh (Whitehead Institute for Medical Research). The primer sets were tested for the ability to produce a single, clear polymerase chain reaction (PCR) product in a 0.8% agarose gel in 1× TBA buffer. Sets fulfilling these criteria were then screened for the level of polymorphism and the suitability for cross-amplification in different odontocetes. One primer of each pair was fluorescently labelled for the detection on an ABI 377 Sequencer.

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Table 1 Primer sequences (5′ to 3′) and characteristics for five polymorphic loci. *primer labelled, T_a = annealing temperature, n = number of chromosomes screened (in *Tursiops aduncus*), H_E = expected heterozygosity, H_O = observed heterozygosity

Locus	Repeat motif	Primer sequences (5′–3′)	T_a (°C)	Size range (bp)	Size of cloned allele	No. of alleles	n	H_E	H_O	GenBank Accession no.
MK3	$(A)_9TAC(GT)_{15}AT(GT)_7$	*TGCATTCATGTAAAGGTGCG CTGCAACTAGAGAAAGCCCG	49	139–171	159	11	672	0.68	0.62	AF237889
MK5	$(TG)_{13}CT(TG)_2CA(TG)_2$ $(TA)_2(TG)_4$	*CTCAGAGGGAAATGAGGCTG TGTCTAGAGGTCAAAGCCTTCC	50	201–221	205	10	710	0.76	0.79	AF237890
MK6	$(GT)_{17}$	*GTCCTCTTTCCAGGTGTAGCC GCCCACTAAGTATGTTGCAGC	50	145–189	147	23	714	0.89	0.87	AF237891
MK8	$(CA)_{23}$	*TCCTGGAGCATCTTATAGTGGC CTCTTTGACATGCCCTCACC	56	87–119	109	11	692	0.71	0.76	AF237892
MK9	$(CA)_{17}$	*CATAACAAAGTGGGATGACTCC TTATCCTGTTGGCTGCAGTG	49	168–180	172	7	456	0.75	0.71	AF237893

Table 2 Amplification characteristics of selected microsatellite loci for two other odontocetes species. n = number of chromosomes screened

Species	Locus	n	No. of alleles
Stenella coeruleoalba	MK5	198	16
	MK6	204	16
	MK9	202	9
Pontoporia blainvillei	MK5	26	4
	MK6	25	5
	MK8	26	4

The PCR was carried out using 30–50 ng template DNA in a 10-μL reaction. The reaction mixture contained 0.5 U of Ampli-*Taq*® polymerase (Perkin Elmer), 5 pmol of each primer, 5 μmol dNTPs (Pharmacia), 15 μmol $MgCl_2$, 0.2 μL DMSO, 0.3 μL of 100× BSA (Bio101), and 1 μL of 10× Ampli-*Taq* buffer (Perkin Elmer). PCRs were carried out on a PTC-100™ Programmable Thermo Controller (MJ Research). We used the following PCR profile to amplify all five loci: 94 °C for 3 min, followed by 10 cycles at 92 °C for 30 s, 60 °C with a 1 °C decrease per step for 30 s, and 72 °C for 60 s. Then followed 24 cycles at 92 °C for 30 s, annealing temperature for 30 s, and 72 °C for 60 s. PCR reactions terminated with 10 min at 72 °C. The annealing temperatures are given in Table 1, which also shows the amplification characteristics of the five loci when tried on *T. aduncus*.

We also tried selected sets of our primers on two other odontocetes; striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea and franciscana dolphins (*Pontoporia blainvillei*) from the Southern coast of Brazil. The number of alleles for each locus is given in Table 2. Our tests show that the loci presented here exhibit polymorphisms in at least two widely divergent families in the suborder Odontoceti (Pontoporiidae and Delphinidae, respectively). Since microsatellite loci have proven to be highly conserved across cetacean species (Schloetterer *et al*. 1991; Valsecchi & Amos 1996), the loci shown here offer the opportunity of expanding the panel of markers suitable for a variety of cetacean population studies.

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