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TECHNICAL NOTE

Isolation and characterization of microsatellite DNA markers for spinner dolphin (*Stenella longirostris*)

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Abstract Practically no studies on the population genetics of the spinner dolphin (*Stenella longirostris*) exist. Seventeen pairs of DNA primers, cloned from an *Mbo I* digestion of *S. longirostris* liver DNA, were selected from a total of 288 sequences. Eight polymorphic microsatellite DNA markers were selected from the 17 primer pairs following amplification of DNA from skin samples of 65 spinner dolphins. Characterization of the polymorphisms revealed between three and nine alleles per loci. The observed heterozygosity ranged from 0 to 0.6032, while the expected heterozygosity ranged from 0.5834 to 0.73. Seven of the eight designed primer pairs amplified DNA from three other delphinid species. There was a marked low observed heterozygosity in the spinner dolphin suggesting a high level of inbreeding within this species in the southern Atlantic.

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Introduction

Microsatellite DNA markers or SSRs (*Simple Sequence Repeats*) have become an important tool in genetic studies of different cetacean populations due to its high polymorphism (Escorza-Treviño et al. 2005; Adams and Rosel 2006; Pomilla and Rosenbaum 2006). Lately, the development of microsatellites for cetaceans has been used for bottlenose dolphins (Caldwell et al. 2002; Rosel et al. 2005), for short-beaked common dolphin (Coughlan et al. 2006), and for striped dolphins (Mirimin et al. 2006). However, in spite of the fact that cetaceans belong to a phylogenetically diverse group, with 40 genera and 84 species, available genetic markers are still scarce.

Spinner dolphins (*Stenella longirostris*) are pelagic delphinids that live mainly in tropical and subtropical waters, with a wide, circumtropical distribution. The genetics of *S. longirostris* is poorly known. To assess the genetic diversity of a spinner dolphin population in Brazil, eight microsatellite markers were developed for this species. These markers were used in the first genetic study with *S. longirostris* in the South Atlantic (Farro 2006).

Materials and methods

Spinner dolphin liver sample for DNA extraction (GenomicPrep™ Isolation Kit, AMERSHAM BIOSCIENCES) was removed from a stranded dolphin found in Fernando de Noronha Archipelago, northeast Brazil (03°50′ S,

32°25′ W) in 1999. DNA (250 ng) was digested with *Mbol* enzyme. Fragments of between 400 and 1,000 bp were selected and cut from 0.8% agarose gel. Band purification was carried out with GFX™ PCR DNA and Gel Band Purification kit (AMERSHAM BIOSCIENCES). Extracted fragments were linked to Short: 5′-CAGCCTAGAGCCGAAT TCACC-3′ or Long: PO_4-5-GATCGGTGAAT TCGGCT CTAGGCC-3′ adapters (10 μM) using a T4 ligase enzyme (3 U/μl).

DNA fragments, linked to adapters, were then selected for cloning by hybridization to biotin-labeled oligonucleotide probes (AC repeats) which were then isolated by binding to Streptavidin-labeled magnetic beads (DYNAL). Cloning was carried out using T_4 ligase and pGEM®-T (EasyVector System, PROMEGA) and *DH5α* competent cells. Following selection of transformed cells containing plasmids with inserts, polymerase chain reactions (PCR) with M13 universal primers were performed. A total of 307 clones were selected, and of these 288 were sequenced using an ABI 3100 DNA sequencer (APPLIED BIOSYSTEMS). Primer sequence selection and design were carried out using Web TROLL (http://wsmartins.net/webtroll/troll.html) and GENERUNNER (http://www.generunner.com) programs.

PCR was carried out using standard conditions with 0.42 μM of primers and 0.75 U of polimerase *Taq* (INVITROGEN). Amplification conditions were 95°C for 5 min, followed by 32 cycles of 30 s each at 94°C, 20 s at the annealing temperature, and 72°C for 20 s; with final extension at 72°C for 10 min. Annealing temperatures varied according to the different primer pairs used.

Fragments were separated in 8% polyacrilamide gels and were visualized using silver-staining. A total of 104 clones containing microsatellite DNA sequences were obtained. From these, 17 pairs of primers were tested for amplifying dolphin microsatellite DNA. The selected primer pairs were used to amplify DNA from 65 spinner dolphin skin samples collected at Fernando de Noronha. POPGENE program, version 1.32 (Yeh et al. 1999), was used to determine observed and expected heterozygosity, number of alleles and Hardy–Weinberg Equilibrium ($P < 0.05$). With GENEPOP, version 3.4 (http://genepop.curtin.edu.au), the linkage disequilibrium between the loci was tested.

Results

Among the designed primer pairs, eight were able to amplify dolphin microsatellite DNA (Table 1). All the amplified loci showed a significant deviation of Hardy–(*P*-value = 0.00, except Slo15 with 0.002), probably due to the high homozygosity present in this spinner dolphin population (Farro 2006). Number of alleles varied from three to nine, and the observed heterozygosity varied from

Table 1 Primer sequences and some characteristics for eight spinner dolphin microsatellites loci

Loci	EMBL accession number	Primer sequence (5′ to 3′)	Repeat motif	T_a (°C)	N_a	N	Size range (bp)	H_o	H_E
Slo1	AM490782	F-CAAACCAAAAGCAAACACACAC	$(AC)_{26}$	63	9	58	140–166	0.138	0.721
		R-CATCTCTATCAGCCATGTCCAA							
Slo3	AM490784	F-CAAGCTCACAATTAGGTCTCCC	$(TC)_9$	54	4	52	270–276	0.000	0.689
		R-CTTCCCTTAACTCCTCCACAGA							
Slo4	AM490785	F-TAAGGTGAGAGGAGGTGGTGAT	$(CA)_6$ $(TA)_2$ CATGC $(AC)_3$ $(ATGC)_2$	55	6	64	212–226	0.156	0.673
		R-CACGGTTGAGAGAATACAGGAA	$(AC)_6$ $(GCAC)_4$ $(AC)_4$						
Slo9	AM490786	F-CCTTCTCCTCCTTCTGTCCTTT	$(TG)_6$ C $(GT)_8$	59	9	62	144–164	0.258	0.726
		R-CTTCCCTCACTTTCCCTACCC							
Slo13	AM490787	F-AAACCTTGACATTTCTTTCCCC	$(CA)_2$ GA $(CA)_4$	63	3	57	226–230	0.000	0.627
		R-TCTGAGTGCTGTGTGTGTGTGT	$(C)_3$ $(CA)_2$						
Slo14	AM490788	F-GCAGGCAGAGAGAAGAAAGAAG	$(GA)_6$ G $(GA)_2$	59	4	65	116–122	0.000	0.583
		R-GTGAAAGGAGGGTGGCATT							
Slo15	AM490789	F-CGTCAAACTCCATCAAGACATC	$(TG)_5$ TACG $(TG)_2$	54	9	63	218–252	0.603	0.73
		R-ATCTCCACCACAAGACACCAC							
Slo16	AM490790	F-GAGGTGAGAGAAAGGAGACGAG	$(GA)_3$ CA $(GA)_4$ CA $(GA)_2$ ... CAGA	54	5	60	158–166	0.100	0.631
		R-GTCCTCGGGTTTAGTTCTTCTG	$(CA)_4$ $(GA)_3$ $(CA)_2$ $(GA)_3$						

Ta, annealing temperature; Na, number of alleles; N, number of genotyped individuals; H_o, observed heterozygosity; H_E, expected heterozygosity; EMBL, European Molecular Biology Laboratory

Table 2 Cross-species amplification data of eight microsatellites developed for spinner dolphin (*Stenella longirostris*)

Locus	*Stenella clymene*	*Sotalia guianensis*	*Pseudorca crassidens*
Slo1	+	+	+
Slo3	+	+	+
Slo4	+	−	+
Slo9	+	+	+
Slo13	−	−	−
Slo14	+	+	+
Slo15	−	+	+
Slo16	−	+	−

(+) amplification; (−) no amplification

0 to 0.6032, while the expected heterozygosity varied from 0.5834 to 0.73, (Table 1). Allele size range varied from 116bp to 276bp. No linkage disequilibrium was detected among the various loci.

In a cross-species DNA amplification (one individual from each species), seven primer pairs from the original eight designed for spinner dolphins also amplified DNA from three other delphinid species (*Stenella clymene*, *Sotalia guianensis*, and *Pseudorca crassidens*, Table 2).

Discussion

These microsatellite primer pairs were the first obtained for *Stenella longirostris*, and studies including the analysis of these markers can lead to a better comprehension of the genetic variability and population dynamics for this species so as to help in the development of conservation management plans.

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References

Adams LD, Rosel PE (2006) Population differentiation of the Atlantic spotted dolphin (*Stenella frontalis*) in the western North Atlantic, including the Gulf of Mexico. Mar Biol 148:671–681

Caldwell M, Gaines MS et al (2002) Eight polymorphic microsatellite *loci* for bottlenose dolphin and other cetacean species. Mol Ecol Notes 2:393–395

Coughlan J, Mirimin E et al (2006) Isolation and characterization of novel microsatellite *loci* for the short-beaked common dolphin (*Delphinus delphis*) and cross-amplification in other cetacean species. Mol Ecol Notes 6:490–494

Escorza-Treviño S, Archer FI et al (2005) Genetic differentiation and intraspecic structure of eastern tropical pacific spotted dolphins, *Stenella attenuata*, revealed by DNA analyses. Conserv Genet 6:587–600

Farro APC (2006) Genetic variability of spinner dolphins *(Stenella longirostris)* revealed through microsatellite markers. PhD Thesis. Universidade Estadual Paulista (UNESP), Botucatu, SP, Brasil

Mirimin L, Coughlan J et al (2006) Tetranucleotide microsatellite *loci* from the striped dolphin (*Stenella coeruleoalba* Meyen, 1833). Mol Ecol Notes 6:493–495

Pomilla C, Rosenbaum HC (2006) Estimates of relatedness in groups of humpback whales (*Megaptera novaeangliae*). Mol Ecol 15:2541–2555

Rosel PE, Forgetta V et al (2005) Isolation and characterization of twelve polymorphic microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). Mol Ecol Notes 5:830–833

Yeh FC, Yang RC, Boyle T (1999) POPGENE version 1.31. Microsoft windows-based freeware for population genetic analysis. University of Alberta, Edmonton, AB, Canada