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TECHNICAL NOTE

Development and characterization of 10 polymorphic microsatellite loci for the blue shark, *Prionace glauca*, and their cross shark-species amplification

Seán Fitzpatrick · Mahmood S. Shivji · Demian D. Chapman · Paulo A. Prodöhl

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Abstract Ten polymorphic nuclear microsatellite loci were developed from a microsatellite enriched genomic library of the blue shark, *Prionace glauca*. The utility of these markers for genetic studies of this globally distributed, heavily exploited, oceanic predator was assessed by screening 120 specimens sampled from six locations throughout the species' range. Both moderately and highly polymorphic marker loci were identified. Three to 35 alleles were found to be segregating per locus (mean 10.1) with observed heterozygosities ranging from 24 to 91%. Evaluation of the cross-species amplification of these markers across 18 additional shark species indicates that these microsatellites are potentially useful for genetic studies of other species of conservation concern.

Keywords Blue shark · *Prionace glauca* · *Carcharhinus* · Polymorphic · Microsatellite · Cross-species

S. Fitzpatrick · P. A. Prodöhl (✉)
School of Biological Sciences, Medical Biology Centre, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK
e-mail: p.prodohl@qub.ac.uk

M. S. Shivji · D. D. Chapman
Guy Harvey Research Institute and Save Our Seas Shark Centre, Oceanographic Centre, Nova South-Eastern University, 8000 North Ocean Drive, Dania Beach, FL 33004, USA

Present Address:
D. D. Chapman
School of Marine and Atmospheric Science, Institute for Ocean Conservation Science, Stony Brook University, Stony Brook, NY 11794-5000, USA

The blue shark, *Prionace glauca*, is the most-wide ranging of the open ocean sharks (Compagno 1984). As an apex predator, *P. glauca* arguably functions as a keystone species and may play a vital role in the stability of pelagic marine ecosystems (Litvinov 2006). Blue sharks make up the highest proportion by species in the global fin trade (Clarke et al. 2006), and given indications of severe regional declines due to heavy fishing, this species is listed as Near Threatened by the International Union for Conservation of Nature (IUCN) Red List. Conservation and management of fisheries requires information regarding population genetic structuring and breeding biology of the species involved. Presently, microsatellite markers provide the best means of obtaining these data for species difficult to study due to the fact that they live in environments that prohibit direct observation. In comparison to many species, however, microsatellite marker development for sharks has proven difficult. This has been attributed to a comparatively low abundance of these loci in the genomes of these ancient fishes (Heist and Gold 1999). Where microsatellite development has been successful, however, they have proven informative for addressing a broad range of conservation and management issues (e.g. Schrey and Heist 2003, Chapman et al. 2004, 2007, DiBattista et al. 2008, Feldheim et al. 2010). Here we describe the isolation and initial characterisation of ten novel *P. glauca* microsatellites, and their cross-amplification and potential utility for the study of additional shark species.

Genomic DNA was extracted from fin tissue of five blue sharks following the protocol described by Taggart et al. (1992). Isolation of microsatellites followed the enrichment protocol of Kijas et al. (1994) with modifications as summarised in Boston et al. (2009) and McInerney et al. (2009). Recombinant cells were screened for microsatellites by southern blot hybridisation with the [P^{32}] end-labelled

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Table 1 Summary details of ten *P. glauca* microsatellites, including: locus ID; microsatellite repeat motif; primer sequences; concentration (pM) and $MgCl_2$ (mM) concentrations; Tm—annealing temperature (°C); number of PCR cycles; allele size range (base pairs); *nA*—number of alleles; Ho and He—observed and expected heterozygosity

Locus ID	Repeat motif	Primer sequence	Conc. (pM)	$MgCl_2$ (mM)	Tm (°C)	No. cycles	Size range (bp)	*nA*	Ho	He
Pgla-01	$(TCC)_7$-$(TCC)_3$ TCG$(TCC)_5$	*F: TTGATCTCGTCCATCTCCTTGTAG	2.0	1.5	60	26	195–210	6	0.24	0.27
		R: ACCTGACGGTCATCAACATCAA								
Pgla-02	$(TCC)_5$TCG$(TCC)_2$ $(TCG)_2$	*F: ACCCGACTCGCCAGGATTCACT	1.0	1.5	56	22	124–148	9	0.77	0.76
		R: CCCGAGTCACTCACCGC								
Pgla-03	$(GGA)_3$AAA$(GGA)_4$TGA$(GGA)_2$	*F: TATGGTGGTGTGCACAAGCAAGAG	1.5	1.5	56	22	180–192	4	0.55	0.60
		R: GACCCTTACCGTCACCAG								
Pgla-04	$(TCT)_4(TCC)_6$	*F: TGCCTCCAGAGGCCTTGGACG	1.5	1.5	55	24	215–224	3	0.60	0.64
		R: TGACCAGCCAGGCGTCCCTGAGG								
Pgla-05	$(GT)_{27}(GA)_{19}$	*F: CAGATTCCTGTGTGGAGCACA	1.5	1.5	56	22	186–260	35	0.91	0.95
		R: CTTCGTGTTGGGGGATAGTGG								
Pgla-06	$(CA)_2(GA)_{10}CA$	F: CTTTCGATGGTCTTTTGATGG	3.0	1.5	56	22	117–141	10	0.28	0.36
		*R: GTAGGAGACAAGCTCGCAGAGAA								
Tailed primers										
Pgla-07	$(TCC)_{14}$	F: CAGGCCCTAGTGACCAAAGT	2.0	1.5	57	22 N	199–232	9	0.55	0.54
		**R: TTTTGGAAGGGGGACGACAT	0.4							
		IRD-CAG_tail: CAGTCGGGCGTCATCA	1.6							
Pgla-08	$(TCC)_7(TCC)_5$	F: CCTTCAACTTCCGGCTGGTGTT	4.0	2.0	60	19 N	179–206	8	0.75	0.68
		**R: CTCTTCCCGCATTGTCATCGTGT	0.8							
		*M13R_tail: GGATAACAATTTCACACAGG	3.2							
Pgla-09	$(GGA)_6(GAA)_5$	**F: AGCCGCTCACTCACTCTGC	4.0	1.5	56	25	133–148	6	0.37	0.36
		R: GATCTGCCGCTTTATTTTTCTTG	0.8							
		*CAG_tail: CAGTCGGGCGTCATCA	3.2							
Pgla-10	$(GA)_2CA(GA)_7$ $(GACA)_3CAGACA$ $(GA)_{13}$	**F: GGGACTGTGAGGCAGCAG	4.0	1.5	55	22	139–159	11	0.83	0.78
		R: GCCCTCTAGGGATGGGTTAC	0.8							
		*Godde_tail: CATCGCTGATTCGCACAT	3.2							

* Fluorescently labelled primer. ** Tailed primer. In four instances screening was performed using tailed primers. In these cases, one of the species-specific primers was synthesised with a short universal flanking sequence on the 5′-end (M13R, CAG, or Godde tails). During PCR amplification, three primers were used in each reaction to amplify these loci, consisting of: the tailed-species-specific primer, the fluorescently labelled complement to the universal tail and the remaining primer of the locus specific pair. The respective concentration of primers and tails, are provided above

Table 2 Summary results from cross-amplification testing of ten *P. glauca* microsatellites across 18 shark species

Common name	Species name	No. sharks screened	No. of alleles observed at each locus									
			Pgla-01	*Pgla*-02	*Pgla*-03	*Pgla*-04	*Pgla*-05	*Pgla*-06	*Pgla*-07	*Pgla*-08	*Pgla*-09	*Pgla*-10
Bignose shark	*Carcharhinus altimus*[a]	1	1	–	1	1	–	1	1	–	1	2
Blacknose shark	*Carcharhinus acronotus*[a]	4	1	3	1	1	1	1	1	–	–	5
Blacktip shark	*Carcharhinus limbatus*[a]	4	2	3	1	2	5	1	–	–	1	4
Blacktip reef shark	*Carcharhinus melanopterus*[a]	5	–	3	1	2	3	1	–	–	–	5
Bull shark	*Carcharhinus leucas*[a]	3	–	1	1	1	3	1	1	–	–	1
Caribbean reef shark	*Carcharhinus perezi*[a]	12	1	2	2	1	10	1	1	1	1	2
Dusky shark	*Carcharhinus obscurus*[a]	4	2	3	1	–	3	1	1	2	1	1
Sandbar shark	*Carcharhinus plumbeus*[a]	3	2	3	–	1	4	–	2	–	1	1
Silky shark	*Carcharhinus falciformis*[a]	4	4	3	1	2	4	1	1	3	2	3
Spinner shark	*Carcharhinus brevipinna*[a]	4	1	2	1	2	2	1	–	–	1	2
Oceanic whitetip shark	*Carcharhinus longimanus*[a]	4	2	4	–	1	1	–	1	1	2	1
Tiger shark	*Galeocerdo cuvier*[a]	4	–	–	–	–	–	–	–	–	–	–
Lemon shark	*Negaprion brevirostris*[a]	4	–	–	–	–	–	–	–	–	–	–
Scalloped hammerhead	*Sphyrna lewini*[b]	3	1	4	–	–	–	–	–	–	1	–
Great hammerhead	*Sphyrna mokarran*[b]	15	1	3	–	–	–	–	–	–	1	–
Smooth hammerhead	*Sphyrna zygaena*[b]	10	1	5	–	–	–	–	–	–	1	–
Great white shark	*Carcharodon carcharias*[c]	5	–	–	–	–	–	–	–	–	–	–
Porbeagle shark	*Lamna nasus*[c]	5	–	–	–	–	–	–	–	–	–	–

"–" indicates PCR was unsuccessful and no product was observed

[a] Family Carcharhinidae

[b] Family Sphyrnidae

[c] Family Lamnidae

oligonucleotides $(GATA)_4$, $(GACA)_4$, $(GGAT)_4$, $(GAA)_5$, and $(GGA)_5$ following Prodöhl et al. (1996). Screening of ~2,500 colonies yielded 182 positives, which were sequenced (Macrogen Inc.) using T3 and T7 universal primers. PCR primer sets for 43 sequences containing microsatellites with sufficiently large, unique flanking regions were designed using PrimerSelect™ (Lasergene 4.0, DNAStar Inc.). From the initial primer sets tested, 16 were found to consistently amplify PCR products of expected size. Six microsatellite loci were found to be monomorphic and omitted from subsequent evaluation. The remaining 10 microsatellites (Table 1) displayed varying levels of polymorphism and were used to screen 120 blue sharks obtained from the Atlantic, Indian, and Pacific Oceans.

PCR amplification for genotyping was conducted in 12 µl reaction volumes containing 1× Promega *Taq* polymerase buffer, 1.5–2.0 mM $MgCl_2$, 100 µM dNTPs, 0.2–4.0 pM of each microsatellite primer (Table 1), 50 ng of template DNA and 0.5 U of Promega *Taq* DNA polymerase. Cycling conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 21–27 cycles of 95°C for 1 min, 54–60°C for 1 min, and 72°C for 1 min. In some instances (i.e. with loci *Pgla*-07 and *Pgla*-08) a nested PCR was required due to the use of fluorescently tailed primers which had different annealing temperatures to the selective primers. Nested PCRs were performed as detailed above, but included an additional five cycles at 95°C for 1 min, 53°C for 1 min, and 72°C for 1 min.

Following PCR, 6 µl of stop solution (95% Formamide, 10 mM NaOH, 10 mM EDTA, 0.01% Pararosaniline) was added to each 12 µl reaction. Reactions were then denatured at 90°C for 5 min, and 0.25 µl loaded onto 25 cm 6% 1× TBE polyacrylamide gels containing 6 M Urea mounted on a Li-Cor dual laser automated DNA analyser. A size ladder for the Li-Cor system (MicroStep-20a from Microzone—UK) was run adjacent to the samples to size allelic fragments. Gels were run at 40 W at a temperature of 50°C for 1.5–2.5 h. Genotyping was conducted using the SAGA genotyping software (Li-Cor Inc.). Control samples of known allele size were used on each run to ensure consistent allele calling.

All 10 *P. glauca* microsatellites resolved exceptionally well allowing consistent and reproducible allele calling. All ten loci were polymorphic, displaying 3–35 alleles per locus (mean 10.1), with observed and expected heterozygosities ranging between 24–91% and 27–95% (Table 1). Tests for deviation from Hardy–Weinberg equilibrium (HWE) and non-random association of alleles among different loci (i.e. linkage disequilibrium) were conducted using GENEPOP '007 (Rousset 2008). All loci conformed to HWE, and no significant associations of alleles between loci were observed. These results highlight the potential utility of these markers for *P. glauca* population and parentage studies. Cross-amplification of *P. glauca* microsatellites was investigated in 18 additional shark species. PCR and cycling conditions for cross-species amplification were identical to those for *P. glauca* amplification. Summary results (Table 2) indicate successful cross-amplification in the more closely related (Carcharhinidae) species, suggesting that these *P. glauca* microsatellites may also be valuable for genetic studies of other shark species of conservation concern.

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