Isolation and characterization of microsatellite loci for storm-petrels

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Abstract

Primers were developed for 10 microsatellite loci for two species of *Oceanodroma* storm-petrels. Variability was tested in 27 *O. castro* and 22 *O. monteiroi* from the Azores, and 24 *O. leucorhoa* from Norway. At least six loci amplified reliably and were polymorphic in each species. The number of alleles per locus averaged 4.6, and observed heterozygosities averaged 0.41. Most primers also yielded polymerase chain reaction products in *O. tethys*, *O. hornbyi* and *Pterodroma phaeopygia*. These loci are being used to assay population genetic structure in storm-petrels.

Keywords: Hydrobatidae, microsatellites, *Oceanodroma*, Procellariiformes

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Whether sympatric populations can form reproductively isolated species is controversial (reviewed in Coyne & Orr 2004). One way sympatric speciation could occur is through segregation of individuals by breeding time (Hendry & Day 2005). At several colonies of band-rumped (*Oceanodroma castro*) and Leach's storm-petrels (*O. leucorhoa*; Procellariiformes: Hydrobatidae), different populations breed in different seasons (Monteiro & Furness 1998). Hypervariable nuclear markers are needed to test whether seasonal populations are genetically isolated species that arose sympatrically.

A genomic library was generated from seven *O. castro* frozen blood samples from Japan (Friesen *et al*. 2007) using enrichment cloning (Glenn & Schable 2005). Five micrograms DNA was digested with the *Alu*I restriction enzyme (Fermentas) to create fragments ranging from 200 to 1200 base pairs. Fragments were dephosphorylated, cleaned using a QIAquick Gel Extraction Kit (Qiagen) and ligated to universal SNX linkers (Glenn & Schable 2005). Ligated product was enriched by hybridization with a cocktail of three biotinylated oligonucleotide probes: $(AC)_{13}$, $(AG)_{13}$ and $(GAA)_8$. Hybridized DNA was isolated using Streptavidin-coated DynaBeads (Dynal Biotech). Microsatellite-enriched DNA was amplified using the SNX-F primer, ligated into PCR 2.1-TOPO vector (Invitrogen), and transformed into TOP 10 One Shot *Escherichia coli* competent cells (Invitrogen) according to the manufacturer's protocol. Cells were grown on ampicillin-treated agar plates and screened for microsatellites using a DIG fluorescence kit (Roche Diagnostics) according to the manufacturer's protocols.

A library of 96 serially enriched clones was compiled, of which 53 were sequenced. Bacterial colonies were lysed by boiling, and the inserts were amplified from ~5 ng DNA in 40 μL 1× buffer with 1.5 mM $MgCl_2$ (Qiagen), 0.15 mM each dNTP, 1 U *Taq* DNA polymerase (Qiagen), and 0.25 μM each M13 forward and reverse primers. Reaction mixtures were denatured at 95 °C for 5 min, followed by 35 cycles of 95 °C for 20 s, 50 °C for 20 s and 72 °C for 60 s, and a final extension at 72 °C for 10 min in a T-Gradient thermal cycler (Biometra). PCR products were sequenced with M13 primers on an ABI 3730XL automated sequencer using ABI BigDye terminator chemistry (Genome Québec).

Forty-six clones contained microsatellites, 20 of which were compound or interrupted repeats. Nine had nine or more simple repeats and sufficient flanking sequence for primer design. Primers for these were designed using the Primer 3 software (Rozen & Shaletsky 2000; Table 1).

A genomic library also was generated from two *O. leucorhoa* frozen tissue samples from Newfoundland, and was screened for dinucleotide repeats following Ibarguchi *et al*. (2000). Twenty-one clones were sequenced, of which only two contained nine or more repeats and sufficient flanking sequence for primer design. Primers were designed for both loci, but PCR products could only be obtained for one (Table 1).

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Table 1 Characteristics of 10 microsatellite loci in three species of seabirds, including repeat motifs, GenBank Accession numbers, primer sequences, numbers of samples that amplified (N_S), number of alleles (N_A), allele size ranges (in base pairs), and expected (H_E) and observed (H_O) heterozygosities. Number of individuals screened is in parentheses after species names. Heterozygosities in bold deviate from Hardy-Weinberg expectations at $\alpha = 0.05$ after Bonferroni corrections for multiple tests. Loci with names beginning with 'Oc' were isolated from *O. castro*; locus Ol10-39 was isolated from *O. leucorhoa*

				O. castro (27)			*O. monteiroi* (22)			*O. leucorhoa* (24)		
Locus	Repeat motif	GenBank Accession no.	Primer sequence (5'–3') (forward/reverse)	N_S N_A	Allele size range	H_E H_O	N_S N_A	Allele size range	H_E H_O	N_S N_A	Allele size range	H_E H_O
Oc28B	$(TG)_9$	FJ238097	GCGTTGACCAGCAAATAGTC	27	181–187	0.50	22	181–191	0.49	6	183	na
			GGAAGGCTACCACAATTTCA	3		0.48	3		0.50	1		
Oc49	$(CA)_{10}$	FJ238098	TGCTTCTGGATTTGTTTTGC	27	175	na	22	171–179	0.44	24	184–187	0.39
			CAAACCGTGTGACCCAGATA	1*			3		0.41	3		**0.17**
Oc51	$(AG)_{16}$	FJ238099	TCCAATGGAAAACAGAGATAGTA	27	289–307	0.71	22	298–306	0.66	23	300–308	0.04
			TATGCTGCCCAACTTCACAG	11		**0.96**	6		**0.95**	2		0.04
Oc63	$(GA)_{17}$	FJ238100	TCACACCAACCTCCATGAAA	27	191–197	0.52	22	178–197	0.81	23	190–206	0.66
			AACGGGGAATATGTGGTTCTT	4		0.48			0.82	4		0.61
Oc64B	$(TG)_{10}$	FJ238101	GCTGGGAAATGCTGAGACA	27	246–252	0.48	22	242–254	0.64	ur	—	—
			GATTTTAGCAGCACTTGGTCA	4		0.48	6		0.73			
Oc79	$(AC)_{13}$	FJ238102	TATTCCTGGCCATGTTTTCC	27	212–214	0.51	22	212–214	0.09	23	204	na
			GCTTCCTTTGTTCAGTTTAGA	2		0.48	2		0.09	1†		
Oc79-2	$(AAG)_7$	FJ238105	AGTGGGATGGGGAGAGAATC	27	224–228	0.04	22	222–234	0.39	ur	—	—
			CATTGGTTGGTTGGTGATGA	3		0.04	4		**0.09**			
Oc84‡	$(AG)_9$	FJ238103	CCTTTTTCCAGGCAGACAAA	27	317–321	0.39	15	317–323	0.25	24	317–325	0.53
			AGTTCAAGGGCAACCTTGTG	3		**0.08**	4		0.27	3		0.58
Oc87B	$(GA)_{12}$	FJ238104	TTTAAGGAACGCAAGTCAGG	27	280–286	0.04	22	276–286	0.76	16	280–294	0.79
			GTGATTCTTGCAGTGGCTTT	3		0.04	6		0.68	9		**0.63**
Ol10-39	$(GT)_{11}$	FJ238106	TTAAGAACAGAGCCTGACTTG	27	135–151	0.80	22	135–153	0.77	24	151–155	0.57
			ACAAAATCTCATGTCCTTGG	8		**0.59**	8		0.59	3		0.50

ur, unreliable amplification; na, not applicable (no variation).
*Variable in a larger sample of *O. castro* (Friesen *et al*. 2007).
†Variable in a larger sample of *O. leucorhoa* (A. Bailie, unpublished data).

The variability of these loci was tested on frozen blood samples from 27 *O. castro* and 22 *O. monteiroi* (Monteiro's storm-petrel; Bolton *et al*. 2008) from the Azores (Friesen *et al*. 2007), and 24 *O. leucorhoa* from Røst, Norway (A. Bailie, unpublished data). Cross-species amplification also was tested on one *O. tethys* (wedge-rumped storm-petrel) feather and one *O. hornbyi* (Hornby's storm-petrel) feather, and six *Pterodroma phaeopygia* (Galapagos petrel; Procellariiformes: Procellariidae; Friesen *et al*. 2006) blood samples. DNA was extracted by protease digestion (Smith *et al*. 2007). For loci developed from *O. castro*, samples were amplified as described in Friesen *et al*. (2007). Amplification was confirmed on 1% agarose gels, then products were sized on a Beckman-Coulter CEQ 8000 automated sequencer with the Beckman-Coulter GenomeLab DNA Size Standard Kit 400. Alleles were scored using Genetic Analysis System Software (Beckman-Coulter). For locus Oc10-39, samples were screened following protocols in Ibarguchi *et al*. (2000).

All 10 loci could be scored reliably in *O. castro* and *O. monteiroi*, and eight could be scored reliably in *O. leucorhoa* (Table 1). One locus that was monomorphic in *O. castro* and one that was monomorphic in *O. leucorhoa* were polymorphic in a broader geographical screening (Friesen *et al*. 2007; A. Bailie, unpublished data). The remaining loci had up to 11 alleles (mean = 4.6). Observed heterozygosities (H_O) were significantly higher than expected heterozygosities for locus Oc51 for both *O. castro* and *O. monteiroi*, suggesting the possibility of nonspecific amplification. Otherwise, there were no consistent deviations from Hardy–Weinberg expected frequencies either within loci or within species (mean $H_O = 0.41$), and no deviations from linkage equilibrium were found after correction for multiple comparisons. Amplification products were obtained for all loci except Oc64B and Oc79-2 for *O. tethys*, *O. hornbyi* and *P. phaeopygia*; all but Oc49 were variable in *P. phaeopygia*.

Currently, we are using these loci to estimate population genetic structure and gene flow in *O. castro*, *O. monteiroi* (Friesen *et al*. 2007) and *O. leucorhoa* (A. Bailie, unpublished data).

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References

Bolton M, Smith AL, Gómez-Díaz E *et al*. (2008) Monteiro's storm-petrel *Oceanodroma monteiroi*: a new species from the Azores. *Ibis*, **150**, 717–727.

Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland, Massachusetts.

Friesen VL, González JA, Cruz-Delgado F (2006) Population genetic structure and conservation of the Galápagos petrel (*Pterodroma phaeopygia*). *Conservation Genetics*, **7**, 105–115.

Friesen VL, Smith AL, Gómez-Díaz E *et al*. (2007) Sympatric speciation by allochrony in a seabird. *Proceedings of the National Academy of Sciences, USA*, **104**, 18589–18594.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.

Hendry AP, Day T (2005) Population structure attributable to reproductive time: isolation by time and adaptation by time. *Molecular Ecology*, **14**, 901–916.

Ibarguchi G, Birt TP, Warheit KI, Boag PT, Friesen VL (2000) Microsatellite loci from common and thick-billed murres, *Uria aalge* and *U. lomvia*. *Molecular Ecology*, **9**, 629–644.

Monteiro LM, Furness RW (1998) Speciation through temporal segregation of Madeiran storm-petrel (*Oceanodroma castro*) populations in the Azores? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **353**, 945–953.

Rozen S, Shaletsky H (2000) Primer 3. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.

Smith AL, Monteiro L, Hasegawa O, Friesen VL (2007) Global phylogeography of the band-rumped storm-petrel (*Oceanodroma castro*; Procellariiformes: Hydrobatidae). *Molecular Phylogenetics and Evolution*, **43**, 755–773.

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Development of compound microsatellite markers in red-tide-causing dinoflagellate *Akashiwo sanguinea* (Dinophyceae)

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Abstract

We isolated 13 polymorphic microsatellites from the red-tide causing dinoflagellate *Akashiwo sanguinea*. These loci were highly variable, with between 2 and 10 alleles per locus, and estimated gene diversity ranging from 0.08 to 0.82. These loci have the potential to reveal genetic structure and estimate gene flow among *A. sanguinea* populations.

Keywords: *Akashiwo sanguinea*, microsatellite, red tide, SSR

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The unarmoured dinoflagellate *Akashiwo sanguinea* (Hirasaka) G. Hansen et Moestrup is a red-tide causing microalgae which frequently predominates marine environments worldwide in the autumn, and has been studied in Hong Kong (Lu & Hodgkiss 2004), the USA (Horner *et al*. 1997; Robichaux *et al*. 1998), Brazil (Domingos & Menezes 1998), the Black Sea (Gómez & Boicenco 2004), Peru (Kahru *et al*. 2004), Japan (Matsubara *et al*. 2007) and Korea (Lee *et al*. 2005). Experimental data has shown that *A. sanguinea* can cause mortality in abalone larva and spat (Botes *et al*. 2003). Several hypotheses have been put forward to account for their range expansion, including natural and/or human-associated dispersal of the vegetative cells. However, no genetic studies have been carried out to reveal population

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