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TECHNICAL NOTES

Isolation and characterization of ten microsatellite loci in Blue-footed (*Sula nebouxii*) and Peruvian Boobies (*Sula variegata*)

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Abstract Ten microsatellite loci were isolated and characterized from Blue-footed (*Sula nebouxii*) and Peruvian Boobies (*S. variegata*). The loci were screened in 24 Blue-footed Boobies and 27 Peruvian Boobies: 8 were polymorphic in Blue-footed Boobies with between 2 and 10 alleles per locus and 9 were polymorphic in Peruvian Boobies with between 2 and 12 alleles per locus. Observed heterozygosity ranged from 0.29 to 0.84. These loci were also tested in Brown Boobies (*S. leucogaster*) and were variably polymorphic. These new loci are currently being used to assess population genetic structure in Blue-footed and Peruvian Boobies and will also be used to examine hybridization between the species.

Keywords Cross-species amplification · Microsatellite · Population genetics · Speciation · Sulid

Introduction

Blue-footed (*Sula nebouxii*) and Peruvian (*S. variegata*) Boobies are large, plunge-diving seabirds in the family Sulidae. They breed in the Eastern Pacific from Mexico to Peru and from Peru to central Chile, respectively, overlapping on two islands in northern Peru (Nelson 1978). Evidence suggests that these sister species did not diverge from their common ancestor via the classic allopatric model of geographic speciation (Friesen and Anderson 1997). Their current distribution suggests instead that they may have diverged with gene flow via the parapatric model of geographic speciation. We isolated highly polymorphic microsatellite markers to address this hypothesis.

Methods

To isolate microsatellite loci, DNA was extracted from blood samples from three Blue-footed Boobies sampled on Isla Lobos de Tierra, Peru, in December 2006, and from three Peruvian Boobies, sampled on Isla Lobos de Afuera, Peru, in December 2007. Genomic libraries for each species were generated using enrichment cloning as in Sun et al. (2009). Basically, DNA from three individuals was mixed, and a total of five micrograms of DNA from each species was digested using the restriction enzymes *Rsa*1 and *Ssp*1 (Fermentas, Burlington, ON) to create DNA fragments ranging in size from 200 to 1,000 base pairs. The methods for dephosphorylation, ligation, enrichment, transformation and bacterial colony screening were carried out as described in Sun et al. (2009).

A library of 312 serially enriched clones was complied for each species; 63 of these were sequenced for Blue-footed Boobies and 92 were sequenced for Peruvian Boobies. Colony treatment, insert amplification, and sequencing of PCR products were carried out as in Sun et al. (2009).

Forty-two Blue-footed Booby clones contained microsatellites, of which 36 were simple dinucleotide repeats, 5 were simple repeats interrupted by one or more base pairs, and 1 was a compound repeat of two different dinucleotide

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Table 1 Characteristics of microsatellite loci for Blue-footed (*Sula nebouxii*) (*BFBO*) and Peruvian Boobies (*S. variegata*) (*PEBO*)

Locus Accession number	Primer sequence 5′-3′	Repeat motif in clone	BFBO					PEBO				
			Size (bp)	*n*	*k*	H_O	H_E	Size (bp)	*n*	*k*	H_O	H_E
Sn2B-68	F: TTCCATGTATAAACACAGACATC	$(CA)_{10}$	168–172	24	3	0.72	0.69	168–174	27	4	0.50	0.58
GU167925	R: CTCTTCTTCAATCCCTGTCA											
Sn2B-83	F: TCTGTTAACCAGAGGAAGGA	$(CA)_{10}$	178–184	24	4	0.64	0.66	178–182	27	3	0.40	0.48
GU167926	R: GAAAGAGGGGTCAGAGAAAT											
Sn2A-90	F: TTCTTTCCTGCTCACCTCTA	$(TC)_9$	204–206	24	2	0.29	0.44	204	27	1	N/A	N/A
GU167927	R: CCATTGGGAATGAGATAGTG											
Sv2A-47	F: GATGTTCCTTCTGGTGACAG	$(CA)_{11}$	291–299	24	5	0.56	0.64	285–297	27	6	0.67	0.81
GU167929	R: GCTCTTAATGACCCTAATG											
Sv2A-53	F: ATCTGCAGCTCCCATATTTA	$(AG)_{22}$	304–330	24	9	0.84	0.86	302–326	27	10	0.73	0.86
GU167930	R: CCATGACAGAAGAGATACACTG											
Sv2B-138	F: ATTATTTGCTCCATCACGTT	$(AC)_{14}$	330–360	24	10	0.83	0.89	322–362	27	12	0.81	0.86
GU167931	R: GTTAGGGGAACATTTGAACA											
Sv2A-26	F: GCACACCTAGCTCCAAATAC	$(TC)_{19}$	346–358	24	6	0.46	0.50	352–364	27	7	0.74	0.78
GU167932	R: TCATCCATCATATTCCTGCT											
Sv2A-152	F: TTCCTTCATCCTGACTCTTG	$(TC)_{16}$	282–284	24	2	0.38	0.50	276–284	27	4	0.77	0.60
GU167933	R: GTAAATCAGCCAAAATGCTC											
Sv2A-2	F: CCACACTTCTGTAAATGCAA	$(CA)_{20}$	238	24	1	N/A	N/A	234–240	27	4	0.70	0.64
GU167934	R: TTTGGTCTTCGATCTTGTTT											
Sn2A-123	F: TAGTTACCACCATGGCTTT	$(GA)_{10}$	176	24	1	N/A	N/A	176–180	27	2	0.33	0.28
GU167928	R: CTGAGCAGGAATCAATCTTC											

Forward primers were tagged with M13 (sequence not included)

F forward primer, *R* reverse primer, *n* number of individuals in which the locus was tested, *k* number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

Table 2 Characteristics of microsatellite loci when tested in the non-focal Brown Booby

Locus	Repeat motif in clone	Size (bp)	*n*	*k*
Sn2B-68	$(CA)_{10}$	170	4	1
Sn2B-83	$(CA)_{10}$	172–180	14	4
Sn2A-90	$(TC)_9$	203	4	1
Sv2A-47	$(CA)_{11}$	289–307	14	8
Sv2A-53	$(AG)_{22}$	302–332	14	4
Sv2B-138	$(AC)_{14}$	329–357	14	10
Sv2A-26	$(TC)_{19}$	346–362	14	8
Sv2A-152	$(TC)_{16}$	268	4	1
Sv2A-2	$(CA)_{20}$	213–237	14	4
Sn2A-123	$(GA)_{10}$	170–184	14	5
Sn2A-36[a]	$(TC)_{10}$	334	4	1
Sn2B-100[a]	$(CA)_{10}$	218–222	14	3
Sv2A-50[a]	$(CA)_{11}$	320	4	1
Sv2B-27[a]	$(CA)_{12}$	290–308	14	3
Sv2A-95[a]	$(TG)_{10}$	179–201	14	3

n number of individuals in which the locus was tested, *k* number of alleles

[a] These primers are not polymorphic in Blue-footed or Peruvian Boobies and are not included in Table 1. The sequences for these primers are as follows: Sn2A-36, *forward* AATGTGTTCTTGAC TTTGACA, *reverse* TTTTTCATCCACATCAAAGG; Sn2B-100, *forward* CATCCTTTCACTATCTTTGTTTG, *reverse* GACTCTAT TGCAGTGGTTCC; Sv2A-50, *forward* ACACAAGCAACTCCA TTCTT, *reverse* CGGTCTATCAGTGTTTCCAT; Sv2B-27, *forward* AGAGTTGTATAAATGTGGGCTA, *reverse* AGCTGTCCTGTTAA ACATCAA; Sv2A-95, *forward* GACTTCTCTCTCCGTTCCTT, *reverse* ATGCATGGTTTTGTGTCTCC. GenBank accession GU167935–39. Forward primers were tagged with M13 (sequence not shown)

motifs. Fourteen clones contained microsatellites with nine or more simple dinucleotide repeats and had enough flanking region for primer design. Primers were designed for ten of these loci using Primer3 (Rozen and Skaletsky 2000). Forty-nine Peruvian Booby clones contained microsatellites, of which 36 were simple, 7 were interrupted, 5 were complex repeats of two different dinucleotide motifs interrupted by one or more base pairs, and 1 was compound. Eighteen clones contained microsatellites with nine or more simple dinucleotide repeats and had enough flanking region for primer design. Primers were designed for ten of these clones using Primer3.

All 20 primer pairs were screened in 24 Blue-footed Boobies and 27 Peruvian Boobies. Loci were amplified using PCR in 5-µl volumes each containing 1× Multiplex mix (Qiagen), 0.15 µM forward and reverse primers, ~5 ng of DNA, and 1 µM of D4 labeled M13 forward primer. Amplifications were performed using a BIOMETRA T-gradient Thermocycler (Biometra Analytik, Goettingen, Germany) and the following temperature profile: 95°C for 15 min, followed by 16 cycles of 95°C for 45 s, annealing temperature for 60 s, and 72°C for 30 s for extension. The annealing temperature started at 60°C and decreased by 0.5°C each cycle for the first 16 cycles. This was followed by a further 20 cycles of 45 s at 95°C, 60 s at 52°C, and 30 s at 72°C with a final extension of 10 min at 72°C. The success of amplifications was confirmed using electrophoresis in 2% agarose gels containing 2% ethidium bromide in 1 mM Tris acetate pH 8.0. DNA fragments were sized using a Beckman–Coulter CEQ™ 8000 genetic analysis system (Core Genotyping Facility, Department of Biology, Queen's University). The program ARLEQUIN Ver. 3.11 (Excoffier et al. 2005) was used to calculate number of alleles and observed (H_O) and expected (H_E) heterozygosity for each locus, and to test for departures from Hardy–Weinberg and linkage equilibrium.

All 20 primer pairs were also amplified in Brown Boobies to test the success of cross-species amplifications. The loci were first screened in four individuals from throughout the range of the species. Loci that were polymorphic in four individuals were subsequently amplified in ten additional individuals.

Results and discussion

Of the 20 primer pairs, 16 reliably amplified loci in Blue-footed or Peruvian Boobies (Table 1). Eight of these loci were polymorphic in Blue-footed Boobies and nine were polymorphic in Peruvian Boobies (Table 1). None of the loci showed significant deviations from Hardy–Weinberg or linkage equilibrium. When tested in a larger sample of individuals spanning the range of each species, all loci presented in Table 1 are polymorphic and informative for population genetic studies of both focal species (Taylor et al. unpubl. data).

The same 16 primer pairs that reliably amplified loci in Blue-footed and Peruvian Boobies did so in Brown Boobies (Table 2). Of the ten primer pairs that amplified polymorphic loci in Blue-footed and Peruvian Boobies, seven were polymorphic in Brown Boobies (Table 2). Five primer pairs that amplified monomorphic loci in Blue-footed and Peruvian Boobies (not included in Table 1) were also tested in Brown Boobies and were variably polymorphic (Table 2).

These new loci will be useful in population genetic studies of the focal species and on a wider range of species within the Sulidae. They are currently being used to assess population genetic structure in, and hybridization between, Blue-footed and Peruvian Boobies.

Zusammenfassung

10 Mikrosatelliten-Loci wurden in Blaufußtölpeln (*Sula nebouxii*) und Guanotölpeln (*S. variegata*) isoliert und charakterisiert. Nach der Durchsicht ergaben sich in 24 Blaufußtölpeln 8 polymorphe *Loci* mit 2 bis 10 Allelen pro *Locus* und in 27 Guanotölpeln 9 polymorphe *Loci* mit 2 bis 12 Allelen pro *Locus*. Die Heterozygotie (observed heterozygosity) variierte dabei zwischen 0,29 und 0,84. Dieselben *Loci* wurden auch im Weißbauchtölpel (*S. leucogaster*) getestet und waren polymorphisch variabel. Diese neuen *Loci* werden gegenwärtig genutzt, um die populationsgenetische Struktur in Blaufußtölpeln und Guanotölpeln zu erfassen. Außerdem werden sie genutzt werden, um Hybridisierung zwischen den Arten zu untersuchen.

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References

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

Friesen VL, Anderson DJ (1997) Phylogeny and evolution of the Sulidae (Aves: Pelecaniformes): a test of alternative modes of speciation. Mol Phylogenet Evol 7:252–260

Nelson BJ (1978) The sulidae: gannets and boobies. Oxford University Press, Oxford

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana, Totowa, pp 365–386

Sun Z, Gomez-Diaz E, Bailie A, Friesen VL (2009) Isolation and characterization of microsatellite loci for storm-petrels. Mol Ecol Resour 9:913–915