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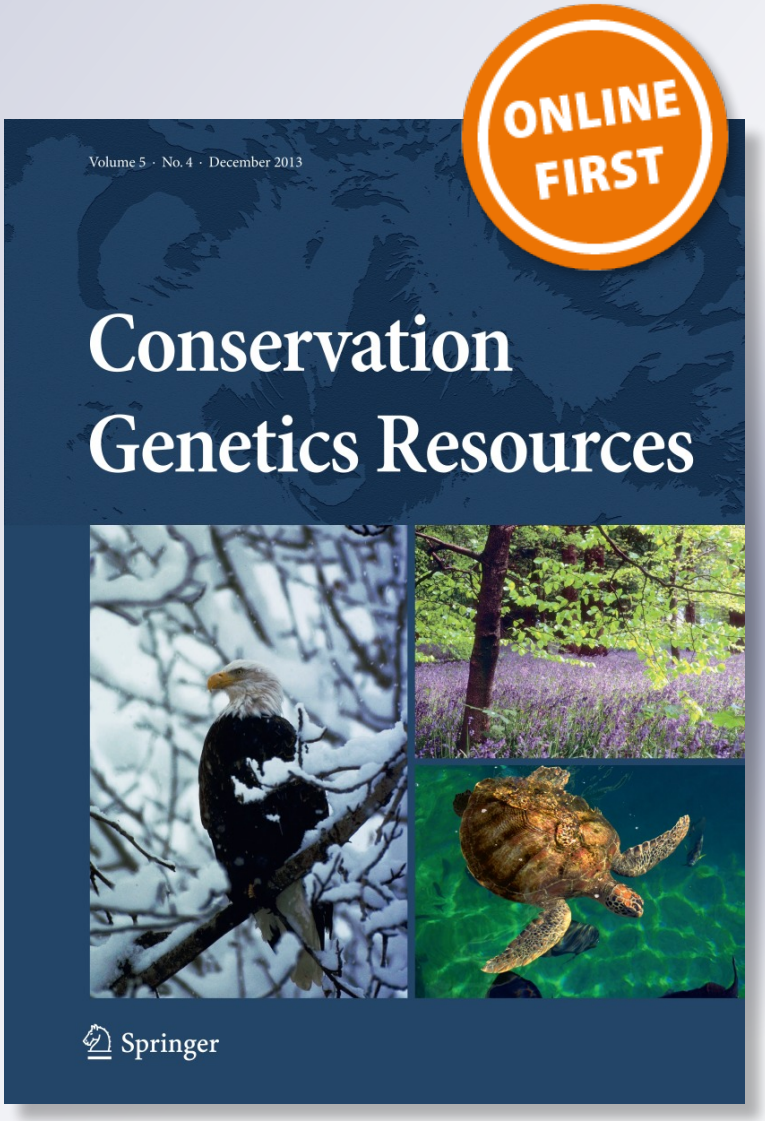

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MICROSATELLITE LETTERS

Development and characterization of 15 polymorphic microsatellite markers for North Pacific albatrosses using paired-end Illumina shotgun sequencing

Luis D. Hernandez · Zachary T. Lounsberry · Paul W. Collins · Robert W. Henry · Seth D. Newsome · Benjamin N. Sacks

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Abstract All three species of North Pacific albatross are vulnerable or near threatened (IUCN 2013) yet microsatellite markers to study their population characteristics are lacking. We employed 250-bp paired-end whole genome shotgun sequencing on an Illumina MiSeq platform to discover microsatellite markers. We selected 16 candidate loci for screening in 36 Black-footed (*Phoebastria nigripes*), 12 Laysan (*P. immutabilis*), and 3 Short-tailed (*P. albatrus*) albatrosses from which we characterized 15 markers.

Keywords Albatross · Illumina · Microsatellite · PAL FINDER · PCR primers · *Phoebastria*

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L. D. Hernandez · Z. T. Lounsberry · B. N. Sacks
Mammalian Ecology and Conservation Unit, Veterinary Genetics Laboratory, University of California, Davis, One Shields Avenue/Old Davis Rd., Davis, CA 95616, USA

P. W. Collins
Santa Barbara Museum of Natural History, 2559 Puesta Del Sol, Santa Barbara, CA 93105, USA

R. W. Henry
Long Marine Laboratory, Center for Ocean Health, Institute of Marine Sciences, University of California, Santa Cruz, 100 Shaffer Rd, Santa Cruz, CA 95060, USA

S. D. Newsome
Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

B. N. Sacks (✉)
Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA
e-mail: bnsacks@ucdavis.edu

North Pacific albatrosses, including Black-footed (BFAL), Laysan (LAAL), and Short-tailed (STAL) albatrosses, were historically hunted for their feathers and eggs, precipitating dramatic population crashes in the early twentieth century. Although conservation efforts have helped to stabilize population numbers, these albatrosses continue to face threats from longline fisheries and rising sea levels (e.g., IUCN 2013). Only one study attempted to use microsatellite markers (derived from distantly related seabirds) to study a North Pacific albatross, and only five markers were polymorphic in that species. The microsatellites described here will provide tools needed to better understand the population structure of these threatened seabirds.

We extracted genomic DNA from muscle tissue of 36 BFAL, 12 LAAL, and 3 STAL using the Qiagen DNeasy blood and tissue kit following the manufacturer's protocol. We prepared sequencing libraries using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs) and NEBNext® Multiplex Oligos for Illumina (New England Biolabs) to individually barcode a BFAL, LAAL, and two STALs, which we sequenced using 250 × 250 paired-end runs on a MiSeq platform (Illumina) at the DNA Technologies and Expression Analysis Cores of the University of California-Davis Genome Center. We screened the resulting, paired-read sequences using PAL_FINDER_v0.02.03 (Castoe et al. 2012). We chose for further investigation 44 potentially amplifiable loci (PAL) including di-, tri-, or tetra-nucleotide repeats that met the following criteria: (1) repeat motif occurs at average frequency (to avoid transposable elements), (2) both forward and reverse primers occurred only once among reads,

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Table 1 Characterization of 15 polymorphic microsatellite loci developed in three species of North Pacific albatross, named according to the species in which the marker was discovered

Locus	Primer sequence 5′ → 3′	Size (bp)	Motif	*n*	N_a	H_o	H_e	*P*
BFAL11	F: 6FAM-AGCTAGGCAGGCATTCAGC	177–189	ATCC	36	4	0.500	0.493	0.761
	R: CAACTGTAAAGGGGGAATGG	169–189		12	6	0.750	0.757	0.502
		177–189		3	4	–	–	–
BFAL14	F: 6FAM-CAAGAAGCTATTATTAAGCTATTTGTTCC	134–146	ATTT	36	4	0.417	0.349	0.761
	R: GGGTGTGAAATCGTTTCTGC	134–142		12	3	0.667	0.620	0.295
		130–142		3	3	–	–	–
BFAL19	F: 6FAM-TGAGCTGCAGTTTACTCTAGGC	248–288	TC	36	18	0.944	0.908	0.772
	R: TGTCTGCTTGTGGTGTTTGG	250–289		12	13	0.750	0.942	0.010
		250–278		3	5	–	–	–
LAAL1	F: PET-TTGGAGCCTGCTGTGTAATG	169–173	TC	36	3	0.278	0.558	0.002*
	R: GTTTGGCTGTAAATTACGTCAGTCTCTC[a]	167–175		12	5	0.333	0.732	0.004
		165–175		3	4	–	–	–
LAAL7	F: NED-CAGCATGACCAAATCCATCC	179–197	AGC	36	4	0.528	0.558	0.947
	R: GTTTGGACACAAATGCCGTTCTTG[a]	200–212		12	4	0.417	0.678	0.186
		191–194		3	2	–	–	–
STAL24	F: VIC-TGTTTTGAAGCCAAAACCAG	182–194	ATAC	36	2	0.194	0.178	1.000
	R: TTCTTTTATGGATGGAGAAATGC	186–190		12	2	0.000	0.159	0.044
		194–198		3	2	–	–	–
BFAL20	F: 6FAM-CCAGGAATGAGATGAATACTCG	156–166	TC	36	6	0.750	0.719	0.825
	R: GCAGTACTGAAACGGAGGAGG	154–172		12	7	0.583	0.652	0.275
		150–154		3	3	–	–	–
BFAL4	F: VIC-TGGGTCAGTTTACCTCTCAGC	214–228	TC	36	8	0.611	0.698	0.149
	R: GTTTGCCATTTTAAACTGTCATTGGC[a]	210–216		12	4	0.417	0.489	0.017
		214–218		3	2	–	–	–
LAAL10	F: VIC-TCCCAGCAGTGAAGCCATAG	140–188	AGT	36	14	0.889	0.908	0.622
	R: GTTTGGCAGGTAAGGAGAACAGAGG[a]	149–179		12	10	0.750	0.902	0.384
		149–170		3	4	–	–	–
LAAL19	F: PET-ATGACCAGTGCCATCAACAG	134–158	ATCC	36	4	0.417	0.353	0.801
	R: TGGCCAGATGTGATTTGAAG	142–166		12	6	0.750	0.815	0.645
		142–162		3	4	–	–	–
STAL18	F: NED-CAGTTCCAAAGAAGGAAAACC	183–204	ATG	36	7	0.778	0.711	0.047
	R: GTTTGGAGTGGTCTTGGGTACAGC[a]	183–204		12	7	0.500	0.826	0.002
		183		3	1**	–	–	–
STAL20	F: PET-CCTGACCAATAATGGCTGTTC	183–203	ATAG	36	6	0.611	0.674	0.867
	R: TTCCAACTGACCCCAAGAAG	187–203		12	5	0.917	0.736	0.959
		191–203		3	4	–	–	–
STAL4	F: 6FAM-TCCCCAGAAAAGTGATGGAG	204–212	TC	36	4	0.528	0.601	0.348
	R: GTTGGAGAACTCCCCTTCTG	192–208		6	5	0.333	0.742	0.047
		196–200		3	2	–	–	–
STAL12	F: VIC-CACTGCTGGGGGCTTTTC	135	TG	36	1	–	–	–
	R: GCCTTAAACTCTGGAGCATTG	135		12	1	–	–	–
		143–145		3	5	–	–	–
STAL8	F: NED-CCCTGCTGGGTTTCATTTC	130	TG	36	1	–	–	–
	R: CATTTGTGCAGTAGGCATGG	130		12	1	–	–	–
		128–132		3	2	–	–	–

For each locus, the first, second, and third rows correspond to BFAL, LAAL, and STAL, respectively, including number of alleles (N_a), observed heterozygosity (H_o), and expected heterozygosity (H_e), with *P* corresponding to tests of Hardy–Weinberg equilibrium

[a] Bases GTTT were added to the 5′ end of sequence

* Significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction

** Locus was found to be monomorphic in *P. albatrus*, possibly due to small sample size

(3) ≥6 repeats, (4) only perfect repeats, and (5) avoid tri- and tetra-repeats composed of 2 or 3 tandem bases, respectively (e.g., TCC, TAAA). We then manually assembled paired reads corresponding to these loci by searching out sequences complimentary to the default primers produced by PAL_FINDER and confirming that paired reads overlapped and had high agreement between their overlapping bases. We then used these contigs to redesign primers in Primer 3 (Rozen and Skaletsky 2000). We PCR-amplified these 44 PALs on the same 4 samples of genomic DNA to confirm amplicon size.

We then used Multiplex Manager to select a subset of 16 of these loci that were predicted to function well together in two multiplex reactions (Holleley and Geerts 2009) and ordered dye-labeled forward primers (Table 1; Electronic Supplementary Material). Using a Qiagen multiplex kit (Qiagen) and an annealing temperature of 60 °C, we screened the loci in multiplex reactions on 51 individuals (BFAL, $n = 36$; LAAL, $n = 12$; STAL, $n = 3$). Of the 16 primer-pairs tested, 13 were polymorphic in BFAL and LAAL and 14 were polymorphic in STALs. We used ARLEQUIN version 3.5.1.3 (Excoffier and Lischer 2010) to calculate descriptive statistics and to test for deviations from Hardy–Weinberg (using 100,000 iterations of a Markov chain simulation) and linkage equilibrium (using 2,000 permutations of Fisher's exact test) in BFAL and LAAL.

After sequential Bonferroni correction for multiple comparisons, one locus in BFAL deviated significantly from Hardy–Weinberg expectations (Table 1), and no locus-pairs deviated significantly from linkage equilibrium. Although low sample size prevented us performing statistical analysis for the STAL, two loci (STAL 8 and STAL 12) found to be monomorphic in the better-sampled species were polymorphic among the three STAL screened. Other than these loci, there was no evidence that markers performed better in the species in which they were discovered than in any other, likely due to their recent shared ancestry.

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