Conservation Genet Resour (2014) 6:765–767
DOI 10.1007/s12686-014-0210-z

MICROSATELLITE LETTERS

Development and characterization of microsatellite markers for the Hawaiian coot, *Fulica alai*, and Hawaiian gallinule, *Gallinula galeata sandvicensis*, through next-generation sequencing

Sarah A. Sonsthagen · Robert E. Wilson · Jared G. Underwood

Received: 7 April 2014 / Accepted: 11 April 2014 / Published online: 24 April 2014

Abstract We used next generation shotgun sequencing to develop novel microsatellite markers for two endangered waterbirds; the Hawaiian coot (*Fulica alai*) and Hawaiian gallinule (*Gallinula galeata sandvicensis*). The 20 loci polymorphic in the Hawaiian coot displayed moderate allelic diversity (average 3.8 alleles/locus) and heterozygosity (average 59.5 %). The 12 loci variable for the Hawaiian gallinule exhibited lower levels of allelic diversity (average 2.4 alleles/locus) and heterozygosity (average 47.5 %). Loci were in linkage equilibrium and only one locus deviated from Hardy–Weinberg equilibrium. These loci are sufficiently variable to assess levels of genetic diversity and will be useful for conservation genetic studies to aid in the management of these endangered waterbirds.

Keywords *Fulica alai* · *Gallinula galeata sandvicensis* · Genetic diversity · Hawaiian coot · Hawaiian gallinule · Microsatellite · Next generation sequencing

The endangered Hawaiian coot and Hawaiian gallinule are endemic to the Hawaiian Islands and both underwent precipitous declines in the early 1900s likely due to the loss and degradation of wetland habitat and introduced predators. Historically both species were common and distributed across the main Hawaiian islands; surveys conducted in the 1950–1960s suggest that numbers were reduced to <1,000 (Hawaiian coot) and 60 (Hawaiian gallinule) individuals throughout Hawaii (USFWS 2011). Population size has increased for the Hawaiian coot (1,777 ± 310 individuals; Underwood et al. 2013). The Hawaiian gallinule's current distribution is now restricted to Oahu and Kauai and recent surveys recorded low numbers (~400 individuals range-wide), though numbers are likely underestimated as Hawaiian gallinules are secretive (Underwood et al. 2013). The microsatellite markers described herein will provide much needed variable nuclear loci to assess the impact of the population declines on genetic diversity of these endangered waterbirds as well as inter-population genetic variation and phylogeographic studies.

Genomic DNA was extracted from blood samples of a Hawaiian coot (USFWS band number 1056-91345) and Hawaiian gallinule (USFWS band number 1196-87722) trapped at James Campbell National Wildlife Refuge, Oahu, Hawaii. We employed next generation sequencing following a shotgun sequencing protocol, using GS Junior Titanium rapid library preparation and sequencing chemistry (Roche, Branford CT). The barcoded libraries were pooled, clonally amplified and sequenced on a Roche 454 GS Junior. The Hawaiian coot library generated 43,955 reads (154 contigs with 37,866 unassembled) and the Hawaiian gallinule library resulted in 36,493 reads (139 contigs and 32,236 unassembled).

Msatcommander 0.8.2 (Faircloth 2008) searched contigs and unassembled reads for microsatellite repeats with ≥6

Electronic supplementary material The online version of this article (doi:10.1007/s12686-014-0210-z) contains supplementary material, which is available to authorized users.

S. A. Sonsthagen (✉) · R. E. Wilson
Alaska Science Center, U.S. Geological Survey, 4210 University Drive, Anchorage, AK 99508, USA
e-mail: ssonsthagen@usgs.gov

R. E. Wilson
Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775, USA

J. G. Underwood
U.S. Fish and Wildlife Service, Pacific Reefs National Wildlife Refuge Complex, Honolulu, HI 96850, USA

Table 1 Characterization of 23 microsatellite loci developed for the Hawaiian coot and Hawaiian gallinule, including repeat motif, primer sequence (universal tail in parentheses), allele size range in base pairs (bp), number of alleles (A), observed (H_O) and expected (H_E) heterozygosity, and GenBank accession numbers

Locus	Repeat Motif	Primer sequence (5′–3′)[a]	Hawaiian coot ($n = 10$)			Hawaiian gallinule ($n = 10$)			Accession no.
			Size (bp)	A	H_O/H_E (%)	Size	A	H_O/H_E (%)	
Fal002	$TA_{(3)}CA_{(8)}$	F: (SP6) CCGTTCTTTGAAGGCGTGG	135–143	3	50/47	141	1	–	KJ658344
		R: ACCCCTGACATCATAGCAC							
Fal003	$CA_{(8)}$	F: (M13R) GCTGGCATCACGTATGAGC	137–139	2	10/10	137	1	–	KJ658345
		R: CAATGAGTCTTGAAGCCTG							
Fal004	$CA_{(4)}GA_{(1)}CA_{(7)}$	F: (M13R) TGTGCAGCAGTTTCCTATCAG	109–121	5	70/63	107	1	–	KJ658346
		R: CATGTTTAGACTGATGGGC							
Fal006	$TA_{(9)}CA_{(2)}$	F: (T7) GTACCTGCATCAGCCTAG	166–176	4	**40/69**	166	1	–	KJ658347
		R: CTGAAAGGTTTCGTCAAGTTC							
Fal007	$TG_{(4)}TA_{(9)}$	F: (SP6) GATAACAACCAGGTAGGGG	106–114[b]	4	80/69	80	1	–	KJ658348
		R: GCTATCCCCACTTGAGC							
Fal008	$CA_{(11)}$	F: (M13R) CCCACATACCTCTGAACC	121–135[c]	5	80/62	137–151	2	30/39	KJ658349
		R: GAGGCTTAATTACGCTTAATG							
Fal010	$CA_{(2)}TA_{(1)}CA_{(11)}$	F: (M13R) AAAGCAAAGTGTCCCAGC	111–119	4	70/73	113–123	3	50/54	KJ658350
		R: GGGGCAGTCTTGCGAGC							
Fal012	$TA_{(8)}CA_{(8)}TG_{(10)}$	F: (SP6) CATAGCATTTGCCTGGC	126–130	3	80/69	106–112	2	60/44	KJ658351
		R: GCAGAGAAGATCAATGTTGC							
Fal014	$GT_{(10)}$	F: (SP6) TATCCCTGCCGCGGAAC	81–95	5	70/81	85–89	3	60/63	KJ658352
		R: CTCCTGGCTCTCAGTGC							
Fal015	$CT_{(2)}GT_{(8)}$	F: (M13R) GTGTTTTTAACAGGGGAGAG	98–100	2	50/48	102	1	–	KJ658353
		R: GTAAAATAGGTTGCATCTGC							
Fal016	$CA_{(5)}TA_{(1)}CA_{(10)}$	F: (SP6) CCCGACAACTTCTACAGG	101–119[c]	7	100/83	105	1	–	KJ658354
		R: GTTGATGTTTGATCCGAGTG							
Fal017	$TA_{(8)}$	F: (SP6) CTGTCCCATTGCATCCTC	133–146[c]	6	90/74	131–141	3	70/67	KJ658355
		R: GGTAGATGTTGGAAGAGGC							
Fal019	$CA_{(8)}$	F: (M13R) CAAACCCAACTGCTACCG	132–140	4	70/63	134–136	2	20/44	KJ658356
		GCCTCCTGTATGTTGTCAG							
Gch002	$TA_{(2)}CA_{(9)}$	F: (M13R) GCATTAAACTCTGAAGAGAGC	157–159	2	80/53	165	1	–	KJ658357
		R: TGTTGATACGGGATCTGTCC							
Gch003	$GT_{(8)}$	F: (M13R) CGTGACTACCATACCTTGTC	178–198	5	40/60	136	1	–	KJ658358
		R: TGCCTGAAACTTCATCTGC							
Gch006	$GT_{(8)}$	F: (T7) GAATGTGTCCTCCAGCTGC	108	1	–	105–114	4	100/72	KJ658359
		R: AGAAGTCCAACGGAGAGGC							
Gch007	$GT_{(8)}$	F: (M13R) GATGCTTCAGTAACACGTG	100–122	4	60/75	100–110	2	10/10	KJ658360
		R: CCTCCAGCAGTGCAACCC							
Gch012	$TC_{(9)}TA_{(5)}$	F: (SP6) TTCTGAATCTGACTTGGTC	153–159	3	80/62	153–157	2	40/34	KJ658361
		R: GAAGGGTTAAGCATGTGATC							
Gch013	$GT_{(9)}$	F: (SP6) TCATCGAGACCCACTGC	–	–	–	142–146	2	50/49	KJ658362
		R: GCTCTGCTGGTGGCAC							
Gch014	$GT_{(11)}$	F: (M13R) GTGCTTCATCTTCCATCGAC	102–114	3	40/57	110	1	–	KJ658363
		R: AAACTCTGGAAGCACAGG							
Gch015	$CA_{(12)}$	F: (M13R) GCACACGCACGCACTTTCTC	130–132	3	20/28	134	1	–	KJ658364
		R: CTTTTCGCACGTAACTCAC							
Gch017	$GA_{(2)}TA_{(1)}GA_{(8)}$	F: (SP6) TGATGTATTGATGCCACAGG	93	1	–	89–95	2	40/34	KJ658365
		R: TTTCCATCGCTCCATCGC							

Table 1 continued

Locus	Repeat Motif	Primer sequence (5′–3′)[a]	Hawaiian coot ($n = 10$)			Hawaiian gallinule ($n = 10$)			Accession no.
			Size (bp)	A	H_O/H_E (%)	Size	A	H_O/H_E (%)	
Gch019	$TA_{(7)}$	F: (M13R) TTCATTAGAGACCCCAGAG R: GCCCTGAAAACTGTCGCTC	94–97	2	10/10	94–100	2	40/34	KJ658366

Values in bold text denote comparisons that deviated from Hardy–Weinberg equilibrium expectation

[a] Universal tail primer sequences; M13R (GGATAACAATTTCACACAGG), SP6 (GATTTAGGTGACACTATAG), and T7 (TAATACGACTCACTATAGGG)

[b] Variable coamplified product at approximately 126–130 bp

[c] Signifies the presence of 1 bp repeat

repeat units and designed primer sets. Microsatellite motifs were identified in 153 reads from the Hawaiian coot and 158 reads from the Hawaiian gallinule. Microsatellite loci with dinucleotide repeat motifs, 20 in both the Hawaiian coot and Hawaiian gallinule, were selected for development and loci were tested for variability and cross species amplification among ten individuals representing each species sampled from James Campbell National Wildlife Refuge. PCR amplifications and thermocycler conditions followed methods described by Sonsthagen et al. (2004). We used Arlequin (v.3.1; Excoffier et al. 2005) to calculate observed and expected heterozygosity and tests for linkage disequilibrium and deviations from Hardy–Weinberg equilibrium (HWE), applying Bonferroni corrections for multiple tests ($\alpha = 0.05$). Of the 40 loci tested for variability, 23 were polymorphic in Hawaiian coot and/or Hawaiian gallinule (Table 1). Genetic diversity metrics for all loci are listed in Table 1. Twenty loci were variable for the Hawaiian coot; the number of alleles ranged from 2 to 7 (mean 3.8) and observed heterozygosity from 10 to 100 % (average 59.5 %). Twelve variable microsatellites were detected in the Hawaiian gallinule; the number of alleles ranged from 2 to 4 (mean 2.4) and observed heterozygosity from 10 to 100 % (average 47.5 %). All loci were in linkage equilibrium and only one locus (Fal006) deviated from HWE expectation. These loci are sufficiently variable to assess levels of genetic diversity, as well as inter-population genetic structure, and will be useful for conservation genetic studies to aid in the management of these endangered waterbirds.

Acknowledgments Support was provided by the U.S. Fish and Wildlife Service and U.S. Geological Survey, Alaska Science Center. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

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

Faircloth BC (2008) Msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94. doi:10.1111/j.1471-8286.2007.01884.x

Sonsthagen SA, Talbot SL, White CM (2004) Gene flow and genetic characterization of northern goshawks breeding in Utah. Condor 106:826–836. doi:10.1650/7448

Underwood JG, Silbernagle M, Nishimoto M, Uyehara K (2013) Managing conservation reliant species: Hawai'i's endangered endemic waterbirds. PLoS One 8:e67872. doi:10.1371/journal.pone.0067872

U.S. Fish and Wildlife (USFWS) (2011) Recovery plan for Hawaiian waterbirds, 2nd revision. U.S. Fish and Wildlife Service, Portland, Oregon