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PRIMER NOTE

Development of 19 microsatellite loci for Swainson's hawks (*Buteo swainsoni*) and other buteos

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Abstract

We developed 26 Swainson's hawk (*Buteo swainsoni*) microsatellite primers from CA, AAT, CATC and GAGAA enriched genomic libraries. Primers were tested in 357 Swainson's hawks from western North America as well as seven other *Buteo* species. These markers will have broad application in investigations of *Buteo* population structure and genetic diversity.

Keywords: Accipitridae, *Buteo swainsoni*, microsatellite, raptor, Swainson's hawk

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The Swainson's hawk (*Buteo swainsoni*), historically one of the most abundant raptors throughout its range across western North America, has experienced significant declines in census population size during the past century (England *et al*. 1997). In order to identify distinct populations for conservation and facilitate management actions, population genetic structure and genetic diversity must be established (Frankham *et al*. 2002). Here we characterize 26 novel microsatellite markers developed in Swainson's hawks and present results of preliminary screening in seven additional species of *Buteo*.

Four libraries enriched with microsatellite motifs (CA, ATT, CATC, GAGAA) were created by Genetic Identification Services (Chatsworth, CA) with DNA extracted from a California Central Valley Swainson's hawk following the methods of Jones *et al*. (2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa*I, *Hae*III, *Bsr* B1, *Pvu*II, *Stu*I, *Sca*I, *Eco* RV). Fragments in the size range of 300–750 bp were adapted and subjected to magnetic bead capture (CPG, Inc.) using biotinylated capture molecules. Libraries were prepared in parallel with Biotin-CA(15), Biotin-AAT(15), Biotin-CATC(8) and Biotin-GAGAA(7) as capture molecules. Captured molecules were amplified and restricted with *Hin*dIII to remove the adapters. Resulting fragments were ligated into the *Hin*dIII site of pUC19. Recombinant molecules were electroporated into *Escherichia coli* DH5α. Recombinant clones were selected randomly for sequencing, and enrichment levels were expressed as the fraction of sequences that contained a microsatellite. Sequences were obtained on an ABI PRISM 377 DNA Sequencer/Genotyper, using ABI PRISM *Taq* dye-terminator cycle sequencing methodology (all Applied Biosystems Inc.).

We used PRIMER 3 (Rozen & Skaletsky 2000) to design 38 primers with melting temperatures of 59–61 °C. QIAGEN DNeasy kits (QIAGEN Inc.) were used to extract DNA from blood samples. Whole blood and contour feathers were collected from a total of 357 Swainson's hawks from two regions (California and Great Basin/Great Plains), six red-shouldered hawks (*Buteo lineatus*), five red-tailed hawks (*Buteo jamaicensis*), five white-tailed hawk (*Buteo albicaudatus*), one broad-winged hawk (*Buteo platypterus*), five ferruginous hawks (*Buteo regalis*), and four rough-legged hawks (*Buteo lagopus*). Blood was drawn via medial metatarsal venipuncture and two feathers were plucked from the breast. Samples were collected from pre-fledged young in nests by permitted raptor biologists as well as from juveniles and adults treated at several wildlife rehabilitation facilities. Extracted DNA from six Galapagos hawks (*Buteo galapagoensis*) was provided by Dr P. Parker. To reduce the cost of initial marker screening, M13 tails were added to primers as in Schuelke's (2000) protocol. The 26 primers that had no more than two alleles per

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Table 1 Characterization of 25 microsatellite loci developed in 357 Swainson's hawks, *Buteo swainsoni*

Locus	Dye label	PCR product size (bp)	Repeat motif	M	N	H_O	H_E	GenBank Accession no.	Primer sequence (5′–3′)
BswA110w	6-FAM	197–233	$(CA)_{18}$	1	13	0.85	0.85	DQ985707	F: ATTTTGAGAGGTGAAGGTCACG R: CAGGTCAGTGAAGGACTCTGC
BswD207w*	NED	215–312	$(GAGAA)_{14}$	1	31	0.47	0.93	DQ985720	F: TGGGAAAAGTAGTTAGGAAGTG R: CTCAGCCAGTCTTGTTGTG
BswD330w*	VIC	145–300	$(GAGAA)_{6}$	1	40	0.50	0.95	DQ985730	F: ATGTCGCTTAATGCATGACTGA R: ACAAGCACTGCAAGGGAGTAGT
BswD122w†	PET	199–299	$(GAGAA)_{10}$	1	24	0.90	0.92	DQ985717	F: GTCAGGCAGTTGGACTAGATGA R: GATGGGGAACTGCTCTAAACAT
BswD312w*	PET	119–209	$(GAGAA)_{21}$	2	18	0.46	0.89	DQ985726	F: ATGCCCAGTCTCCCAAAG R: TAGGTCCCTTCCAAATGAAATA
BswA204w	NED	133–177	$(CA)_{16}$	2	18	0.82	0.82	DQ985708	F: GCAGAAGGAAATGTGTTTGGTT R: TAAGAAACCAGGTGGCATTAGG
BswA317w	PET	369–391	$(CA)_{17}$	2	11	0.76	0.79	DQ985712	F: CTGAAAATGTCACCACAACAAA R: TGAGTAAGCACAGGAGATGGAT
BswD210w†	VIC	183–328	$(GAGAA)_{14}$	2	26	0.95	0.94	DQ985721	F: TTAACAAGTCCAAATGCTGGAT R: TTGGAATAAATGGTCATTGTAGGT
BswD220w	6-FAM	265–335	$(GAGAA)_{11}$	2	15	0.81	0.87	DQ985722	F: TAACTTTTGGTCAGCCCTGAAT R: TCTGTGGCACTGCAATGAAT
BswA303w	PET	226–250	$(CA)_{15}$	3	12	0.80	0.82	DQ985710	F: ACTGAATAAGCAGAGGGCAAAA R: TGGCACTTCCATAGTCAATCAG
BswD123w*	6-FAM	225–280	$(GAGAA)_{10}$	3	12	0.41	0.79	DQ985718	F: CAGCCCTGAAGTGGTTAG R: GCCTACAGTGAATGTGATTG
BswB111aw	NED	188–208	$(CATC)_{7}$	3	6	0.40	0.44	DQ985713	F: TCATCCCAATGCAGTTCTCA R: CACTGGCATGAATGGACAGA
BswD234w	VIC	198–268	$(GAGAA)_{17}$	3	13	0.84	0.84	DQ988163	F: GGAATTGCATAGGTCAAACACA R: CTGTGCAACATATTATTTCCCTTG
BswD310w†	PET	211–246	$(GAGAA)_{24}$	4	31	0.93	0.94	DQ985725	F: GAACAATTTGGGATACACTGA R: TAATGCCATGATGTTATCAGAC
BswD235w*	VIC	203–298	$(GAGAA)_{20}$	4	19	0.45	0.89	DQ985724	F: ATAACACAATGCCCAGTCT R: AAGCCTTGATTTTCTTGC
BswD313w†	6-FAM	205–280	$(GAGAA)_{19}$	4	14	0.85	0.84	DQ985727	F: CTGCACCTTTCTTCTTATGC R: GCTGAGGTCTGAATTTTTACC
BswB220w	NED	136–172	$(AAT)_{9}$	5	11	0.78	0.80	DQ985714	F: GGCTTTTCTGATTGAATTAGGG R: CACAAACTGTTGCCTGAACTTT
BswB221w	PET	206–224	$(AAT)_{4}$	5	7	0.66	0.67	DQ985715	F: TAACTTCGACACAGGGTAGCAA R: TGGGAGAGTGTTTGTGCTCTTA
BswD327w	FAM	265–315	$(GAGAA)_{9}$	5	11	0.77	0.82	DQ985729	F: ATGGTCCACTAGAATGTTTGAC R: TCTCCCTATGTTACGTTAGCAT
BswD223w*	VIC	197–305	$(GAGAA)_{11}$	5	22	0.45	0.91	DQ985723	F: GTGGGAATGAATGACCTTGAGT R: CTGAAGTGGTCAGACAGTTGGA
BswA302w	NED	201–227	$(CA)_{16}$	6	12	0.89	0.83	DQ985709	F: CGAAGTTGTGCAATCTCATTTC R: CTTGCTTTCACAATTTGCAGTC
BswA312w	NED	136–168	$(CA)_{21}$	6	15	0.92	0.89	DQ985711	F: GGCAGAAATCAGCAGCATAAAT R: CCACTCCCTCATGAAACAGATT
BswD107w†	VIC	168–358	$(GAGAA)_{14}$	6	49	0.95	0.96	DQ985716	F: CCATCTCTTGGTCCTGTTT R: CTACAATCCTGTCTGGACATG
BswD127w†	PET	128–183	$(GAGAA)_{9}$	6	19	0.87	0.89	DQ985719	F: CAGGGTGGACAGACAGGTAG R: GTGAGGCAGTTGGACTTGAT
BswD324w†	FAM	210–460	$(GAGAA)_{27}$	6	23	0.89	0.91	DQ985728	F: AAAAGGATTGAAGGAGTTGG R: CCCTGTTGTGCATCTTTG

*Possible null alleles detected.
†Irregular repeat, not conforming to stepwise mutation model. M, multiplex panel; N, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

individual and product of expected size were then traditionally fluorescently dye labelled and combined into multiplex groups.

Each individual was genotyped at 26 microsatellite loci in polymerase chain reactions (PCR) carried out in 13 μL volumes containing 1× PCR buffer II (Applied Biosystems), 2.5 mM $MgCl_2$, 0.2 mM dNTP (Fisher Scientific), 0.5 U Thermoprime Plus DNA Polymerase (ABgene), 1 μL DNA (20–60 ng) and various concentrations of forward fluorescently dye-labelled (Applied Biosystems Inc.) and reverse primers (BioSource) ranging from 0.05 to 0.15 μM. Thermal cycling parameters were as follows: 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 58 °C for 45 s and 72 °C for 45 s, and a final-30 min extension at 72 °C followed by 15 °C until further use. PCR products were separated with an ABI PRISM 3730 DNA Analyzer (Applied Biosystems Inc.). PCR products were visualized and scored with STRAND version 2.3.69 (Toonen & Hughes 2001).

Among the 38 primers tested, 26 amplified in Swainson's hawks with no more than two alleles per individual, one of which was monomorphic. The number of alleles per locus ranged from six to 49 with an average of 18.9. Expected heterozygosity ranged from 0.44 to 0.96 while the observed heterozygosity ranged from 0.40 to 0.95 (ARLEQUIN version 3.01, Excoffier *et al*. 2005) (Table 1). Hardy–Weinberg equilibrium and linkage disequilibrium were tested within two presumptive North American populations (GENEPOP version 3.4; Raymond & Rousset 1995). Following Bonferroni correction for multiple tests (P value = 0.00015), six loci (BswD207w, BswD330w, BswD312w, BswD123w, BswD235w, BswD223w) significantly deviated from HWE (heterozygote deficit, $P < 0.0001$) in both presumptive populations (Table 1), possibly due to null alleles. Of the 325 pairwise comparisons, 12 were significant in one population and 19 in another; no significant pairs occurred in both populations. The 19 polymorphic loci displaying no heterozygote deficiency have probabilities of identity of 9.09×10^{-29} and 1.39×10^{-9}, respectively, using the Hardy–Weinberg equilibrium and sibling probability calculations (GENECAP version 1–2, Wilberg & Dreher 2004). Cross-amplification tests in seven sister taxa are summarized in Table 2. None of these primers showed overlap with

Table 2 Cross-amplification of 25 microsatellite loci in seven raptor species. *N*, number of alleles; —, indicates no product amplified; *, indicates more than two bands in same individuals; *n*, number of individuals; range, size of product in base pairs. Products have not been sequenced

	Buteo regalis (*n* = 5)		*Buteo lagopus* (*n* = 3)		*Buteo jamaicensis* (*n* = 5)		*Buteo albicaudatus* (*n* = 5)		*Buteo galapagoensis* (*n* = 6)		*Buteo platypterus* (*n* = 1)		*Buteo lineatus* (*n* = 6)	
Locus	*N*	range	*N*	range	*N*	range	*N*	range	*N*	range	*N*	Range	*N*	range
BswA110w	3	209–213	1	211	3	211–215	3	209–217	2	215–219	1	215	2	215–217
BswD207w	—	—	—	—	4	220–235	6	203–238	3	217–227	1	244	7	231–336
BswD330w	7	165–230	5	170–220	4	152–180	5	150–240	3	148–158	1	145	3	145–180
BswD122w	6	300–330	4	285–315	9	222–292	5	238–263	4	209–234	1	297	6	314–339
BswD312w	5	158–183	2	188–203	5	129–164	3	133–143	1	133	1	138	1	162
BswA204w	1	142	2	142–144	3	142–146	3	143–153	2	161–163	1	143	1	152
BswA317w	1	373	4	371–393	5	369–379	4	367–373	1	383	2	375–385	1	375
BswD210w	7	285–325	5	278–303	9	262–347	5	185–200	3	229–249	2	210–295	7*	204–224
BswD220w	6	238–328	4	393–343	4	215–330	5	262–297	2	280–290	2	282–292	4	292–312
BswA303w	3	236–240	3	236–242	7	232–248	3	236–242	1	246	2	236–240	1	240
BswD123w	2	216–221	2	226–236	2	226–236	6	251–276	2	250–255	—	—	3	257–272
BswB111aw	4	192–204	3	184–200	4	196–208	2	184–192	1	196	2	188–196	1	196
BswD234w	4	222–237	4	237–262	5	207–232	3	203–218	3	193–203	2	198–208	5	223–248
BswD310w	6	281–326	4	300–350	6	222–262	3	180–210	6	215–260	1	214	5	233–263
BswD235w	5	242–267	3	272–307	5	214–249	3	219–229	—	—	1	224	1	219
BswD313w	7	238–303	2	283–303	4	250–275	5	280–355	5	220–250	1	270	1	230
BswB220w	3	145–154	4	148–160	4	152–161	4	142–154	2	145–148	1	155	4	148–163
BswB221w	1	206	1	212	3	206–215	1	195	1	212	2	212–215	1	203
BswD327w	4	284–309	1	269	4	269–299	2	269–289	1	280	2	284–289	2	269–274
BswD223w	5	248–273	3	258–268	7	235–320	4	323–358	1	227	1	239	4	239–254
BswA302w	1	219	2	219–221	2	219–227	1	200	1	219	2	213–215	1	215
BswA312w	4	132–144	4	132–156	7	140–162	1	136	1	150	2	150–152	4	148–156
BswD107w	8	192–307	5	210–305	7	277–352	6	168–203	6	217–242	2	291–306	7	184–219
BswD127w	4	157–187	4	162–172	4	157–172	4	148–163	3	137–147	2	199–219	4	157–172
BswD324w	8	238–338	4	278–318	10	210–460	9	220–285	3	230–250	2	248–288	8	308–348

previously published *Buteo* primers (a BLAST search found no alignments with any falconiform). These primers may assist in genetic investigations of multiple *Buteo* species.

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