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TECHNICAL NOTE

Characterization of ten microsatellite loci in the Broad-tailed Hummingbird (*Selasphorus platycercus*)

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Abstract The Broad-tailed Hummingbird (*Selaphorus platycercus*) breeds at higher elevations in the central and southern Rockies, eastern California, and Mexico and has been studied for 8 years in Rocky Mountain National Park, Colorado. Questions regarding the relatedness of Broad-tailed Hummingbirds banded together and then recaptured in close time proximity in later years led us to isolate and develop primers for 10 polymorphic microsatellite loci. In a screen of 25 individuals from a population in Rocky Mountain National Park, the 10 loci were found to have levels of variability ranging from two to 16 alleles. No loci were found to depart from linkage disequilibrium, although two loci revealed significant departures from Hardy–Weinberg equilibrium. These 10 microsatellite loci will be applicable for population genetic analyses, investigation of mating systems and relatedness, and may help gain insight into the migration timing and routes for this species.

Keywords Broad-tailed Hummingbird · Family relationships · Microsatellites · Population genetics · *Selasphorus platycercus*

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The Broad-tailed Hummingbird (*Selasphorus platycercus*) breeds in the foothills, montane valleys, and subalpine meadows of southern and central Rocky Mountains, eastern California, and Mexico (Calder and Calder 1992). Their mating system has been described as promiscuous (Woodbury and Sugden 1938; Hering 1947; Barash 1972; Calder 1991). The exact timing and routes of migration between wintering and breeding grounds are unknown (Calder and Calder 1992).

We have studied the timing of migration and demographics of Broad-tailed Hummingbirds for 8 years in Rocky Mountain National Park, Colorado. Our observations and circumstantial evidence led to the hypothesis that adult female Broad-tailed Hummingbirds remain with recently fledged young for weeks or longer after fledging and that some females captured and banded together and then recaptured again in close time proximity in later years might be related (i.e., mother and daughter). No information on such relatedness exists in published literature. To investigate this hypothesis and to potentially shed light on questions related to migration (i.e., where birds caught during migration or on the wintering grounds may have come from), we isolated and designed primers for 10 polymorphic microsatellite loci.

Genomic DNA was extracted from muscle tissue of a Broad-tailed Hummingbird carcass using the Promega Wizard DNA Purification Kit following the manufacturer's instructions (Promega Corporation) and used to isolated microsatellites following the technique developed by St John and Quinn (2008). Captured fragments were amplified and cloned using PCR-Script® Amp cloning kit (Stratagene) following manufacturer's protocol. The isolation yielded 84 clones that were subsequently sequenced.

A colony touch protocol was used to isolate the plasmid DNA. Using a sterile pipette tip, the colony was transferred

Table 1 Characterization of 10 polymorphic microsatellite loci developed for the Broad-tailed Hummingbird

Locus	GenBank accession no.	Primer sequence	T_A (°C)	Repeat of cloned allele	A	N	Allele size range	H_E	H_O	F(null) alleles
HumB1	HQ316946	F: M13-GTCCTACAGTCCTCTCCTCTCC R: AGGTGCTTGCTGCTGCATGCTC	60	$[(CA)_4GA]_2CAGA(CA)_9$	5	25	157–165	0.601	0.560	0.0278
HumB2	HQ316947	F: M13-TCTAGGTCACCAAGAGATTCCCTG R: CAGATTCAGTGAAGAAGTCTACCAAGG	60	$(CA)_{11}$	12	25	164–186	0.908	0.840	0.0241
HumB3	HQ316948	F: M13-CACGTCCAGAAATGTAAAGAGGGA R: GAATTCATCTGGACTGCAGCTAGA	60	$(CA)_{15}$	10	25	168–186	0.758	0.720	0.0036
HumB6	HQ316949	F: M13-CCTTCCTCTGGCTCCCTACA R: TCCAAGCACCCACAGACAAGA	60	$(CAT)_{21}$	16	22	178–232	0.940	0.818*[a]	0.0579
HumB8	HQ316950	F: M13-GGAGGTGGCAACGGAAAGA R: GTGAGCTCTTGGCACTCTG	58	$(CAT)_{23}$	12	25	128–180	0.891	0.440*[a]	0.3353
HumB9	HQ316951	F: M13-TTCTGCACAGGCACTGAG R: CATGACAAGGCCCATATTCC	60	$(GATA)_7$	8	24	120–148	0.750	0.625[a]	0.0801
HumB10	HQ316952	F: M13-CTCTTCGGTGTAAGCAACAG R: GCACTTACTTTACTCTTCCAC	52	$(CAT)_{20}$	14	15	120–158	0.926	0.667[a]	0.1472
HumB11	HQ316953	F: M13-CCTCAGGTCTTCAACCAAGTA R: GGCCACAAAAGCACAGTATGA	60	$(CAT)_5$	2	24	157–161	0.082	0.083	0.0122
HumB12	HQ316954	F: M13-CTAGTAGCAGTAATAGTAG R: TTGGAGGCATAGAACATC	52	$(GTA)_2ATA(GTA)_6$	4	21	145–155	0.331	0.333	0.0158
HumB15	HQ316955	F: M13-CCAGATCCTTTGCTGTGCAGG R: CTACCTGATTCCGTGGAACCAG	60	$(GATA)_8$	11	25	165–193	0.859	0.680[a]	0.1088

Annealing temperature (T_A), number of alleles (A), number of individuals screened (N), expected (H_E) and observed (H_O) heterozygosities are reported

F(null) refers to frequency of null alleles

* Significant deviation from Hardy–Weinberg equilibrium ($\alpha = 0.05$)

[a] Frequency of null alleles >0.05

to a tube containing 100 μl T.E (10 mM Tris, 0.1 mM EDTA, pH 8.0) and heated to 100°C for 10 min. A 1 μl aliquot was used as the template in a 25 μl polymerase chain reaction (PCR) as in St John et al. (2005). The PCR products were purified for sequencing by the addition of 5 U exonuclease I (USB) and 0.5 U shrimp alkaline phosphatase (USB) and a subsequent 37°C incubation for 30–45 min. These two enzymes were denatured by a 15 min 80°C incubation. Sequencing was performed using 4 μl prepared template and a Quickstart kit (Beckman Coulter) following manufacturer's protocol except that half reaction volumes (10 μl) were used. Sequencing reactions were run on the CEQ8000 XL DNA Analysis System (Beckman Coulter).

Primers were designed for 14 loci using the software PRIMER 3 (Rozen and Skaletsky 2000) and screened for polymorphism using DNA isolated from one or two central tail feathers from 25 Broad-tailed Hummingbirds captured in Rocky Mountain National Park. DNA was extracted using the user-developed protocol available from QIAGEN to accompany their DNeasy Blood and Tissue Kit. Ten of these primer pairs were polymorphic, amplified well with the feather DNA and were consistent and unambiguous for scoring purposes. Screening PCRs were performed in 12.5 μL reactions containing 125 μM of each dNTP, 1X GoTaq Flexi Buffer (Promega), 1.25 mM $MgCl_2$, 0.03 μM M13-tailed forward primer (following Boutin-Ganache et al. 2001), 0.5 μM non-tailed reverse primer, 0.5 μM M13 dye-labeled primer with Beckman Coulter dyes D2, D3 or D4 (Sigma), and 0.5 U of *Taq* DNA polymerase (Promega). The amplification conditions were as follows: 94°C for 2 min, then 35 cycles of 94°C for 40 s, annealing temp (Table 1) for one min and 72°C for one min, with a final extension at 72°C for 10 min. The PCR products were run on the CEQ8000 XL DNA Analysis System (Beckman Coulter). All loci were run with the S400 size standard (Beckman Coulter) and analyzed using the Frag 3 default method associated with the CEQ8000 XL DNA Analysis System.

For each polymorphic locus, we calculated observed heterozygosity (H_O), expected heterozygosity (H_E) and null allele frequencies using CERVUS 3.0 (Marshall et al. 1998). GENEPOP version 3.4 (Raymond and Rousset 1995) was used to test for evidence of linkage disequilibrium and deviations from Hardy–Weinberg equilibrium. The number of alleles per locus ranged from 2 to 16, and single locus heterozygosities ranged from 0.331 to 0.940 (Table 1). Significant deviations from Hardy–Weinberg equilibrium ($P > 0.05$) were observed at 2 loci and high null allele frequencies (>0.05) were detected at 5 loci (Table 1). We tested 45 pairwise comparisons and found no evidence for genotypic linkage disequilibrium between any set of paired loci after a sequential Bonferroni was applied ($P < 0.0002$). These microsatellite loci will enable researchers to investigate family groups and relatedness of Broad-tailed Hummingbirds and may be useful in gaining more information about the timing and migratory routes for this species.

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