

Fifteen polymorphic microsatellite loci from Jamaican streamertail hummingbirds (*Trochilus*)

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Abstract We isolated and characterized 15 microsatellite loci from the endemic Jamaican streamertail hummingbird *Trochilus polytmus*. Loci were screened in 12 individuals of both *T. polytmus* and its sister species *T. scitulus*, also a Jamaican endemic. The number of alleles per locus ranged from 2 to 10, observed heterozygosity ranged from 0 to 1, and the probability of identity values ranged from 0.038 to 0.663. These new loci provide tools for characterizing the narrow hybrid zone between the two species.

Keywords Black-billed streamertail · Hummingbird · Jamaica · Microsatellite · PCR primers · SSR · STR · Red-billed streamertail · *Trochilus polytmus* · *Trochilus scitulus*

The genus *Trochilus* is represented by two species of sexually dimorphic hummingbirds endemic to the oceanic island of Jamaica. The Black-billed Streamertail (*T. scitulus*), which has an entirely black bill, is restricted to the extreme eastern tip of the island. The better known Red-billed Streamertail (*T. polytmus*) occurs widely in the forested remainder of Jamaica except for arid areas along the southern coast (Gill et al. 1973). Streamertails have a promiscuous mating system typical of trochiline hummingbirds (Gosse 1847; Schuchmann 1999). Males perform courtship displays that emphasize bill color, lengthened crown feathers, and their dramatically elongated rectrices (Gill et al. 1973; Graves 2009; Schuchmann 1978).

From a male *T. polytmus* (United States National Museum of Natural History catalog number 633628) we extracted total DNA from approximately 25 mg of ethanol-preserved pectoral muscle tissue using a DNeasy tissue kit (Qiagen, Valencia, California). DNA was then serially enriched twice for microsatellites using 3 probe mixes (mix 2 = (AG)₁₂, (TG)₁₂, (AAC)₆, (AAG)₈, (AAT)₁₂, (ACT)₁₂, (ATC)₈; mix 3 = (AAAC)₆, (AAAG)₆, (AATC)₆, (AATG)₆, (ACAG)₆, (ACCT)₆, (ACTC)₆, (ACTG)₆; mix 4 = (AAAT)₈, (AACT)₈, (AAGT)₈, (ACAT)₈, (AGAT)₈) following Glenn and Schable (2005). Briefly, the DNA was digested with restriction enzyme *RsaI* (New England Biolabs) and simultaneously ligated to double-stranded SuperSNX linkers (SuperSNX24 Forward 5'-GTTTAAGGCCTAGCTAGCA GCAGAATC and SuperSNX24 Reverse 5'-GATTCTGCT AGCTAGGCCTTAAACAAAA). Linker-ligated DNA was

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denatured and hybridized to biotinylated microsatellite oligonucleotide mixes, which were then captured on magnetic streptavidin beads (Dyna). Unhybridized DNA was washed away and remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SuperSNX24 as a primer, and cloned with TOPO-TA Cloning Kits (Invitrogen). Inserts from a total of 192 clones were PCR amplified and sequenced with M13 forward and reverse primers using the BigDye Terminators v3.1 (Applied Biosystems) and ABI-3130xl capillary sequencer. Sequences from both strands were assembled and edited in Sequencer 4.6 (Genecodes). Microsatellites were identified using MsatCommander version 0.8.1 (Faircloth 2008) and primers designed with Primer3. One primer from each pair was modified on the 5' end with an engineered sequence (CAG tag 5'-CAGTCGGGCGTCATCA-3') to enable use of a third primer in the PCR (identical to the CAG tag) that was fluorescently labeled for detection.

Twenty-seven primer pairs were tested for amplification and polymorphism using DNA obtained from four individuals of each species. PCR amplifications were performed in a 12.5 μ l volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μ g/ml BSA, 0.4 μ M unlabeled primer,

0.04 μ M tag labeled primer, 0.36 μ M universal dye-labeled primer, 1.2 mM MgCl₂, 0.8 mM dNTPs, 0.5 units JumpStart Taq DNA Polymerase (Sigma), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs (Don et al. 1991) encompassing a 10°C span of annealing temperatures ranging between 65–55°C or 55–45°C were used for the amplification (see Table 1). Cycling parameters were 20 cycles of 96°C for 30 s, highest annealing temperature (decreased 0.5°C per cycle) for 30 s, and 72°C for 30 s; and 20 cycles of 96°C for 30 s, lowest annealing temperature for 30 s, and 72°C for 30 s. PCR products were run on an ABI-3130xl sequencer and sized with Naurox size standard prepared as described in De Woody et al. (2004) except that unlabeled primers started with GTTT. Results were analyzed using GeneMapper version 3.7 (Applied Biosystems). Fifteen of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed variability of these 15 loci in 12 specimens of each species. The *T. polytmus* samples came from three populations, none more than 130 km apart, whereas the *T. scitulus* samples were collected from four populations, none more than 11 km apart (Table 1). Conditions and

Table 1 Sampling localities for specimens of *Trochilus* examined in this study

USNM catalog no	Species	Locality
636170	<i>T. polytmus</i>	JAMAICA: Trelawny Parish; Windsor (18°22'N, 77°40'W)
636172	<i>T. polytmus</i>	JAMAICA: Trelawny Parish; Windsor (18°22'N, 77°40'W)
636174	<i>T. polytmus</i>	JAMAICA: Trelawny Parish; Windsor (18°22'N, 77°40'W)
636176	<i>T. polytmus</i>	JAMAICA: Trelawny Parish; Windsor (18°22'N, 77°40'W)
636178	<i>T. polytmus</i>	JAMAICA: Trelawny Parish; Windsor (18°22'N, 77°40'W)
633624	<i>T. polytmus</i>	JAMAICA: Portland Parish; Somerset Falls (18°12'N, 76°33'W)
633625	<i>T. polytmus</i>	JAMAICA: Portland Parish; Somerset Falls (18°12'N, 76°33'W)
633626	<i>T. polytmus</i>	JAMAICA: Portland Parish; Somerset Falls (18°12'N, 76°33'W)
633628	<i>T. polytmus</i>	JAMAICA: Portland Parish; Somerset Falls (18°12'N, 76°33'W)
635678	<i>T. polytmus</i>	JAMAICA: Portland Parish; Fellowship (18°08'N, 76°28'W)
635679	<i>T. polytmus</i>	JAMAICA: Portland Parish; Fellowship (18°08'N, 76°28'W)
635683	<i>T. polytmus</i>	JAMAICA: Portland Parish; Fellowship (18°08'N, 76°28'W)
633544	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°03'N, 76°24'W)
633545	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°03'N, 76°24'W)
633530	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°02'N, 76°23'W)
633531	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°02'N, 76°23'W)
633533	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°02'N, 76°23'W)
633534	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°02'N, 76°23'W)
633535	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°02'N, 76°23'W)
633536	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°02'N, 76°23'W)
633537	<i>T. scitulus</i>	JAMAICA: Portland Parish; Millbank (18°02'N, 76°23'W)
633602	<i>T. scitulus</i>	JAMAICA: Portland Parish; Cambridge backlands (18°07'N, 76°23'W)
633609	<i>T. scitulus</i>	JAMAICA: Portland Parish; Cambridge backlands (18°07'N, 76°23'W)
636133	<i>T. scitulus</i>	JAMAICA: Portland Parish; Hartford on Ecclesdown road (18°05'N, 76°21'W)

Table 2 Details of 15 polymorphic microsatellite loci for *Trochilus*

Locus/acc no	Primer sequence 5'→3'	Repeat motif	T _a (°C)	Size (bp)	N	k	H _o	H _e	PI
Tro2	F: AGTCTGAGCCCAATACTGCC	(AAC) ₇	65	178–181	9	2	0.000	0.198	0.663
	R: ^a CGAGGAATGGAGTAGGCG			178–181	10	2	0.400	0.320	0.514
Tro3	F: ^a TGCAAACAACCTCTGAGCCC	(AAGT) ₅	65	348–364	12	5	0.833	0.747	0.107
	R: GACCCTATGGGACCATGCC			348–392	11	5	0.818	0.756	0.097
Tro4	F: ^a TGGAGGAGGGACATTGCTG	(AACT) ₅	65	203–211	11	3	0.545	0.525	0.285
	R: CTGGTGTGGCTTGATGGAG			193–211	10	6	0.600	0.705	0.129
Tro5	F: ^a CCACCTGTTCTCTGTTGGC	(AGAT) ₁₃	65	190–223	10	8	1.000	0.810	0.060
	R: TGGACCTGTCAAGTTGAGGC			190–223	10	9	0.900	0.855	0.038
Tro6	F: TGAACACACAGCATCTTTGCTC	(AAAC) ₅	65	440–460	11	10	0.818	0.835	0.049
	R: ^a TTTGCCTGGAAGAAACACC			444–456	6	5	0.833	0.736	0.049
Tro10	F: ^a TGGAGGACAGTGGCAAAGG	(AGAT) ₅	65	238–264	11	6	0.182	0.723	0.124
	R: AGTCTGCTGGCCTTCTGTG			238–253	9	4	0.444	0.636	0.203
Tro11	F: ^a TTTGCATGGCGTCTCGTG	(AC) ₁₂	65	273–283	11	6	0.545	0.773	0.086
	R: AGACAGGAACATAAACATCTCTGC			277–281	9	3	0.222	0.642	0.203
Tro13	F: CACTGACCCAAGACATCAAAGG	(AC) ₄ CG(AC) ₈	65	293–316	10	7	0.600	0.555	0.217
	R: ^a GTCCTGGGCATCTGCAAAC			293–318	10	8	0.800	0.735	0.217
Tro15	F: GGTTCCTGCAAGCACAAAGG	(AGAT) ₇ (AGAC) ₈	65	288–329	12	9	0.333	0.799	0.060
	R: ^a CCGCTGCTTCTGAATGGTG			288–333	10	10	0.700	0.790	0.062
Tro17	F: ACCACAGAGAGAAGAAGCAAG	(AAAC) ₆	65	338–345	11	3	0.455	0.368	0.442
	R: ^a CTTAAAGCCACCGCTCTCG			341–349	10	3	0.600	0.540	0.285
Tro18	F: ^a GGTCTCCAGAAGGAGGGTG	(AG) ₁₄	55	392–409	11	4	0.091 ^b	0.591	0.285
	R: CCACCTCCAGTCCAGAAG			392–418	10	5	0.300	0.660	0.175
Tro19	F: TTCAGTCCTGTCCCACTGC	(CA) ₅ GAG(CA) ₁₃	65	339–358	9	5	0.556	0.451	0.331
	R: ^a CTTGACTTTGCTATGAAACCC			339–358	11	4	0.545	0.492	0.331
Tro20	F: ATCCTTACTGCCAACTGCG	(AAAC) ₅	65	283–293	10	4	0.500 ^b	0.715	0.135
	R: ^a GCCAGAATATTACAAACAGTTCC			283–293	10	4	0.300 ^b	0.655	0.171
Tro21	F: AGAGGCTTAGGAGGGAGGG	(ATCT) ₁₀	65	365–405	9	8	0.333	0.802	0.063
	R: ^a CCCAGATCCTTTGCTGTGC			358–407	10	10	0.800	0.870	0.063
Tro23	F: ^a TGTGCATATAACAAGCAAGCAC	(AAAC) ₅	65	347–359	10	2	0.400	0.480	0.386
	R: GACTGCACTGGAAATCTCACG			347–359	9	2	0.000	0.346	0.488

For each locus the information in the top row refers to *T. scitulus* and the second row refers to *T. polytmus*. The number of individuals genotyped is *N*; *T_a* corresponds to highest annealing temperature for touchdown thermal cycling; size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; *k* is number of alleles observed; *H_o* and *H_e* are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus

^a Indicates CAG tag (5'-CAGTCGGGCGTCATCA-3') label; ^b indicates significant deviations from Hardy–Weinberg expectations after Bonferroni corrections

characteristics of the 15 loci are given in Table 2. We estimated number of alleles per locus (*k*), observed and expected heterozygosity (*H_o* and *H_e*), probability of identity (PI), and tested for deviations from Hardy–Weinberg equilibrium (HWE) using GenAIEx v6.0 (Peakall and Smouse 2006). Only two loci showed significant deviations from expectations under HWE after Bonferroni correction for multiple comparisons. Using GENEPOP version 4.0 (Rousset 2008) we detected no linkage in *T. scitulus* or *T. polytmus* among 105 paired loci comparisons after Bonferroni corrections for multiple comparisons. *Trochilus scitulus*, with an estimated population of 6,000–12,000

individuals, has one of the smallest geographic ranges among western Hemisphere birds. These microsatellite loci will permit researchers to assess the conservation status of this range-restricted species and to measure population genetic variability, the degree of genetic structure, and the amount of introgression occurring between *T. scitulus* and *T. polytmus*.

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