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Eight Polymorphic Microsatellite Loci Developed and Characterized From Townsend's Big-Eared Bat, *Corynorhinus Townsendii*

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PERMANENT GENETIC RESOURCES

Eight polymorphic microsatellite loci developed and characterized from Townsend's big-eared bat, *Corynorhinus townsendii*

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Abstract

Two of the five subspecies of the western big-eared bat, *Corynorhinus townsendii*, are listed as federally endangered with the remaining three being of conservation concern. Knowing the degree of connectivity among populations would aid in the establishment of sound conservation and management plans for this taxon. For this purpose, we have developed and characterized eight polymorphic microsatellite markers.

Keywords: *Corynorhinus townsendii*, microsatellite, Townsend's big-eared bat

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Townsend's big-eared bat, *Corynorhinus townsendii*, is a North American bat of the family Vespertilionidae. There are five subspecies in the USA and Mexico (Piaggio & Perkins 2005) and two, *Corynorhinus townsendii townsendii* and *Corynorhinus townsendii pallescens*, are listed as species of Special Concern or sensitive species by state and federal agencies (Pierson *et al*. 1999), while *Corynorhinus townsendii ingens* and *Corynorhinus townsendii virginianus* are federally listed as Endangered. Although many local, state and federal agencies have developed management plans for *C. townsendii* that include monitoring and protection, little is known about their population structure or connectivity.

Population-level genetic data would significantly increase our understanding of *C. townsendii*. Only five of 15 microsatellite primers designed for other microchiropteran species (Burland *et al*. 1998; Vonhof *et al*. 2002) amplified and were variable in *C. townsendii* (Piaggio *et al*. in press). To increase the number of markers for this species, we developed and characterized eight new microsatellite loci.

Tissue samples were obtained from 25 individuals from Colorado (*C. t. pallescens*) and from 29 individuals from Idaho (*C. t. townsendii*). One individual from Colorado was used in the enrichment and development process. We targeted these localities because most current population-level research of *C. townsendii* is focused on these subspecies. We developed a microsatellite library following methods adapted from Glenn & Schable (2005). Genomic DNA was digested with the enzyme *Rsa*I and fragments were ligated using double-stranded SNX-24 linkers. This library was hybridized to 12 biotinylated microsatellite oligonucleotide probes with dinucleotide and trinucleotide repeats (e.g., GT, CA, TG, CAC and CAG). Hybridized fragments were captured on streptavidin-coated Dynabeads (Dynal Biotech). Microsatellite-enriched fragments were amplified and cloned with the TOPO TA cloning kit (Invitrogen). Insert sequences from 96 colonies were obtained with M13 forward and reverse primers and visualized on an ABI 3730xl genetic analyser (Applied Biosystems). Forty-one clones had recognizable microsatellite sequences, of which 88% (36) had adequate flanking regions to design primers, which was accomplished with Staden package (Staden *et al*. 1998), TROLL (Castelo *et al*. 2002; Martins *et al*. 2006), and web-based Primer 3 (Rozen & Skaletsky 2000) software packages.

Polymerase chain reactions (PCR) were carried out using 0.5 μL each of 10 μM 5′ fluorescently end-labelled primers (Table 1), 3.0 μL nanopure water, 5.0 μL ReddyMix (ABgene), and 1.0 μL of DNA (6–25 ng DNA/μL). The thermal profile

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Table 1 Characteristics of the eight microsatellite loci that were developed and optimized from *Corynorhinus townsendii*

Locus/primer name	Primer sequence (5′–3′); F, forward; R, reverse†	Repeat motif	Size range (bp)	N_A per population	N_A Total	H_O	H_E	Null allele freqs.
Coto_B02F_B02R	F: NED-CCAGCTAGAAGTTGAGAGTCAGA	$(TC)_{14}(AC)_{12}$	CO:150–206	CO:14	18	CO:0.88	CO:0.85	CO:0.000
	R: GTCTCTTGTCACACTTTCTGTCC		ID:152–184	ID:13		ID:0.86	ID:0.91	ID:0.0154
Coto_G07F_G07R	F: HEX-GATGAAGATTCAGCTTATGATGC	$(GT)_9$	CO:314–324	CO:6	6	CO:0.52	CO:0.64	CO:0.063
	R: AGCCCTCTATTTCATACCACAGT		ID:316–324	ID:4		ID:0.45	ID:0.43	ID:0.000
Coto_C02F_H08R	F: FAM-CACCCAGTTGAGAACTATTTGAC	$(GT)_{24}$	CO:171–193	CO:7	8	CO:0.76	CO:0.79	CO:0.009
	R: TTGAAGGGACTAAATGAACTGAA		ID:185–195	ID:6		ID:0.38	ID:0.66*	ID:0.162§‡
Coto_G12F_B11R	F: HEX-TGCAAGTCTTAACTCACCTCATT	$(AC)_{23}$	CO:236–296	CO:17	20	CO:0.76	CO:0.92*	CO:0.076§
	R: CCACTCCCCTAGTTTTCATCTAC		ID:238–268	ID:11		ID:0.86	ID:0.87	ID:0.000
Coto_H10F_E11R	F: FAM-AGGCAAACTTTCTTACAGTTGA	$(GT)_{20}$	CO:242–282	CO:13	13	CO:0.72	CO:0.87*	CO:0.072
	TCTTCTTCCATTTTCCTTCAC		ID:250–272	ID:7		ID:0.48	ID:0.77*	ID:0.157§‡
Coto_E09F_B10R	F: HEX-CTACCCTTCCTCTCTTCTTTCTG	$(TG)_{20}(GA)_{13}$	CO:191–235	CO:13	16	CO:0.96	CO:0.88	CO:0.000
	R: ATTTCTCCCTATCTCCATCACTC		ID:203–229	ID:11		ID:0.76	ID:0.86	ID:0.056
Coto_F09F_F10R	F: FAM-GAGAAGGAAGAGAAACTGGTGTT	$(AC)_{23}$	CO:192–222	CO:10	12	CO:0.64	CO:0.84	CO:0.101§
	R: TACTAAAGAACCTTGACAGTGGC		ID:192–220	ID:11		ID:0.83	ID:0.88	ID:0.020
Coto_G02F_H10R	F: FAM-AGAGTGCTTTTATGGGCAAAT	$(GT)_{20}$	CO:188–208	CO:10	11	CO:0.88	CO:0.84	CO:0.000
	R: TGCTTGTAGTTCCCTTTCCTT		ID:172–204	ID:10		ID:0.90	ID:0.83	ID:0.000

†GenBank Accession nos EU262763–EU262770. N_A, mean number of alleles per locus; H_O, observed and H_E, expected heterozygosities; *, indicates significant deviation from Hardy–Weinberg Equilibrium after Bonferroni correction (Rice 1989). Null allele frequencies are based on Brookfield2 estimates from Micro-Checker software; §indicates significant evidence of null alleles with 95% confidence intervals; and ‡indicates significance with 99% confidence interval.

for all loci was an initial denaturation at 94 °C for 2 min (B02, G07 and C02H08), 3 min (G12B11, H10E11 and E09B10) or 4 min (F09F10 and G02H10) followed by 35 cycles of 94 °C for 30 s, annealing at 51 °C (G12B11 and H10E11), 52 °C (E09B10, F09F10 and G02H10) or 55 °C (B02, G07 and C02H08) for 45 s, and extension at 72 °C for 45 s. Cycling was followed with a 7-min extension at 72 °C (B02, G07 and C02H08) or a 30-min extension at 60 °C. Of the 36 primer pairs that were designed and tested, eight pairs amplified and were variable in both populations.

PCR products were genotyped on an ABI 3130 genetic analyser and analysed with STRand software (Hughes 1998; Locke *et al*. 2000). Genotypic disequilibrium between pairs of loci was tested using FSTAT 2.9.3 (Goudet 2001). Hardy–Weinberg Equilibrium (HWE), number of alleles and expected and observed heterozygosities were estimated in Arlequin (Excoffier *et al*. 2005) and each locus was tested for null alleles using Micro-Checker (van Oosterhout *et al*. 2004). We found no evidence of linkage disequilibrium between loci. The number of alleles ranged from six to 20 per locus (Table 1). Two loci in each population demonstrated significant deviations from HWE (Table 1) after sequential Bonferroni correction (Rice 1989); however, only locus Coto_H10F_E11R violated HWE in each population. Moderate (0.05–0.20, Chapuis & Estoup 2007) null allele frequencies were found at some loci (Table 1) which could be the result of a Wahlund effect or the presence of true null alleles, although we have no evidence of the latter since all individuals yielded amplification products (i.e. we found no null homozygotes). These eight new markers, plus previously characterized markers developed from other Vespertilionids (Piaggio *et al*. in press), now make it possible to undertake detailed population genetic studies of *C. townsendii*.

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References

Burland TM, Barrrat EM, Racey PA (1998) Isolation and characterization of microsatellite loci in the brown long-eared bat, *Plecotus auritus*, and cross-species amplification within the family vespertilionidae. *Molecular Ecology*, **7**, 133–140.

Castelo AT, Martins WS, Gao GR (2002) Tandem repeat occurrence locator. *Bioinformatics*, **8**, 634–636.

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, **24**, 621–631.

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

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.

Goudet J (2001) FSTAT, *Version 2.9.3, A program to estimate and test gene diversities and fixation indices (updated from goudet 1995)*. University of Lausanne, Switzerland. Available from URL: http://www.unil.ch/izea/softwares/fstat.html.

Hughes S (1998) *STRand Nucleic Acid Analysis Software*. Regents of the University of California, Davis, California. Available from URL: (http://www.vgl.ucdavis.edu/STRand).

Locke M, Baack E, Toonen RJ (2000) *The STRand Manual*. Available from URL: (http://www.vgl.ucdavis.edu/STRand/docs/STRand_manual.pdf).

Martins W, de Sousa D, Proite K, Guimarães P, Moretzsohn M, Bertoli D (2006) New softwares for automated microsatellite marker development. *Nucleic Acids Research*, **34**, E31.

van Oosterhout C, Hutchison WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535.

Piaggio AJ, Navo KW, Stihler CW (in press) Intraspecific comparison of population structure, genetic diversity, and dispersal among three subspecies of Townsend's big-eared bats, *Corynorhinus townsendii townsendii*, *C. t. pallescens*, and the endangered *C. t. virginianus*. *Conservation Genetics*. doi: 10.1007/s10592-008-9542-0.

Piaggio AJ, Perkins SL (2005) Molecular phylogeny of North American long-eared bats (Vespertilionidae: *corynorhinus*): inter- and intraspecific relationships inferred from mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution*, **37**, 762–775.

Pierson ED, Wackenhut MC, Altenbach JS *et al*. (1999) *Species Conservation Assessment and Strategy for Townsend's Big-eared Bat* (Corynorhinus townsendii townsendii *and* corynorhinus townsendii pallescens). Idaho Conservation Effort. Idaho Department of Fish and Game, Boise, Idaho.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rozen S, Skaletsky H (2000) Primer 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. The Humana Press Inc., Totowa, New Jersey.

Staden R, Beal KF, Bonfield JK (1998) The Staden Package. In: *Computer Methods in Molecular Biology, Bioinformatics Methods and Protocols* (eds Misener S, Krawetz S), Vol. 132, pp. 115–130. The Humana Press Inc., Totowa, New Jersey.

Vonhof MJ, Davis CS, Fenton MB, Strobeck C (2002) Characterization of dinucleotide microsatellite loci in big brown bats (*Eptesicus fuscus*), and their use in other North American verspertilionid bats. *Molecular Ecology Notes*, **2**, 167–170.