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Jay F. Storz
University of Nebraska - Lincoln, jstorz2@unl.edu

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Published in *Molecular Ecology* 9:12 (December 2000), pp. 2199–2201; doi 10.1046/j.1365-294X.2000.105324.x
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http://www3.interscience.wiley.com/journal/117989598/home

Variation at tri- and tetranucleotide repeat microsatellite loci in the fruit bat genus *Cynopterus* (Chiroptera: Pteropodidae)

J. F. Storz

Department of Biology, Boston University, 5 Cummington Street, Boston, MA, 02215, USA

Abstract

There is considerable uncertainty surrounding the taxonomic relationship between *Cynopterus sphinx* and *C. brachyotis*, and the status of the many named forms within *C. sphinx* (Storz & Kunz 1999). Polymorphic microsatellite markers for cynopterine fruit bats would greatly aid efforts to elucidate species boundaries and genetic correlates of morphological variation within species. To assess levels of variation in *C. sphinx* and *C. brachyotis*, microsatellite genotypes were obtained for a total of 731 bats (620 *C. sphinx* and 111 *C. brachyotis*).

Species in the fruit bat genus *Cynopterus* (Chiroptera: Pteropodidae) are widely distributed across the Indomalayan region (Corbet & Hill 1992). The two most geographically widespread members of the genus are the short-nosed fruit bat (*Cynopterus sphinx*) and the lesser dog-faced fruit bat (*C. brachyotis*). There is considerable uncertainty surrounding the taxonomic relationship between *C. sphinx* and *C. brachyotis*, and the status of the many named forms within *C. sphinx* (Storz & Kunz 1999). The availability of polymorphic microsatellite markers for cynopterine fruit bats would greatly aid efforts to elucidate species boundaries and genetic correlates of morphological variation within species. The primary motivation for developing microsatellite markers for *C. sphinx* was to investigate the influence of polygynous mating and harem social organization on population genetic structure (Storz *et al*. 2000a,b). Efforts are also underway to investigate comparative levels of geographical differentiation in body size and microsatellites in Indian populations of *C. sphinx* (see Storz *et al*. 2000c).

Genomic DNA was isolated from wing-membrane biopsy samples of *C. sphinx* using QIAamp extraction columns (Qiagen). Microsatellite loci were isolated from three genomic libraries enriched for tri- and tetranucleotide repeat motifs following the methods of Jones *et al*. (2000). Following partial digestion with a combination of seven blunt-end restriction endonucleases, size-selected genomic fragments (350–650 bp) were ligated to 20 bp oligonucleotide adapters that contained a *Hin*dIII restriction site. Genomic fragments were subjected to magnetic bead capture using the following 5′-biotinylated oligonucleotides: ATG_8, $CATC_8$, and $TAGA_8$ (Integrated DNA Technologies). Captured fragments were Polymerase chain reaction (PCR)-amplified using primers complementary to the adapter sequences. The resultant products were ligated into the *Hin*dIII restriction site of the plasmid pUC19. Recombinant plasmids were transfected into *Escherichia coli* strain DH5α by electroporation. Colonies were screened according to the protocol of Jones *et al*. (2000). Following PCR amplification, a total of 27 clones in the size range 350–650 bp were sequenced using Prism Cycle Sequencing kits and labelled dNTP's (Applied Biosystems). Sequences were resolved on an ABI 373 automated sequencer (Applied Biosystems).

All clone sequences contained at least one microsatellite locus. Primers were designed for a total of 21 microsatellite loci using the program Designer PCR version 1.03 (Research Genetics). Primer pairs were tested by amplifying DNA from eight individual *C. sphinx* sampled from various localities in peninsular India. Sixteen primer pairs amplified variable PCR products, as revealed by electrophoresis in 3.5% agarose gels followed by ethidium-bromide staining. Nine primer pairs that yielded the most consistent results were selected for further testing, and the forward primer of each pair was fluorescently labelled with 6-FAM, TET, or HEX (Applied Biosystems). PCR was performed using 20 μm of each primer, 5 mm dNTP's, 25 mm $MgCL_2$, 0.012 U of AmpliTaq DNA polymerase (Applied Biosystems), 10× PCR buffer (100 mm Tris-HCl buffer, pH 8.3, 500 mm KCl), ddH_2O, and 10 ng of template DNA in a total reaction volume of 15 μL. Thermal cycling was performed in a GeneAmp PCR System 9700 (Applied Biosystems)

Table 1. Primer sequences and characteristics of nine tri- and tetranucleotide repeat microsatellite loci used in the genetic analysis of *Cynopterus sphinx* from Pune, India (18°32′ N, 73°51′ E). Repeat numbers refer to cloned alleles and plus signs denote sequence interruptions between tracts of ≥2 repeat units. T_a, annealing temperature; *n*, number of bats genotyped per locus; N_A, number of alleles per locus; H_O, observed heterozygosity; H_E, expected heterozygosity. Loci CSP-4, CSP-6, and CSP-7 segregated subsets of alleles that differed by 2 bp rather than 4 bp. It is not known whether this was due to interruptions within the array of tetranucleotide repeats or insertions/deletions in flanking sequences

Locus	Primer sequences (5′–3′)	Repeat motif	$T_{a\ (°C)}$	Allele size range	*n*	N_A	H_O	H_E	GenBank accession no.
CSP-1	F: GGGGAAACAAAGGAAAAGT R: AGAAAAGTGAGACCTGACAGAG	$(ATC)_{2+4+5+3}$	55	191–218	431	9	0.73	0.71	AF289705
CSP-2	F: CCCGATGATGGATTTCTAC R: CTGGGCTGTAATAAGTGCTC	$(ATC)_{3+13+2}$	57	113–134	431	7	0.78	0.74	AF289706
CSP-3	F: AACACCACCACCACCACTA R: TGTGGCAACAACTCAGACA	$(ATC)_8$	57	95–107	431	5	0.37	0.38	AF289707
CSP-4	F: GAGAGGACTCCGTTCTTTTAGA R: ATGGATGGGTGACAGATGA	$(CATC)_{12}$	57	139–163	431	10	0.79	0.78	AF289708
CSP-5	F: CATTTGTGGTAACTTGTGATG R: ACAGCAGTGAAACTTCCTCT	$(ATGG)_8(ACGG)_4$	55	110–170	431	12	0.76	0.73	AF289709
CSP-6	F: TGAGGAGTGTTCCCGAGTA R: AAAAATCCCAACGCACAG	$(CATC)_{10}$	55	127–219	431	14	0.81	0.85	AF289710
CSP-7	F: CCACAAGAAACCCAATACTAAC R: CTTCCTAGCCCCACAATC	$(TATC)_{3+8}$	57	231–265	431	17	0.82	0.82	AF289711
CSP-8	F: CCAGGTGTTATGGGTTGA R: TGAGGTGTTGGGAGTTTG	$(TAGA)_{3+3+5+11}$	57	150–202	420	14	0.75	0.74	AF289712
CSP-9	F: GGTCCCTCTGCTCTTCAG R: AGCATGGGGAATATAGTCAAG	$(TAGA)_{3+7}$	57	278–298	431	5	0.49	0.47	AF289713

Table 2. Summary statistics for five tri- and tetranucleotide repeat microsatellite markers used in the genetic analysis of *Cynopterus sphinx* (from localities <18° N latitude) and *C. brachyotis* in peninsular India. *n*, number of bats genotyped per locus; N_A, number of alleles per locus; H_O, observed heterozygosity; H_E, expected heterozygosity. In both species, locus CSP-7 segregated multiple alleles with lengths that differed by 2 bp, even though the cloned allele was a $(TATC)_n$ repeat.

	Cynopterus sphinx (southern localities)					*Cynopterus brachyotis*				
Locus	Allele size range	*n*	N_A*	H_O	H_E	Allele size range	*n*	N_A	H_O	H_E
CSP-1	191–224	189	12	0.79	0.86	176–227	111	12	0.61	0.69
CSP-2	119–134	189	6(7)	0.74	0.71	101	20	1	0	0
CSP-5	130–190	189	11(16)	0.82	0.81	110–166	111	11	0.29	0.34
CSP-7	227–285	189	21	0.78	0.84	229–263	111	17	0.72	0.86
CSP-9	286–302	189	5(6)	0.55	0.60	270–282	111	4	0.45	0.49

*Numbers in parentheses refer to numbers of alleles observed in the complete sample of *C. sphinx*, from Pune and the southern localities (*n* = 620 bats).

under the following conditions: initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55–57 °C (Table 1) for 45 s, and extension at 72 °C for 50 s (with a final extension at 72° for 2 min 30 s). Allele sizes were quantified using an ABI Prism 377 automated sequencer and analysed using genescan software (PE Applied Biosystems).

To assess levels of variation in *C. sphinx* and *C. brachyotis*, microsatellite genotypes were obtained for a total of 731 bats (620 *C. sphinx* and 111 *C. brachyotis*). A total of 431 adults and juveniles of *C. sphinx* from a single population in Pune, India (Storz *et al*. 2000b) were genotyped at all nine loci (Table 1). A total of 185 known mother–offspring pairs were examined, and no genotypic mismatches were observed at any locus.

Using a subset of five microsatellite loci, an additional 189 *C. sphinx* that were sampled from localities in southwestern India (see Storz *et al*. 2000c), and 111 *C. brachyotis* that were sampled from high-elevation wet forest sites in the Western Ghats were genotyped. In the total sample of *C. sphinx* (*n* = 620), mean number of alleles per locus was 12.4 (range = 6–21; Table 2). Although preliminary screening of 20 individuals indicated that CSP-2 was monomorphic in *C. brachyotis*, the remaining four loci segregated 4–17 alleles. Relative to *C. sphinx*, homologous loci in *C. brachyotis* segregated alleles that were generally shorter in length (Table 2). These markers should open up many new opportunities for studying the population biology and phylogeography of Old World fruit bats.

Acknowledgments

Funding was provided by the National Geographic Society, the Lubee Foundation, and the National Science Foundation (DEB 97–01057). I thank H. R. Bhat, J. Balasingh, G. Marimuthu, P. T. Nathan, A. A. Prakash, and D. P. Swami Doss for assistance with field collections.

References

Corbet, G. B. & Hill, J. E. (1992) *The mammals of the Indomalayan Region: a systematic review*. British Museum Publications, Oxford University Press, Oxford.

Jones, K. C., Levine, K. F., & Banks, J. D. (2000) DNA-based genetic markers in black-tailed and mule deer for forensic applications. *California Fish and Game,* **86**, in press.

Storz, J. F., Balasingh, J., & Bhat, H. R., et al. (2000c) Clinal variation in body-size and sexual dimorphism in an Indian fruit bat, *Cynopterus sphinx* (Chiroptera: Pteropodidae). *Biological Journal of the Linnean Society,* **71**, in press.

Storz, J. F., Balasingh, J., Nathan, P. T., Emmanuel, K., & Kunz, T. H. (2000a) Dispersion and site-fidelity in a tent-roosting population of the short-nosed fruit bat (*Cynopterus sphinx*) in southern India. *Journal of Tropical Ecology,* **16**, 117–131.

Storz, J. F., Bhat, H. R., & Kunz, T. H. (2000b) Social structure of a polygynous tent-making bat, *Cynopterus sphinx* (Megachiroptera). *Journal of Zoology* (London), **251**, 151–165.

Storz, J. F., & Kunz, T. H. (1999) Cynopterus sphinx. *Mammalian Species,* **613**, 1–8.