*Eight novel polymorphic microsatellites in the hispid pocket mouse (*Chaetodipus hispidus*) and cross-amplification in other Perognathinae species (Rodentia: Heteromyidae)*

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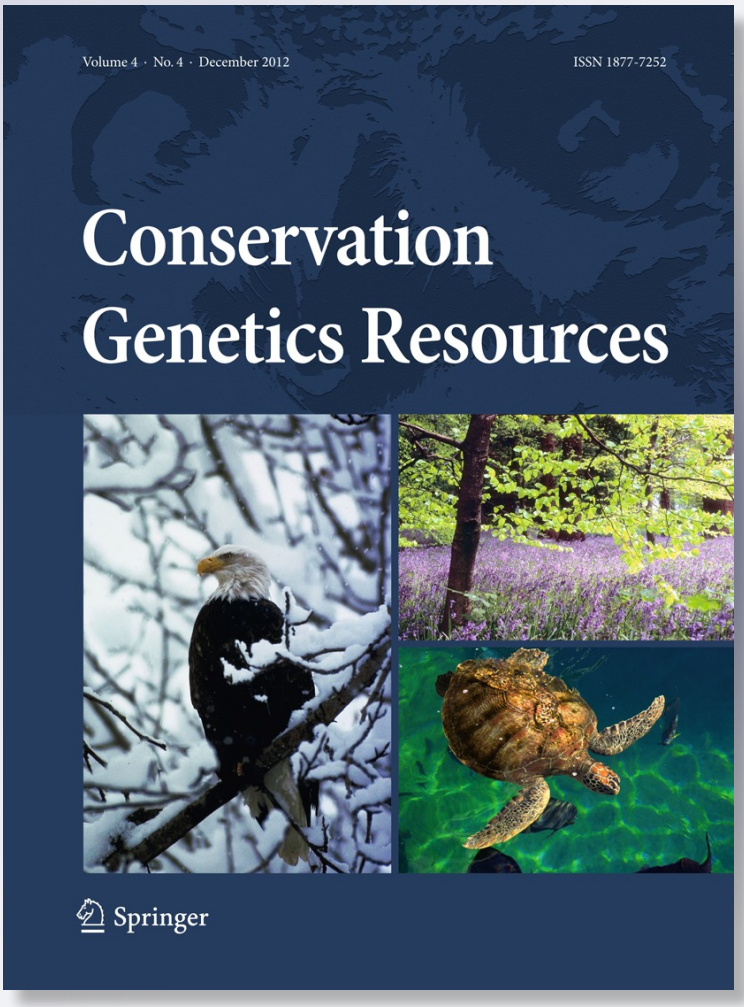

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TECHNICAL NOTE

Eight novel polymorphic microsatellites in the hispid pocket mouse (*Chaetodipus hispidus*) and cross-amplification in other Perognathinae species (Rodentia: Heteromyidae)

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Abstract Twenty-eight nuclear microsatellites were isolated for the hispid pocket mouse, *Chaetodipus hispidus*, and characterized in 33 individuals from four genetically distinct groups throughout the geographic range of the species. Dinucleotide and trinucleotide microsatellite repeat motifs were screened, and of the 28 primer pairs created, eight amplified and were polymorphic. After correction for multiple tests, no loci deviated significantly from Hardy–Weinberg expectations. Working primers also were tested in other closely related species found within the subfamily Perognathinae. The microsatellite markers characterized in this study will be beneficial towards future population genetic research within the hispid pocket mouse and other Perognathinae rodents.

Keywords *Chaetodipus hispidus* · Heteromyidae · Microsatellites · Population genetics · Rodentia

The hispid pocket mouse, *Chaetodipus hispidus* (Heteromyidae: Rodentia), occupies a large geographic range extending from North Dakota (Geluso and Wright 2010) to México. Across this extensive geographic distribution are several barriers to gene flow, such as the Deming Plains (Hunt 1983), Balcones Escarpment (Gehlbach 1991), and Southern Coahuila filter-barrier (Baker 1956). A recent phylogeographic study of *C. hispidus* found four genetically distinct groups whose geographic limits coincided with these major geographic features (Andersen and Light 2012). Based on these results, Andersen and Light (2012) revised the systematics within the hispid pocket mouse, recognizing the four genetic groups as three subspecies and one *incertae sedis*. Although this study elucidated the evolutionary history of this widespread species, it used only maternally-inherited markers (i.e., mitochondrial data) and inclusion of nuclear markers (i.e., microsatellites) are necessary to provide a better understanding of the population structure and dynamics within *C. hispidus* as they relate to historical biogeography. Herein, we characterize eight microsatellite markers for the hispid pocket mouse and determine the utility of these loci across several Perognathinae (heteromyid subfamily which includes the genera *Chaetodipus* and *Perognathus*) species.

Creation of the enriched genomic library followed the protocol outlined in Welborn et al. (2012). DNA fragments were hybridized with di- and tri-oligonucleotides, incubated with streptavidin-coated magnetic M-280 Dynabeads (Invitrogen), and rinsed. This enriched DNA was increased in quantity through PCR amplification and cleaned with a PCR purification kit (QIAGEN Inc.; Valencia, California). Cleaned products were ligated into pCR®2.1 TOPO® vectors (Invitrogen) and transformed into *Escherichia coli* (One Shot® TOP10 Chemically Competent Cells, Invitrogen). Cells were dispersed onto X-Gal/LB/agar plates treated with ampicillin and incubated overnight at 37 °C. Positive clones (white) were sent to the University of Florida DNA Sequencing Core Laboratory (Gainesville, Florida) for sequencing with the M13 forward primer.

Sequences were edited using SEQUENCER 4.1 (Gene Codes) and screened for microsatellites. Twenty-eight primer pairs were developed using Primer3 (http://frodo.wi.mit.edu/primer3/) and

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Table 1 Summary data for 8 polymorphic microsatellite loci characterized for the hispid pocket mouse, *Chaetodipus hispidus*

Locus	Primer sequence (5′–3′)[a]	Repeat Motif	Size (bp)[b]	N	N_A	Range	H_E	H_O	P_{HW}
Chis01	AATTAAGGGCAGGAATAGGC CACACACAGAAAGAGTGAAGAGG	$(AC)_{21}$	193	28	16	193–243	0.824–0.924	0.625–0.833	0.103–0.469
Chis04	GCCGGCTGATTTTGTGAA GGGTTAGACTTCCACCAAGG	$(TG)_{20}$	227	33	15	227–265	0.883–0.948	0.714–1.000	0.131–0.474
Chis05	TGGGAAAAGAAGGCAGTGG TTTCAACCCCTATTGGATGC	$(GT)_{19}$	173	33	13	173–245	0.725–0.902	0.286–0.889	0.008–0.869
Chis11	AACTTCCACATATGGGACTGG TGCACAACTGTGTTTGTTGC	$(AC)_7$	191	33	11	191–217	0.503–0.857	0.429–0.556	0.019–1.000
Chis14	CATTCCATCACCCAAAATCC TCAAGGAATTGTTCTCATATACCC	$(CA)_{15}$	238	30	16	236–276	0.767–0.956	0.625–0.889	0.072–0.771
Chis15	ATCACGTTTCTGTCTACAGTGC AAAACTTAACTACCTTTGTTGTGG	$(CA)_9$	250	32	8	250–284	0.440–0.782	0.286–0.556	0.169–0.443
Chis18	CAGCGTAAGCAGGAAAGTCC TGGACTGTTAATTTATACAAATGTTGG	$(CA)_{18}$	231	25	16	229–283	0.867–0.934	0.600–1.000	0.177–1.00
Chis22	GACTACTTGCTTAGCATTTATGAGACC GGACTGTAATGACAATTTGAAACG	$(AC)_{21}$	245	25	15	241–275	0.893–0.934	0.750–1.000	0.313–1.000

N is the number of individuals assayed, N_A is the number of alleles detected, *Range* refers to size range in base pairs of alleles, including 21-bp "tail" sequence, H_E is the range of expected heterozygosity between the populations, H_O is the range of observed heterozygosity between the populations, P_{HW} represents the range of probabilities of deviation from the expectations of Hardy–Weinberg equilibrium between the populations

[a] Primer sequences are forward (top) and reverse (bottom)

[b] Clone size in base pairs (bp) of the allele in the sequenced clone

tested for amplification and polymorphisms. Polymerase-chain-reactions (PCR) amplifications followed Karlsson et al. (2008) and were performed in 10 μL reactions containing 3.7 μL Emerald Master Mix (Takara Bio Inc.), 4.25 μL water, 0.5 μL fluorescently dye-labeled "tail" primer (6-FAM; 5′-GCCTCG TTTATCAGATGTGGA-3′; 10 μM), 0.05 μL forward primer with additional "tail" sequence (Integrated DNA Technologies; 10 μM), 0.5 μL reverse primer (10 μM), and 1 μL DNA. Amplified DNA from each PCR reaction was combined with a 400 HD Rox size-standard DNA ladder (Applied Biosystems) and electrophoresed on an ABI PRISM 377 DNA Sequencer (Applied Biosystems). Sizes of microsatellite fragments were visualized in GENESCAN v. 3.1.2 (Applied Biosystems) and assessed using GENOTYPER v. 2.5 (Applied Biosystems). Of the originally developed 28 primer pairs, 8 were amplified successfully and polymorphic (Table 1).

The 8 polymorphic loci were assessed in 33 *C. hispidus* specimens from four genetically distinct groups identified in Andersen and Light (2012). These groups corresponded to *C. h. conditi* ($n = 8$), *C. h. hispidus* ($n = 9$), *C. h. paradoxus* North ($n = 7$), and *C. h. paradoxus* South ($n = 9$). Criteria for breaking *C. h. paradoxus* into 2 groups were based on the large geographic range (roughly 1,450 km) and well supported phylogenetic subclades (Andersen and Light 2012). The *insertae sedis* genetic group from central México was not assessed due to insufficient sample size. GENEPOP v. 2.5 (Rousset 2008) and Arlequin v. 3.5 (Excoffier et al. 2005) were used to calculate number of alleles for each locus and expected heterozygosity (H_E), observed heterozygosity (H_O), probability of conformance to Hardy–Weinberg equilibrium (P_{HW}), and linkage-disequilibrium for each genetic group (Table 1). After correction for multiple tests (Rice 1989), no microsatellite loci deviated significantly from Hardy–Weinberg expectations. Additionally, no loci exhibited significant signs of linkage disequilibrium.

These eight microsatellite markers also were tested against 11 other Perognathinae species. Amplification success was fairly high within other *Chaetodipus* species (>63 %); however, there was a decreased level of amplification success within *Perognathus* (13–38 %; Table 2). Although *Chaetodipus* and *Perognathus* once belonged in the same genus (Hafner and Hafner 1983), recent studies support that these two genera are quite distinct and diverged from each other over 20 million years ago (Hafner et al. 2007). The failure of many of these *Chaetodipus*-specific microsatellite markers to amplify in *Perognathus* species is therefore not surprising. However, even the limited amplification success within *Perognathus* indicates the microsatellite markers characterized in this study will be helpful for future research regarding the population

Table 2 Cross amplification of *Chaetodipus hispidus* microsatellite loci across 11 pocket mouse species

	Chis01	Chis04	Chis05	Chis11	Chis14	Chis15	Chis18	Chis22
Chaetodipus baileyi	−	+	+	+	+	−	−	+
Chaetodipus californicus	−	+	+	+	+	+	+	+
Chaetodipus eremicus	+	+	+	+	+	−	+	+
Chaetodipus formosus	−	−	+	+	+	+	+	+
Chaetodipus intermedius	+	+	+	+	−	+	−	+
Chaetodipus spinatus	+	+	+	+	+	+	−	−
Perognathus flavescens	−	−	+	+	−	−	−	−
Perognathus flavus	−	−	+	+	−	−	−	−
Perognathus longimembris	+	−	+	+	−	−	−	−
Perognathus merriami	−	−	−	+	−	−	−	−
Perognathus parvus	+	−	−	−	−	−	−	−

Successful amplification (*plus* signs; failed amplifications are indicated by *minus* signs) was determined by generation of PCR products of the expected size (determined by agarose gel electrophoresis)

genetics of the hispid pocket mouse as well as other Perognathinae rodents.

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