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Sarah R. Welborn, Mark A. Renshaw & Jessica E. Light

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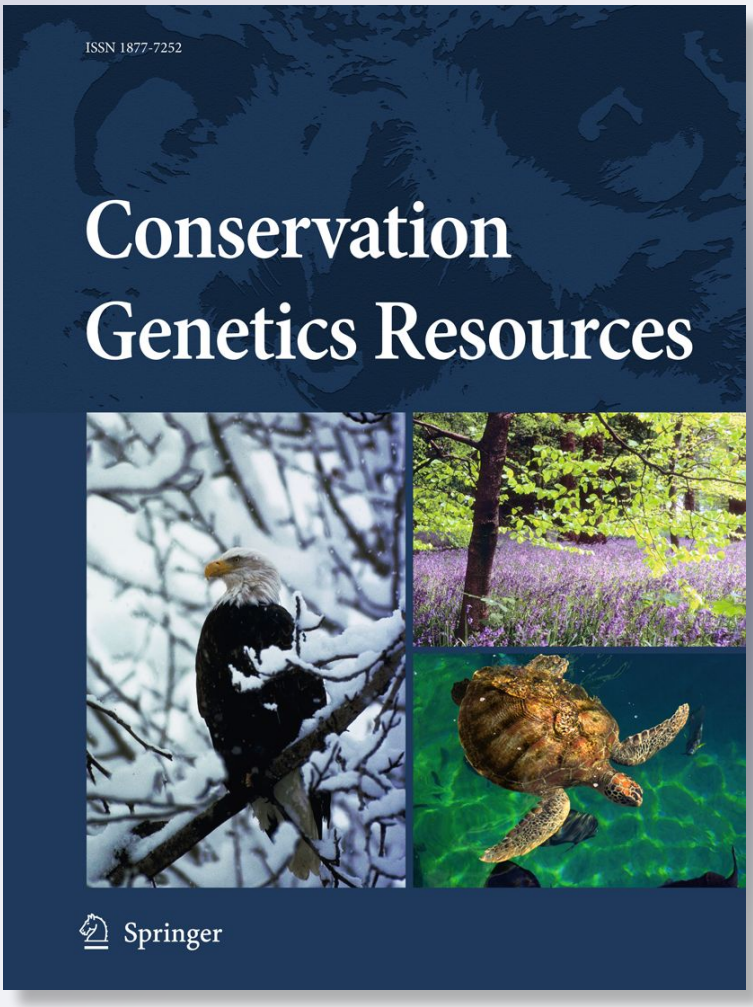

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TECHNICAL NOTE

Characterization of 10 polymorphic loci in the Baird's pocket gopher (*Geomys breviceps*) and cross-amplification in other gopher species

Sarah R. Welborn · Mark A. Renshaw · Jessica E. Light

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Abstract Ten polymorphic microsatellite loci were isolated and characterized for the Baird's pocket gopher, *Geomys breviceps*. Each locus was screened in 40 individuals from 4 different populations found throughout College Station, Texas. The number of alleles per locus ranged from 2 to 16 and observed heterozygosity ranged from 0.100 to 1.000. Deviation from Hardy–Weinberg and linkage disequilibrium was also analyzed. These 10 microsatellite primers, as well as 4 previously described primers developed for *Thomomys*, also were tested for amplification in additional species of pocket gophers. These microsatellite loci will provide useful tools in population genetic studies of *G. breviceps*, as well as other pocket gopher species.

Keywords *Geomys breviceps* · Pocket gophers · Geomyidae · Microsatellites · Population genetics · Rodentia

Pocket gophers (Rodentia: Geomyidae) are solitary, fossorial New World rodents, and are highly modified for an underground life. Modifications include fur lined cheek pouches for food transportation, increased muscle mass at the anterior ends of their bodies, small eyes, reduced ears, and elongated claws for digging. Pocket gophers spend the majority of their lives in their burrow systems (Sulentich et al. 1991), have low vagility (Sudman et al. 2006), and are often distributed in isolated populations (Patton 1972). These characteristics are relatively rare among mammals and little is known about the population genetics of solitary species with isolated populations (Lacey 2001). Thus, designing microsatellite loci for pocket gopher species may provide markers that can be used to gain a better understanding of population dynamics in these rare, solitary species with low vagility. Herein, we develop microsatellites for the Baird's pocket gopher, *Geomys breviceps*, and determine the utility of these loci across several pocket gopher species.

Total DNA was extracted from each individual using the DNeasy Tissue Kit (QIAGEN Inc.; Valencia, California) and digested with the restriction enzymes *Rsa*I and *Hae*III (New England Biolabs). Fragments of 500–1,000 base pairs were dissected from an agarose gel, purified with a DNA gel extraction kit (QIAGEN Inc.; Valencia, California), and adaptors were attached to each fragment with T4 DNA ligase (Promega). DNA fragments were hybridized with di, tri, and tetra oligonucleotides, incubated with streptavidin-coated magnetic M-280 Dynabeads (Invitrogen), and rinsed. This enriched DNA was increased in quantity though PCR amplification and cleaned with 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/IPTG plates treated with ampicillin and incubated overnight at 37°C. Positive clones (white) were picked using sterilized toothpicks and placed into 96 well culture plates filled with LB (treated with ampicillin). Plates were covered and incubated overnight at 37°C to increase culture density. Subsamples of the library were placed into new 96 well culture plates filled with LB (with ampicillin), incubated overnight, frozen in a −80°C freezer and sent to the University of Florida DNA Sequencing Core Laboratory

S. R. Welborn · M. A. Renshaw · J. E. Light (✉)
Department of Wildlife and Fisheries Sciences,
Texas A&M University, College Station, TX 77843, USA
e-mail: jlight2@tamu.edu

Table 1 Summary data for 10 polymorphic microsatellite loci characterized for the Baird's pocket gopher, *Geomys breviceps*

Locus	Primer sequence (5′–3′)[a]	Repeat motif	Size (bp)[b]	N[c]	N_A[d]	Range[e]	H_E[f]	H_O[g]	P_{HW}[h]
Gbr06	CTCATCGCTGGGGAGAGA CTTGGATCTGGGGATCCTTT	$(CA)_{15}$	221	40	10	234–288	0.689–0.789	0.600–1.000	0.052–0.503
Gbr09	TGGCTCAAGTGAGAGCATCA GGAGGAGGAACAAGCAATCA	$(CA)_{18}$	214	40	11	210–252	0.616–0.857	0.700–1.000	0.027–0.633
Gbr10	TAGTGCATGCTCTGGCTTTG AAATGCCCTCCAGAAGGAAC	$(CA)_{19}$	235	40	11	216–282	0.552–0.847	0.500–0.700	0.103–1.000
Gbr14	GGACCTGGTGACACTGGTTT TTCTTATGCACCCCCTTTCA	$(GT)_{14}$	203	40	5	201–308	0.526–0.668	0.600–1.000	0.006–1.000
Gbr15	CTCTCCCTCAGCTCAGCAGT GTGTCCAGCCCAGTTATGCT	$(GT)_{14}$	212	40	14	222–258	0.789–0.800	0.700–0.900	0.127–0.928
Gbr25	CCTGGGAGACTAGCATGAGG CACAAGAAAGCCAGAAGTGC	$(GT)_{27}$	227	40	16	237–260	0.621–0.842	0.600–0.800	0.212–0.593
Gbr26	TGGAATCACCAACAAGCAGA TAACAGGTGTGAGGCGACAG	$(CA)_{20}$	240	40	16	250–274	0.763–0.873	0.300–0.900	0.005–0.469
Gbr27	TGATGACACGCTGACTTTCC TGGAGGTGTAGCTCAAGTGG	$(GT)_{10}$	229	40	5	243–258	0.189–0.532	0.200–0.700	0.479–1.000
Gbr33	GTGGTAGTGGTGGTGTTTGC ACACTGGAGTGTCTCATGTGG	$(AAGG)_{14}$	227	40	12	228–280	0.689–0.821	0.300–0.800	0.009–0.884
Gbr36	CCACCAGAGAAATCAAAGAAGG AGCCACTGCTCAACTTCAGG	$(AGGC)_4$	173	40	2	193–197	0.100–0.505	0.100–0.800	0.172–1.000

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

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

[c] N is the number of individuals assayed

[d] N_A is the number of alleles detected

[e] Range refers to size range in base pairs of alleles

[f] H_E is the range of expected heterozygosity between the populations

[g] H_O is the range of observed heterozygosity between the populations

[h] P_{HW} represents the range of probabilities of deviation from the expectations of Hardy-Weinberg equilibrium between the populations

(Gainesville, Florida) to be sequenced using ABI Prism BigDye Terminator cycle sequencing protocols (Applied Biosystems, Foster City, CA, USA).

Sequences were screened for microsatellites and 35 primer pairs were developed using Primer3 (http://frodo.wi.mit.edu/primer3/) and tested for amplification and polymorphisms. PCR amplifications 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 (56-FAM; 5′-GCCTCGTTTATCAGATGT GGA; 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. All polymerase chain reactions of microsatellite loci were accomplished as described by (Karlsson et al. 2008). PCR products were loaded onto a polyacrylamide gel and run using an ABI Prism 377 DNA Sequencer (Biosystematics Center, College Station, TX, USA). Results were analyzed using the Genescan® 400 ROX-Size Standard (Applied Biosystems) and Genescan 3.1.2 (Applied Biosystems). Allele sizes were called using the Genotyper® software, version 2.5 (Applied Biosystems). Of the originally developed 35 primer pairs, 10 were amplified successfully and were polymorphic (Table 1).

Variability of the 10 polymorphic loci was assessed in 40 *G. breviceps* specimens from 4 different populations (10 pocket gophers per population) found in the western portion of College Station, TX, USA. Specimen voucher information (museum numbers and collection localities) is available from Jessica E. Light. (all specimens are housed in the Texas Cooperative Wildlife Collection at Texas A&M University). We estimated the number of alleles per locus (N_A) across all 40 individuals, while expected heterozygosity (H_E), observed heterozygosity (H_O), probability of deviation from the expectations of Hardy–Weinberg equilibrium (P_{HW}), and linkage disequilibrium were estimated on a per population basis (Arlequin 3.5.1.2; Excoffier et al. 2005). Bonferroni correction (Rice 1989) was utilized

Table 2 Success amplifying *Geomys breviceps* (designed here) and *Thomomys* (Steinberg 1999) microsatellite loci across 18 pocket gopher species

	Gbr06	Gbr09	Gbr10	Gbr14	Gbr15	Gbr25	Gbr26	Gbr27	Gbr33	Gbr36	Tm1	Tm2	Tm6	Tm7
Geomys attwateri	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Geomys bursarius	+	+	−	+	+	+	+	+	+	+	+	+	+	+
Geomys personatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Geomys pinetus	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Geomys texensis	+	+	−	+	+	−	+	+	+	+	−	+	−	−
Cratogeomys castanops	+	+	−	−	+	+	+	+	+	+	+	+	−	+
Cratogeomys fumosus	−	+	−	+	+	−	+	+	+	+	+	+	+	+
Cratogeomys perotensis	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Cratogeomys planiceps	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Orthogeomys grandis	+	+	−	+	+	−	+	−	+	+	+	+	+	+
Orthogeomys hispidus	+	+	−	+	+	−	+	+	+	+	+	+	+	+
Orthogeomys underwoodi	+	+	−	+	+	+	−	+	−	+	+	−	+	+
Pappogeomys bulleri	−	+	−	+	+	+	+	+	+	+	+	+	+	+
Thomomys atrovarius	+	+	+	−	+	+	+	+	−	+	+	+	+	+
Thomomys bottae	−	−	−	+	−	−	+	−	+	−	+	+	+	+
Thomomys talpoides	+	+	−	+	+	+	−	−	−	+	+	+	+	+
Thomomys umbrinus	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zygogeomys trichopus	+	+	+	+	+	+	+	+	+	−	+	+	+	−

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). The 4 *Thomomys* primers (Steinberg 1999) all amplified and were polymorphic for *G. breviceps*

when calculating both Hardy-Weinberg and linkage disequilibrium. The number of alleles per locus ranged from 2 to 16 and observed heterozygosity ranged from 0.100 to 1.000 (Table 1). Following Bonferroni correction, none of the loci deviated significantly from Hardy–Weinberg expectations. The loci Gbr10, Gbr15 and Gbr27 showed signs of linkage disequilibrium and only Gbr36 showed signs of null alleles (GENEPOP v4.0; Rousset 2008).

The 10 polymorphic microsatellite loci designed here, as well as 4 previously described *Thomomys* primers (Steinberg 1999), were tested for amplification in 18 additional pocket gopher species (Table 2). The *Geomys* primers had fairly high amplification rates in the majority of the species tested, except for *T. bottae* and *T. talpoides* (Table 2). The Steinberg (1999) primers also successfully amplified in all species except *G. texensis* (Table 2). These microsatellite loci can be useful in future analyses investigating population dynamics of *G. breviceps* as well as other pocket gopher species.

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