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TECHNICAL NOTE

Isolation and characterization of 17 polymorphic microsatellite loci in the kangaroo mouse, genus *Microdipodops* (Rodentia: Heteromyidae)

Stacey L. Lance · Jessica E. Light · Kenneth L. Jones · Cris Hagen · John C. Hafner

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Abstract We isolated and characterized 17 microsatellite loci from kangaroo mice, *Microdipodops megacephalus* and *M. pallidus*. Loci were screened in 24 individuals from 21 general localities across their distributional range in the Great Basin Desert. In total, the number of alleles per locus ranged from 4 to 16, observed heterozygosity ranged from 0.333 to 1, and the probability of identity values ranged from 0.013 to 1. These loci provide new tools for examining the biogeographic history and population dynamics of *Microdipodops* in the context of molecular ecology.

Keywords *Microdipodops* · Kangaroo mouse · Microsatellite · PCR primers · SSR · STR

Kangaroo mice, *Microdipodops megacephalus* and *M. pallidus*, are sand-obligate rodents endemic to the Great Basin Desert of western North America. These two species diverged roughly 8 million years ago (Hafner et al. 2007) and subsequently encountered large-scale climatic fluctuations during the late Pleistocene and Holocene. As a result of these climatic events in conjunction with restricted habitat preferences, populations of both *M. megacephalus* and *M. pallidus* are often isolated. In fact, several recent studies have revealed that some of these isolated populations may represent unique genetic clusters (Hafner et al. 2006, 2008) resulting in conservation concerns for both *M. megacephalus* and *M. pallidus*. Microsatellite markers will be used to help elucidate how *Microdipodops* populations responded to past climatic changes as well as gain a better understanding of the interactions among extant populations of *M. megacephalus* and *M. pallidus*.

We extracted total DNA from one individual of *M. megacephalus*, using the DNeasy tissue kit protocol (Qiagen, Valencia, CA) following manufacturers protocols. We followed the enrichment procedure of Glenn and Schable (2005) with some exceptions. Briefly, DNA was digested with restriction enzyme *Rsa*I (New England Biolabs), ligated to double-stranded linkers, denatured and hybridized to biotinylated microsatellite oligonucleotide 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 = $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(AGAT)_8$), then captured on magnetic streptavidin beads (Dynal). Unhybridized DNA was washed away and remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SimpleX4 as a primer. There were two primary changes to the Glenn and Schable (2005) protocol. First, a new linker was used (SimpleX-4 Forward 5′-AAAAG CAGCAGCGGAATC and SimpleX-4 Reverse 5′- pGAT TCCGCTGCTGC). Second, the enriched libraries were sequenced on a 454 using titanium chemistry following standard Roche 454 library protocols (454 Life Sciences, a Roche company, Branford CT). Sequences were subjected to

S. L. Lance · C. Hagen
Savannah River Ecology Laboratory, Aiken, SC 29802, USA

J. E. Light
Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843, USA

K. L. Jones
Georgia Genomics Facility, University of Georgia, Athens, GA 30602, USA

J. C. Hafner (✉)
Moore Laboratory of Zoology and Department of Biology, Occidental College, Los Angeles, CA 90041, USA
e-mail: hafner@oxy.edu

a 3′ quality trim where only one base in the last 25 bases of the sequence contains a quality score less than 20 or alternatively contains one ambiguous base. CAP3 [33] was then used to assemble sequences at 98% sequence identity using a minimal overlap of 75 bp. Along with singlets, contigs of two or three sequences were searched for the presence of microsatellite DNA loci using the program 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′-CAG TCGGGCGTCATCA-3′) to enable use of a third primer in the PCR (identical to the CAG tag) that was fluorescently labeled for detection.

Forty-eight primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals of *M. megacephalus*. PCR amplifications were

Table 1 Details for 17 polymorphic microsatellite loci developed for *Microdipodops megacephalus* and screened for both *M. megacephalus* and its sister species, *M. pallidus*

Locus	Primer sequence 5′–>3′	Repeat motif	Size (bp)	N	k	H_o	H_e	PI
Mime2	F: AGAGGCATGGACATTGGTG	(ATCT)^13	224–260	15	9	0.800	0.840	0.044
	R: *GCTTGACTTATCAGTGTCTGGC		228–256	9	8	0.778	0.821	0.055
Mime3	F: AGAGTGCAAGCTCACGGAC	(AGAT)^15	351–383	15	7	0.533	0.787	0.078
	R: *TCCTTGCCCAAATCTGCAC		363–415	7	7	0.571	0.837	0.048
Mime4	F: AGGGTTAAGCACCCAGGC	(AGAT)^14	271–305	15	8	0.667	0.811	0.062
	R: *TGGTGGTTGTAGACACTAAGG		275–299	9	5	0.556	0.765	0.095
Mime5	F: CAGGGACAGTGCCCAAGTC	(ATCT)^11	241–295	15	11	0.867	0.869	0.031
	R: *CATGCTCTTCCCAATGAAAC		225–295	8	10	0.875	0.883	0.025
Mime10	F: *CACAAAGGGAGAAGAAATTCGG	(AGAT)^12	286–306	14	8	0.500	0.742	0.097
	R: TCCATATTAGATTGCTTTGGTCCC		266–294	9	7	1	0.796	0.070
Mime11	F: *CACTTCAGTTACAATCCATAGGAC	(ATCT)^14	264–296	14	6	0.714	0.786	0.079
	R: CTGCTTGGCAGGAGTTGAG		280–324	8	8	1	0.789	0.068
Mime12	F: *CCAGAAGCATCATTTGGGC	(ATCT)^14	185–219	14	7	0.786	0.778	0.082
	R: TGGCTTTGAGAGTCTAACATTGG		205–235	8	10	1	0.883	0.025
Mime21	F: *GACAGCCTCAGGTTTATGGC	(AAGG)^15	296–330	14	8	0.357†	0.832	0.048
	R: TCTTCTAGTCTGTTGCATTGGTC		300–330	9	7	0.333	0.778	0.080
Mime24	F: *GCAGGTCATTCAGGTTGCC	(AGAT)^15	257–346	15	16	0.867	0.916	0.013
	R: CTCAGCAAACTCCAAGCAGG		266–336	9	12	0.778	0.889	0.22
Mime26	F: *GGAAAGGAAAGTGTGCTCATC	(ATCT)^15	263–287	15	7	0.667	0.822	0.055
	R: TCTCAAACATAGGTCTCTTGTTCC		259–279	9	6	0.667	0.722	0.113
Mime29	F: *TGGATGTAGCTCCTATAGCACAG	(ATCT)^13	316–380	15	11	0.867	0.873	0.028
	R: AGACTCTTGAACAGGATTTAGGC		316–396	8	9	0.750	0.805	0.056
Mime32	F: *TGTGCTTAATCTGCTGGTTG	(ATCT)^12	239–267	15	8	0.867	0.831	0.050
	R: AGGATGTTCTGCCAGTCCC		259–271	9	4	0.778	0.722	0.130
Mime33	F: *TGTTGCGACGCTGTGTATTC	(ACAT)^11	210–266	15	11	0.667	0.860	0.033
	R: GCCTCTCTTGCTTATTATTGGCTG		210	8	1	0.000	0.000	1.0
Mime35	F: *TTTGGGAGGATCTTATGTAGAGG	(ATCT)^13	194–210	15	5	0.933	0.767	0.093
	R: AGACTCATCTTTACCTAGTAGCAATTC		186–298	9	7	0.778	0.698	0.120
Mime36	F: CGCTGTCAGGATATGCACC	(ATCT)^15	258–312	15	12	0.733	0.849	0.037
	R: *GCTCAGGGTGGCTTTGAAC		N/A	9	0	N/A	N/A	N/A
Mime39	F: GCATTAAGTGCTTTGAATTCCC	(ATCT)^12	273–293	15	6	0.933	0.738	0.108
	R: *CCTGGACATATGCATTCCAC		275–301	7	6	0.857	0.694	0.124
Mime48	F: TGTTTGCATTTGGTCCTAGATTAC	(ATCT)^15	259–285	12	7	0.500	0.778	0.077
	R: *TTGAGCCTCTGCTCGTAGG		269–293	7	7	0.714	0.837	0.048

The number of individuals genotyped is N; 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. Results in the top row are for *M. megacephalus* and in the bottom row for the congener *M. pallidus*

* Indicates CAG tag (5′-CAGTCGGGCGTCATCA *-3′) label

† Indicates significant deviations from Hardy–Weinberg expectations after Bonferroni corrections

performed in a 12.5 µL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 µg/ml BSA, 0.4 µM unlabeled primer, 0.04 µM tag labeled primer, 0.36 µM universal dye-labeled primer, 3.0 mM $MgCl_2$, 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 were used for all loci. Touchdown cycling parameters consisted of 20 cycles of 96°C for 30 s, highest annealing temperature of 65°C (decreased 0.5°C per cycle) for 30 s, and 72°C for 30 s; and 20 cycles of 96°C for 30 s, 55°C 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 DeWoody et al. (2004), except that unlabeled primers started with GTTT. Results were analyzed using GeneMapper version 3.7 (Applied Biosystems). Seventeen of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed the variability of 17 polymorphic loci in 24 specimens (15 *M. megacephalus* and 9 *M. pallidus*) that came from a total of 21 general localities across the distributional range of *Microdipodops* in the Great Basin Desert. Specimen voucher information (museum numbers and collection localities) is available from J.C.H. Conditions and characteristics of the 17 loci are given in Table 1. We estimated number of alleles per locus (k), observed and expected heterozygosity (H_o and H_e), probability of identity (PI) using GenAlEx v6.0 (Peakall and Smouse 2006). Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0 (Rousset 2008). For *M. megacephalus* the number of alleles per locus ranged from 5 to 16, observed heterozygosity ranged from 0.357 to 0.933, and the probability of identity values ranged from 0.013 to 0.107. For *M. pallidus*, 16 of 17 microsatellite loci amplified although one locus was monomorphic. Of the remaining 15 loci, the number of alleles per locus ranged from 4 to 12, observed heterozygosity ranged from 0.333 to 1.0, and the probability of identity values ranged from 0.022 to 1.0. After Bonferroni correction for multiple comparisons only one locus showed significant deviations from expectations under HWE and no linkage disequilibrium was detected for any of the 136 paired loci comparisons. These microsatellite loci may be useful in future analyses to determine how past climatic changes may have affected populations of *M. megacephalus* and *M. pallidus* and assess population dynamics within these two rare species.

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