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Five Hundred Microsatellite Loci for *Peromyscus*

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Abstract

Mice of the genus *Peromyscus*, including several endangered subspecies, occur throughout North America and have been important models for conservation research. We describe 526 primer pairs that amplify microsatellite DNA loci for *P. maniculatus bairdii*, 467 of which also amplify in *P. polionotus subgriseus*. For 12 of these loci, we report diversity data from a natural population. These markers will be an important resource for future genomic studies of *Peromyscus* evolution and mammalian conservation.

Keywords

microsatellite; *Peromyscus maniculatus*; *Peromyscus polionotus*; SSR; STR; PCR primers

As genomic markers, tools and techniques are becoming increasingly accessible, a handful of new “model” systems have emerged, allowing us to begin to better understand the evolution of organismal diversity (Abzhanov et al. 2008). One of these emerging models is *Peromyscus* (Cricetidae: Neotominae), arguably the most diverse and well-studied group of non-commensal rodents. Mice of the genus *Peromyscus* are ubiquitous throughout North America with more than 50 species distributed across a variety of habitats (Hall 1981). The most widespread species, the deer mouse *Peromyscus maniculatus*, has been the subject of conservation, ecological and evolutionary studies starting in the early 1900’s (e.g., Osgood 1909; Dice 1940; Sullivan 1977; Jimenez et al. 1994; Storz et al. 2007) and is considered a model organism for such research (Dewey and Dawson 2001). *Peromyscus maniculatus* is comprised of more than 65 subspecies that display a wide range of morphological and behavioral variation (King 1968; Hall 1981). In addition, as a carrier of both Lyme disease (Spielman et al. 1985) and hantavirus (Childs et al. 1994), *P. maniculatus* recently emerged as a health concern and warrants continued research and monitoring for environmental and human health effects.

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The *maniculatus* species group includes several geographically peripheral taxa, including its sister species, the oldfield mouse *Peromyscus polionotus* (Blair 1950; Avise et al. 1983; Bradley et al. 2007). Blair (1950) proposed that *P. polionotus* split from *P. maniculatus* in the Pleistocene, and populations of *P. polionotus* were isolated to the southeastern states and along the Gulf and Atlantic coasts. *P. polionotus* has sixteen subspecies (Hall 1981), eight of which are 'beach mice' that occur along the sandy dunes and barrier islands of the Atlantic and Gulf coast (Bowen 1968; Bowen and Dawson 1977). Six of these eight subspecies are classified as endangered or threatened and one, *P. p. decoloratus*, is now considered extinct (USFWS 1999). Subspecies of *P. polionotus* exhibit broad variation in pelage coloration (Bowen 1968; Mullen and Hoekstra 2008) and frequently match their local substrate (Belk and Smith 1996; Mullen et al. *in review*). This matching of pelage to substrate is a classical example of local adaptation, the molecular mechanisms of which are just now being understood (Hoekstra et al. 2006; Steiner et al. 2007).

Both *P. maniculatus* and *P. polionotus* are intriguing species in which to study population processes as well as genome evolution within and between species. Specifically, microsatellite DNA loci are often used to elucidate genetic patterns in populations, such as migration rates, population structure, and evolutionary history (Hedrick et al. 2001) as well as to describe genomic or chromosomal variation (e.g., Womack and Kata 1995; Payseur and Nachman 2000). More importantly, however, microsatellites, unlike other genetic markers, are highly variable and can easily be assayed across a wide number of closely related species. Therefore, having a large number of microsatellite markers can be extremely useful in both population genomic and genetic mapping studies (Stinchcombe and Hoekstra 2008). These approaches can be used to identify genes relevant to disease as well as genomic regions contributing to phenotypic variation, the latter a topic of great interest to evolutionary, ecology and conservation biologists.

Here we describe over 500 microsatellite markers that amplify in *Peromyscus*. This large set of microsatellites contributes to a growing number of genomic resources (Mullen et al. 2006; Glenn et al. 2008) for studies of *Peromyscus* evolution.

A total of eight genomic libraries for *P. m. bairdii* (N=6) and *P. p. subgriseus* (N=2) were constructed and enriched for microsatellites following Glenn and Schable (2005). All mice were obtained from *Peromyscus* Genetic Stock Center (University of South Carolina). DNA was extracted using Qiagen DNeasy kits, digested with restriction enzyme *Rsa*I (New England Biolabs), and simultaneously ligated to double-stranded SuperSNX linkers (SuperSNX24 Forward 5′-GTTTAAGGCCTAGCTAGCAGCAGAATC and SuperSNX24 Reverse 5′-GATTCTGCTAGCTAGGCCTTAAACAAAA). Linker-ligated DNA was denatured and hybridized to biotinylated microsatellite oligonucleotides, which were 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 SuperSNX24 as a primer, the enrichment process was repeated, and the DNA was cloned with TOPO-TA Cloning Kits (Invitrogen). Inserts were isolated from clones using bacterial colony PCR with M13 primers and sequenced using the same primers on an ABI-3130xl sequencer. Sequences from both strands were assembled and edited in Sequencer 4.1 (Genecodes). Microsatellites were identified either by searching sequences by eye or exporting sequences to Ephemeris 1.0 (available at http://www.uga.edu/srel/DNA_Lab/programs.htm).

Sequences of more than 3000 clones from these eight genomic libraries were used to design 1077 primer pairs. An additional 78 primer pairs were developed from expressed sequence tags (EST libraries) from *P. maniculatus* (Glenn et al. 2008). PCR primers were designed using Oligo 6.67 (Molecular Biology Insights) or Primer Premier 5 (Premier Biosoft International).

One primer in each pair was modified on the 5′ end to include an engineered sequence (CAG tag: 5′-CAGTCGGGCGTCATCA-3′), allowing the use of a third oligo in the PCR (complementary to the CAG tag) that is fluorescently labeled for detection.

All primer pairs were then tested in *P. maniculatus* or *P. polionotus* individuals. PCR amplifications were 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, 0.08μM tag labeled primer, 0.36μM universal dye-labeled primer, 2 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 units JumpStart Taq DNA Polymerase (Sigma), and 20–40 ng DNA template]. Each primer pair was tested in at least one PCR protocol; we used touchdown thermal cycling programs (Don et al. 1991), encompassing a 10° C span of annealing temperatures ranging between 65-55°C, 60-50°C, 58-48°C or 55-45°C (Table S1). Cycles were 95°C for 3min; 5 cycles of 95°C for 30s, highest annealing temperature for 30s, and 72 °C for 30s; 21 cycles of 96°C for 30s, highest annealing temperature (decreased 0.5°C per cycle) for 30s, and 72°C for 30s; and 15 cycles of 96 °C for 30s, lowest annealing temperature for 30s, and 72°C for 30s. PCR products were then run on an ABI-3130xl sequencer and analyzed using GeneMapper version 4.0 (Applied Biosystems). Of these 1077 primer pairs, we found that 526 amplified a product of the correct estimated size in *P. maniculatus* and 467 in *P. polionotus* using one of the three PCR protocols (Table S2). We also tested a subset of these primers (N = 192) in beach mice, and found 110 successfully amplified.

After determining which microsatellite loci could be amplified, we next genotyped several individuals of *P. maniculatus* (N = 4–14) and *P. polionotus* (N = 1–7) to characterize intra- and inter-specific size variation at these loci (Table S2). Of the 526 loci, 393 were polymorphic in *P. maniculatus* and 203 of 467 were polymorphic in *P. polionotus* (Table S2). In our sample, 230 markers had non-overlapping size distributions between *P. maniculatus* and *P. polionotus*, and 37 were diagnostic between the two *P. polionotus* subspecies, *P. p. subgriseus* and *P. p. leucocephalus*.

To test the utility of these markers for molecular studies of natural populations, we assayed 12 markers in a single population (N = 20) of *P. maniculatus luteus* from Nebraska (Cherry Co., Schlagel Creek State Wildlife Management Area, N42°42.7′/W100°37.1′). For each locus, we report the number of alleles, the observed and expected heterozygosity, and any departure from Hardy-Weinberg equilibrium (Table 1). Standard diversity indices were calculated, and χ^2 tests were performed using GeneAlEx version 6 (Peakall and Smouse 2006). Pairwise linkage disequilibrium between all 12 markers was calculated using GENEPOP version 3.4 (Raymond and Rousset 1995); no markers showed significant linkage ($p > 0.05$). These results suggest that the markers reported here will be useful for studies of wild *Peromyscus*, but that reasonably large numbers of primer pairs should be screened for any given population so that loci exhibiting evidence of null alleles (e.g., loci 172, 280, 437, and 451; Table 1) can be avoided.

This study reports a large number of microsatellite loci for *Peromyscus*. Based on previous studies, it is likely that many of these markers will also amplify in additional *Peromyscus* species (Prince et al. 2002) as well as other cricetid rodents. Moreover, even those markers that are not polymorphic in lab strains of *Peromyscus* are likely to be polymorphic in natural populations (Mullen et al. 2006). Thus, this collection of markers will be useful for studies of ecological genetics in a large number of rodent taxa. Perhaps more importantly, these loci also can be used to further develop the *Peromyscus* linkage map (Steiner et al. 2007; Ramsdell et al. 2008). Specifically, because species of *Peromyscus* breed well in the lab (Sumner 1915; Dewey and Dawson 2001), are interfertile (Watson 1942) and vary in a large number of traits that are ecologically, behaviorally and biomedically relevant (King 1968), these markers can be used in quantitative trait locus mapping experiments to identify the genetic architecture, and eventually the genes, responsible for a wide diversity of fitness-related traits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Number of alleles, observed (H_O) and expected (H_E) heterozygosity and Hardy-Weinberg equilibrium (HWE) test results for 12 loci surveyed in a natural population of *P. maniculatus* (N = 20).

Pmbw locus	No. of alleles	H_0	H_E	HWE (χ^2 p-value)
172	7	0.368	0.817	< 0.001
280	9	0.350	0.794	< 0.001
282	12	0.800	0.874	ns
346	6	0.737	0.679	ns
385	11	0.900	0.831	ns
390	14	0.800	0.898	ns
397	12	0.650	0.700	ns
410	8	0.700	0.723	ns
437	16	0.529	0.913	< 0.01
441	18	0.895	0.916	ns
447	11	0.789	0.845	ns
451	14	0.667	0.893	< 0.001