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PRIMER NOTE

# Sixty polymorphic microsatellite markers for the oldfield mouse developed in *Peromyscus polionotus* and *Peromyscus maniculatus*

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**Abstract**

**We isolated and characterized 60 novel microsatellite markers from the closely related oldfield mouse (*Peromyscus polionotus*) and deer mouse (*Peromyscus maniculatus*) for studies of conservation, ecological, quantitative and population genetics. We assessed all 60 markers in a wild population of *Peromyscus polionotus rhoadsi* (*N* = 20) from central Florida and found an average of nine alleles per marker and an observed heterozygosity ($H_O$) of 0.66 (range = 0.00–1.00). These polymorphic markers contribute to the growing number of genomic resources for *Peromyscus*, an emerging model system for ecological and evolutionary research.**

*Keywords*: deer mouse, microsatellites, oldfield mouse, *Peromyscus*, Rodentia, simple sequence repeats

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Members of the genus *Peromyscus* are the most abundant small mammals in North America. Because of their wide distribution, *Peromyscus* exhibits extensive morphological, physiological and behavioural variations both within and among populations and species (King 1961). As a result, natural populations of *Peromyscus* have been intensively studied and considered to be a model organism for studies of ecology and behaviour (Musser & Carleton 1993; Dewey & Dawson 2001). Despite the wealth of ecological information from *Peromyscus*, the limited number of genetic markers has restricted the utility of genomic approaches for understanding the genetic basis of ecologically relevant phenotypes (Schlotterer 2002; Feder & Mitchell-Olds 2003).

One species in particular, the oldfield mouse (*Peromyscus polionotus*), presents a unique opportunity in which to investigate genetic and morphological evolution. *Peromyscus polionotus* is found throughout the southeastern US primarily in oldfields, characterized by open, sandy habitat. *Peromyscus polionotus* has also colonized the primary dunes and barrier islands on the Gulf coast of Florida and Alabama as well as coastal dunes in northeastern Florida. In these regions, there are several *P. polionotus* subspecies (termed 'beach mice') originally described based on their cryptic dorsal pelage (Sumner 1926; Bowen 1968). Six of the seven beach mouse subspecies are classified as endangered or threatened (US Fish and Wildlife Service, 1985; Oli *et al*. 2001). Thus, studies of population structure and genomic variability may identify targets for conservation efforts as well as lead to deeper insights into the molecular mechanisms underlying adaptive morphological variation in the wild.

Microsatellites were cloned from an enriched partial genomic library following the method of Schable *et al*. (2002), which was adapted from Hamilton *et al*. (1999). DNA from a single *Peromyscus maniculatus bairdii* (BW) and a single *Peromyscus polionotus subgriseus* (PO) adult was extracted from tail snips using a QIAGEN DNeasy Kit. Genomic DNA was digested with *Hae*III restriction enzyme, and 300–700 bp fragments were ligated to SNX linkers using T4 ligase. Linked genomic fragments were enriched for $(AC)_{13}(AG)_{12}(AAC)_8(AAT)_{12}(AGAT)_8$ and $(AAAT)_8$ repeats

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by hybridization to biotinylated microsatellite oligonucleotides and then captured on streptavidin-coated magnetic beads. Fragments were recovered by polymerase chain reaction (PCR) with linker-specific primers and ligated into pBluescript II KS+ (Stratagene). These products were transformed into XL10 Gold *Escherichia coli* and colonies screened for inserts using M13 primers. In all, 288 clones were sequenced, and 60 polymorphic microsatellite markers were identified. PCR products were sequenced using BigDye version 3.0 (Applied Biosystems) on an ABI 377-XL sequencer. Sequences from both strands were edited in SEQUENCHER 3.1.1 (GeneCodes). Primers for PCR were developed using OLIGO 6.60 (Molecular Biology Insights).

PCR primers were designed using two different approaches; primers sets for microsatellites designed in the two species, PO or BW, require different reaction conditions. All PCRs were performed in a 15-μL volume using an Eppendorf Mastercycler gradient thermal cycler. For PO reactions, we used 30 ng of template DNA, 10× *Taq* buffer with 1.5 mM $MgCl_2$ (Eppendorf), 0.3 μL of 10 μM dNTPs, 0.6 μM each of a fluorescently labelled forward primer, unlabelled reverse primer and 0.15 U *Taq* DNA polymerase (Eppendorf). PCRs with BW primers were prepared as above with the following exception: one of the microsatellite primers was synthesized with a known CAG (5′-CAGTCGGGCGTCATCA-3′) or M13R sequence (5′-GGAAACAGCTATGACCAT-3′) attached to the 5′ end. This sequence tag allows for the binding of a fluorescently labelled probe to the PCR product (HEX-labelled M13R probe and 6-FAM-labelled CAG probe) for detection on the ABI 3100 sequencer (Boutin-Ganache *et al*. 2001). The PCR master mixes used in this system included 0.06 μM of the sequence-tagged primer, 0.6 μM of the untagged primer and 0.54 μM of the fluorescently labelled probe.

Two types of cycling parameters for PCR were used: classical (one annealing temperature) and touchdown (successively lower annealing temperatures). The classical PCR parameters were as follows: 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, 10 s annealing and 72 °C for 1 min, and a final extension at 72 °C for 5 min. Touchdown PCR parameters were: 94 °C for 90 s, followed by 21 cycles of denaturation at 94 °C for 30 s, annealing for 30 s and 72 °C for 1 min. The initial annealing temperature was decreased by 0.5 degrees for each of 20 cycles. An additional 15 cycles were performed as follows: 94 °C for 30 s, followed by 30 s at the last temperature and 72 °C for 1 min. The final extension occurred at 72 °C for 5 min. Primer-specific annealing temperatures are given in Table 1.

For the population study, 20 individuals of *P. p. rhoadsi* were captured in Lake Louisa State Park in central Florida. Genomic DNA was isolated from tail snips using a QIAGEN DNeasy Kit. All microsatellites were scored on an ABI 3100 sequencer using a ROX 400HD ladder. Results were analysed using the GENEMAPPER version 3.5 software (Applied Biosystems). Table 1 summarizes the genetic variability for the 60 microsatellite loci. We calculated observed ($H_O$) and expected ($H_E$) heterozygosities using MSA (Microsatellite Analyser) version 1 (Dieringer & Schlotterer 2002). Polymorphism was observed in 57 of the 60 loci for the Lake Louisa population, but the additional three microsatellites were polymorphic in other populations of *P. polionotus*. The number of alleles observed across polymorphic loci ranged from two to 18 (average = 9 per locus); observed heterozygosity ranged from 0.00 to 1.00 (average = 0.66) and expected heterozygosity ranged from 0.15 to 0.97 (average = 0.76). Tests of Hardy–Weinberg equilibrium and linkage disequilibrium (LD) were calculated using GENEPOP version 3.4 (Raymond & Rousset 1995). After Bonferroni correction for multiple tests, eight loci were found to depart significantly from Hardy–Weinberg expectations (adjusted *P* value = 0.00072; Table 1) and showed heterozygote deficiency, suggesting the presence of null alleles at these loci or that other factors such as sampling error or natural selection are responsible for this pattern. Two of the pairwise tests for LD (Bw3-29 vs. Po2-40 and Po3-59 vs. Po3-72) were significant after sequential Bonferroni correction (Rice 1989). Overall, the level of microsatellite variation observed here is greater than that reported for captive populations of *P. polionotus*, but similar to wild-caught populations of *P. maniculatus* (Table 2). Together, these microsatellite markers exhibit a wide array of genetic variability, and therefore subsets of these markers may be useful for studies ranging from paternity analyses to phylogeography.

Importantly, microsatellites developed in one species of *Peromyscus* can often be amplified and are polymorphic in other species of *Peromyscus* (Prince *et al*. 2002; Chirhart *et al*. 2005). Previously, 46 microsatellites have been characterized for use in different species of *Peromyscus* (Table 2); combined with the markers reported here, over 100 microsatellites are now available for use in *Peromyscus*.

The microsatellite loci characterized here are a promising set of genetic markers for detailed intraspecific studies, phylogeographical analyses and genetic mapping in *Peromyscus*. Specifically, these markers can be used to generate data that may aid conservation management decisions as well as to understand the genetic basis of morphological variation in the endangered beach mice. In addition, these microsatellites contribute to a growing number of variable markers for genomic investigations in *Peromyscus*, an emerging model for studies in ecological and evolutionary genetics.

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**Table 1** Primer sequences, GenBank Accession no., repeat motif, annealing temperature ($T_a$) and genetic variability measures for 60 microsatellite loci from *Peromyscus maniculatus bairdii* (BW) and *Peromyscus polionotus subgriseus* (PO)

| Name | GenBank no. | Repeat motif | Sequence 5′–3′ | $T_a$ (°C) | Size range | *A* | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|
| Bw-TBX | DQ103268 | $(AC)_{23}$ | F: (**M13R**)CATGTGTGTGCATGAATATATGC<br>R: CATGTGTGTGCATGAATATATGC | 58 | 380–412 | 15 | 0.80 | 0.93 |
| Bw2-1 | AF526098 | $(TG)_{23}$ | F: GCCTATGGAAACCTTTAGT<br>R: (**CAG**)GACAAGATTTTTAAACAGGA | TD55 | 126–160 | 9 | 0.82 | 0.81 |
| Bw2-25 | AF526102 | $(TG)_{22}$ | F: CTGTTGGGGAAACTTGAA<br>R: (**M13R**)ATTTACCGCCATCCT | TD55 | 223–251 | 9 | 0.65 | 0.82 |
| Bw2-65 | AF526108 | $(CT)_{21}(GT)_{30}$ | F: (**M13R**)GCCTCTGCGTCTTC<br>R: CAGCGTGGTATGTATTGAC | TD55 | 183–243 | 19 | 0.90 | 0.96 |
| Bw2-76 | AF526111 | $(AG)_{26}$ | F: (**CAG**)GGGCTGAAATCAATAA<br>R: ATTCATTGGCTGTTGTTA | TD55 | 197–201 | 3 | 0.55 | 0.42 |
| Bw2-110 | AF526099 | $(CA)_{21}$ | F: (**M13R**)CCCAAAATCAAATCAATC<br>R: GGGCTGTTGATCTATGTG | TD55 | 108–128 | 7 | 0.70 | 0.83 |
| Bw2-SREL | AF526118 | $(GA)_6$ | F: (**M13R**)CTATCTCTGCCTCTTACCAT<br>R: TCGTGGATAATTACTTGGAC | TD55 | 153 | 1* | | |
| Bw3-12 | AF526119 | $(TATG)_6$ | F: (**CAG**)ACCATTCATTCACATCT<br>R: TCATTCCAGACAAGAGTT | TD55 | 128–140 | 4 | 0.63 | 0.59 |
| Bw3-15 | AF526120 | $(CTAT)_{17}$ | F: (**CAG**)TCTCCAGGTGGTCAAT<br>R: TGGCAGTAGATCAATAAC | 48 | 287–307 | 5 | 0.67 | 0.69 |
| Bw3-29 | AF526123 | $(TGTC)_{10}(TC)_8$ | F: (**M13R**)CCTTTATGCTTCAGACT<br>R: CCCACAAACATAATAGAC | TD55 | 90–106 | 3 | 0.55 | 0.63 |
| Bw3-46 | AF526125 | $(AACA)_6$ | F: (**CAG**)TTCTCCTTCTTGGGCTAAA<br>R: GGCCCTAGAACCTTACTGT | TD55 | 287–307 | 6 | 0.83 | 0.76 |
| Bw4-1 | AF526127 | $(CTTT)_{13}$ | F: (**CAG**)TGGTTAAGGATTTCTCGT<br>R: TGCTGAAGATGAGAATAGG | 58 | 143–169 | 9 | 0.90 | 0.79 |
| Bw4-5 | AF526155 | $(CAAA)_6$ | F: (**CAG**)GCAAGAGAAACAAGTGGAAT<br>R: CCCAAGCAACTGATACATGA | TD55 | 147–151 | 2 | 0.32 | 0.27 |
| Bw4-7 | AF526159 | $(ATAC)_6(CA)_{21}$ | F: (**CAG**)CACAGACAAGCAGACATAC<br>R: GGCTCATCTTAACTCTT | 53 | 345–395 | 10 | 0.60 | 0.76 |
| Bw4-8 | AF526162 | $(ATGT)_{12}$ | F: (**CAG**)TCTAGCTGCGTTCC<br>R: CCACTCACAAACGCATAC | 58 | 247–273 | 12 | 0.74 | 0.88 |
| Bw4-13 | AF526132 | $(AGAT)_{18}$ | F: (**M13R**)GACAGACAGACGGACG<br>R: ACTGTGTCATGGCATTAT | TD55 | 158–226 | 9 | 0.47† | 0.84 |
| Bw4-18 | AF526139 | $(CTAT)_{32}(CTGT)_8$ | F: (**CAG**)TCCTCCTTTTTGTATTC<br>R: GTGGGTGACTGAGTAAGATA | 50 | 382 | 1* | | |
| Bw4-28 | AF526144 | $(TCTA)_{15}$ | F: TAATCCAGGTGTATCTAATCT<br>R: (**M13R**)CCCAGTATTGCTAGTCT | TD65 | 291–399 | 22 | 0.89 | 0.97 |
| Bw4-40 | AF526153 | $(AGAT)_7$ | F: (**CAG**)CCAAGAGTCTCGTTAAA<br>R: CACCACTAATTATGTCTTC | TD55 | 266–282 | 5 | 0.74 | 0.75 |
| Bw4-45 | AF526154 | $(CTTT)_{19}(CT)_{19}(CCTT)_4$ | F: (**M13R**)ATGGCCTGCCTACCTCA<br>R: AGGGGAAGTGAAAAGCTACA | TD70 | 257–387 | 16 | 0.67† | 0.94 |
| Bw4-54 | AF526156 | $(TATG)_4(TGTA)_{10}(CA)_9$ | F: CCCGCCCTATGTATGT<br>R: (**M13R**)GAGGCCCCTAGTTGATTC | TD65 | 179–213 | 9 | 0.56 | 0.79 |
| Bw4-63 | AF526158 | $(TC)_6$ | F: (**CAG**)CAGCAAGGCGTAACAGT<br>R: AGAAATAAGGCAAGGACTCA | TD65 | 134–136 | 2 | 0.65 | 0.50 |
| Bw4-74 | AF526160 | $(CAGA)_5$ | F: GACAAATATCAGCCAATCAAG<br>R: (**M13R**)GTCCTAAGAGTTTGCCTAAG | TD65 | 358–362 | 2 | 0 | 0.10 |
| Bw4-84 | AF526163 | $(AACC)_6(AATC)_{10}$ | F: AGCTTTAAAACTCAACC<br>R: (**CAG**)CCAGCAAGTTGTCCT | 48 | 137–157 | 4 | 0.20 | 0.41 |
| Bw4-93 | AF526165 | $(CCTT)_{10}(CTTT)_{23}$ | F: (**CAG**)GACATTTAAAAAGGACTG<br>R: CCCTCTTGATTCCACAC | 53 | 282–378 | 16 | 0.53† | 0.93 |
| Bw4-110 | AF526128 | $(AAAG)_{16}(AG)_4$ | F: (**M13R**)GAGGCCAGAGGAATTG<br>R: AAGTCAGATCCCCATTAC | TD55 | 198–234 | 7 | 0.60 | 0.72 |
| Bw4-112 | AF526129 | $(AGAT)_{13}$ | F: (**CAG**)GGCAGTGCATTCATGGTAA<br>R: TGAGTCCCCAGTTGTATGTA | 58 | 251–279 | 10 | 0.70 | 0.87 |
| Bw4-129 | AF526131 | $(AAGG)_{11}$ | F: (**M13R**)AAAAACAAAAGACGCATCAC<br>R: GGGCTGCATCACTGACTA | TD60 | 236–304 | 9 | 0.25† | 0.88 |
| Bw4-137 | AF526134 | $(ATAG)_9(GATA)_{16}$ | F: (**CAG**)GGCTTGGTGGATTAATG<br>R: ATGCCAGAGCTGTTATAC | TD65 | 170–222 | 15 | 0.89 | 0.93 |
| Bw4-143 | AF526135 | $(CGTG)_4$ | F: (**CAG**)GGCAGATCATAGGTTAAAC<br>R: AGACTAGCCAAGGTTACATA | TD60 | 153–157 | 2 | 0.30 | 0.26 |
| Bw4-151 | AF526136 | $(AGG)_5$ | F: GAGGGAGATAAATATAGGA<br>R: (**CAG**)CTTGGAGGGAACATTG | TD55 | 196–214 | 4 | 0.50 | 0.48 |
| Bw4-178 | AF526138 | $(ATAG)_{13}$ | F: CCGTTTTTCTTACTCA<br>R: (**CAG**)CAAAACAGTGGGTCAA | TD55 | 282–344 | 12 | 0.18† | 0.86 |

**Table 1** *Continued*

| Name | GenBank no. | Repeat motif | Sequence 5′–3′ | $T_a$ (°C) | Size range | *A* | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|
| Bw4-186 | AF526140 | $(TTTA)_4(TTCA)_6$ | F: GCCCAGAGTGTGTCATGTAG | 52 | 226–266 | 9 | 0.80 | 0.76 |
| | | | R: **(CAG)**TTCCAACTCAGCAGGTAGA | | | | | |
| Bw4-188 | AF526141 | $(GTTT)_4$ | F: CCCGACAGGTAAGGTTTT | TD55 | 286–294 | 2 | 0.53 | 0.51 |
| | | | R: **(CAG)**TATGTGTGGGCAGGTTGAA | | | | | |
| Bw4-192 | AF526142 | $(ATGA)_{13}$ | F: TACTTACTGGCAGTCAACA | 53 | 170 | 1* | | |
| | | | R: **(CAG)**CAATACCTCCATGCAATACT | | | | | |
| Bw4-200 | AF526143 | $(ATCT)_5(GTCT)_6$ | F: **(CAG)**GCACATTTCTCCTTCTAAGC | TD60 | 423–463 | 11 | 0.84 | 0.88 |
| | | | R: GACCACCTGATGAGCATAGAT | | | | | |
| Bw4-234 | DQ103269 | $(TGAA)_6TAAC(AAAT)_4$ | F: **(CAG)**ATTCCAACTCAGCAGGTAGA | TD55 | 226–268 | 10 | 0.80 | 0.84 |
| | | | R: GCCCAGAGTGTGTCATGTAG | | | | | |
| Bw4-245 | AF526145 | $(AGAT)_{16}$ | F: **(CAG)**TGTTGTTCTATTGGGTATT | TD55 | 288–316 | 8 | 0.55 | 0.78 |
| | | | R: TTTGCTGGTTTTACTATTGT | | | | | |
| Bw4-249 | AF526147 | $(AAGG)_8(AG)_{12}(AAAG)_{27}$ | F: **(M13R)**CAGGACAGGGCAACTA | TD55 | 216–304 | 20 | 0.55 | 0.79 |
| | | | R: TGAGATGTGCTATATGGCTTA | | | | | |
| Bw4-251 | AF526148 | $(CA)_{20}(TA)_6$ | F: GGGAGAGAAAAGACTATA | TD55 | 248–252 | 3 | 0.65 | 0.66 |
| | | | R: **(CAG)**TCTTGGGAATAATCTA | | | | | |
| Bw4-260 | AF526149 | $(AGAT)_{18}$ | F: **(CAG)**CAAAGAGGAGGGAACACC | 50 | 226–254 | 7 | 0.75 | 0.78 |
| | | | R: GCCAGGGAGTATCAAGTTCA | | | | | |
| Bw4-276 | AF526150 | $(TTCC)_{18}$ | F: AGAGCCAACACAATAGTAT | TD55 | 200–280 | 9 | 0.11† | 0.89 |
| | | | R: **(M13R)**AAGGAAAAGATTAGTAAGGT | | | | | |
| Bw4-SREL | AF526166 | $(TGGA)_{13}$ | F: **(CAG)**CCAACAAGAGTCTATATTC | TD55 | 182–206 | 7 | 0.70 | 0.84 |
| | | | R: TATCCATTGGTTCATCTATC | | | | | |
| BwC-28 | AF526168 | $(AC)_{15}$ | F: **(CAG)**GGCACCTGTAACTCTAGCTC | 50 | 214–232 | 6 | 0.89 | 0.83 |
| | | | R: GGCAGTGTGATGGTTAAGA | | | | | |
| Po-17 | AF380234 | $(TTTG)_9$ | F: **(6-FAM)**TCTGTAAACCCAAACTCATAA | 62 | 196–216 | 5 | 0.74 | 0.78 |
| | | | R: GGGGGAGAGAACTGAC | | | | | |
| Po-25 | AF380236 | $(GA)_{24}$ | F: **(HEX)**GGACAGCCAGGACTGTTACAC | 58 | 116–170 | 15 | 0.58† | 0.93 |
| | | | R: CCCACTCATCTCAATGCC | | | | | |
| Po-31 | DQ103263 | $(GA)_{26}$ | F: **(HEX)**TTTCAGTGGCTCTCATGGTTA | 58 | 267–291 | 10 | 0.75 | 0.86 |
| | | | R: AGCTTTCTTCTTCCCAACTA | | | | | |
| Po-43 | DQ103264 | $(TG)_{24}$ | F: **(6-FAM)**GGTTGGGGCTAGTGAGT | TD55 | 122–136 | 8 | 0.95 | 0.86 |
| | | | R: CTTCCTCCTGATGGGATCCAC | | | | | |
| Po-71 | AF380240 | $(AC)_{10}(AG)_{32}$ | F: **(HEX)**CAGCCAGAACAAAATAGCACT | 58 | 228–270 | 16 | 0.90 | 0.95 |
| | | | R: AGCTTCATGCCTCCTATATTC | | | | | |
| Po-98 | DQ103265 | $(AG)_{42}$ | F: **(6-FAM)**ACACTGCCTCAAAGAACTCAC | TD60 | 237–277 | 14 | 0.69 | 0.93 |
| | | | R: GCACTTTGGGACCTTATG | | | | | |
| Po-99 | AF380241 | $(AG)_{21}$ | F: **(HEX)**CGAAATGGAGATGGACGAA | TD60 | 215–239 | 12 | 1.0 | 0.89 |
| | | | R: CTTTCAAACTCAGCGACTCAA | | | | | |
| Po-116A | DQ103267 | $(CT)_{30}(TG)_{15}$ | F: **(HEX)**GATAGCCAGGACTGTTACAC | 58 | 130–182 | 14 | 1.0 | 0.92 |
| | | | R: CCACAGCCATGTACAGC | | | | | |
| Po2-5 | AF380243 | $(GA)_{16}$ | F: **(6-FAM)**GAAGTAAGGAAAAGGGGGAAA | TD55 | 242–282 | 12 | 0.68 | 0.87 |
| | | | R: CTCCCAGCCAGGCGATCCAAT | | | | | |
| Po2-23 | AF380244 | $(AGAT)_{16}(AGAC)_6$ | F: **(HEX)**CAGATATGATAGATAGATAGG | 53 | 249–303 | 11 | 0.88 | 0.90 |
| | | | R: TGATCAGAACAATAGCAATA | | | | | |
| Po2-33 | DQ103267 | $(TG)_{19}(CG)_5$ | F: **(6-FAM)**CCATGACCTCCACATAGAGA | 47 | 179–181 | 2 | 0.15 | 0.45 |
| | | | R: CAAGTAGTCCTCATTCACTCC | | | | | |
| Po2-40 | AF380245 | $(AC)_{25}$ | F: **(HEX)**AGGGTTGACCTCTAGCC | 62 | 96–116 | 8 | 0.90 | 0.85 |
| | | | R: TGGATGACTGAGTGGACCTAA | | | | | |
| Po3-30 | AF380246 | $(AG)_{16}$ | F: **(6-FAM)**AATCGGCTGTCGCTGATCTA | TD60 | 206–254 | 10 | 0.89 | 0.90 |
| | | | R: CGGGCACGGAGTACCTC | | | | | |
| Po3-59 | AF380247 | $(AC)_{21}$ | F: **(HEX)**CAGGGCAGCCAAAGTTACA | 58 | 141–159 | 7 | 0.79 | 0.79 |
| | | | R: GGGCTGGAGGGAATTAGTGT | | | | | |
| Po3-68 | AF380248 | $(TG)_{28}$ | F: **(6-FAM)**GTAGTCTGAGAAGCGAAAGG | 58 | 247–287 | 13 | 0.85 | 0.93 |
| | | | R: TTTATTTGGGTCAGCTCGAC | | | | | |
| Po3-72 | AF380249 | $(AC)_{11}$ | F: **(6-FAM)**AAACCGGTGAGATGAGTGTG | TD55 | 223–233 | 6 | 0.30† | 0.71 |
| | | | R: CTGAATTCTCCCGTTGTC | | | | | |
| | | | | | **Average** | **9** | **0.66** | **0.76** |
| | | | | | SD | +/−5 | +/−0.24 | +/−0.20 |

The location of the CAG or M13R sequence tag is provided on the BW primer sequence, and for direct labelling of the PO primer, the dye is given. Annealing temperature ($T_a$) represents the optimal annealing temperature; TD, touchdown PCR protocol followed by the initial annealing temperature. *A*, number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity. *denotes three microsatellite markers that did not show variability in this population, although are known to be variable in other populations of *P. polionotus*, and were excluded from calculations of *A*, $H_O$ and $H_E$. †, loci that deviate from Hardy–Weinberg expectations.

**Table 2** Comparison of microsatellite markers developed in *Peromyscus*

| Species* | Study source | ‡ | Alleles† | $H_O$ | Reference |
|---|---|---|---|---|---|
| *P. polionotus rhoadsi* | Wild population | 60 | 9 | 0.659 | This study |
| *P. polionotus ammobates* | Wild population | 5 | 3 | 0.660 | Wooten *et al*. 1999 |
| *P. polionotus subgriseus* | *Peromyscus* Genetic Stock Center | 11 | 2 | 0.398 | Prince *et al*. 2002 |
| *P. polionotus subgriseus* | Outbred colony | 3 | 5 | 0.521 | Eklund & Ober 2000 |
| *P. maniculatus* | Wild population | 11 | 11 | 0.644 | Chirhart *et al*. 2000 |
| *P. leucopus* | Wild population | 6 | 7 | 0.578 | Schmidt 1999 |

*Species in which microsatellite variability was surveyed.
†Average number of alleles per marker.
‡Number of microsatellite markers.

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