PERMANENT GENETIC RESOURCES

Ten polymorphic microsatellite markers for the pygmy rabbit (Brachylagus idahoensis)

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Abstract

We developed 10 polymorphic microsatellite loci for the pygmy rabbit (Brachylagus idahoensis). Nine of the 10 loci amplified reliably and had a low frequency of null alleles. Number of alleles per locus ranged from four to 12, and observed and expected heterozygosities ranged from 0.26 to 0.89 and from 0.63 to 0.88, respectively. These loci will be useful in determining population genetic structure and assessing patterns of gene flow in the pygmy rabbit.

Keywords: Brachylagus idahoensis, gene flow, Genetic Identification Services, microsatellite, Oryctolagus cuniculus, pygmy rabbit

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The pygmy rabbit (Brachylagus idahoensis) is the smallest rabbit in North America and the only species in the genus Brachylagus. Pygmy rabbits occur in the Great Basin and neighbouring intermountain regions in the western USA (Green & Flinders 1980), but are confined to areas with mature sagebrush (Artemisia spp.) and relatively deep soils (Orr 1940). In 2003, the Columbia Basin population of the pygmy rabbit was listed under the US Endangered Species Act as a Federally Endangered distinct population segment (Federal Register 2003), and concern about the genetic diversity and persistence of populations throughout the rest of the species’ range is increasing. An evaluation of genetic diversity and population genetic structure is important for informing conservation and management decisions for this species. Microsatellite markers can be one important tool for examining genetic diversity and population genetic structure. Although microsatellite markers developed for the distantly related European rabbit (Oryctolagus cuniculus; Rico et al. 1994; Mougel et al. 1997; Surridge et al. 1997) have been used for pygmy rabbits (K.I. Warheit, personal communication), markers specific to pygmy rabbits currently are not available. Therefore, we isolated and developed primers for 10 microsatellite markers in the pygmy rabbit.

Microsatellite libraries were developed by Genetic Identification Services, Chatsworth, California, USA. Methods for DNA library construction, enrichment and screening followed Jones et al. (2002). Genomic DNA from two rabbits was extracted from kidney tissue using a QIAGEN tissue kit (QIAGEN), and was partially restricted with seven blunt-end cutting enzymes (RsaI, HaeIII, BsrBI, PvuII, StuI, Scal, EcoRV). Fragments between 300 bp and 750 bp were ligated to double-stranded adaptor sequences and subjected to magnetic bead capture (CPG), using biotinylated capture molecules. Libraries were prepared in parallel using biotin-CA(15), biotin-ATG(12), biotin-CATC(8) and biotin-TAGA(8) as capture molecules according to the manufacturer’s protocol. Microsatellites were isolated following Jones et al. (2002). Sequences were obtained on an ABI PRISM 377 (Applied Biosystems) using ABI PRISM Taq dye terminator cycle sequencing methodology.

Primers were designed for 40 microsatellite-containing clones using designerpCR version 1.03 (Research Genetics) and tested for polymorphism against library DNA and DNA from seven pygmy rabbits. DNA was extracted using the PUREGENE DNA Extraction Kit (Gentra Systems). Microsatellite loci were amplified in 10-μL reactions consisting of 2 mM MgCl₂, 0.2 mM each dNTPs, 0.3 μM each primer, 0.025 U/μL BioTaq DNA Polymerase (Bioline USA), and 0.2 ng/μL template DNA. Samples were amplified in a RoboCycler Gradient 96R thermal
cyclers (Stratagene) by an initial denaturation (94 °C, 3 min), followed by 35 cycles of denaturation (94 °C, 40 s), annealing (55 °C, 40 s), and extension (72 °C, 30 s), and a final extension at 72 °C for 4 min. Primers were labelled using either NED, HEX or FAM (Applied Biosystems). Amplification products were separated on polyacrylamide gels in an ABI PRISM 377 DNA sequencer and sized using GeneScan-500 LIZ size standard (Applied Biosystems) and GENEMAPPER version 3.7 (Applied Biosystems). We estimated expected and observed heterozygosities, and number of alleles at each locus using the program GENEPop version 3.1 (Raymond & Rousset 1995). We tested for deviations from Hardy–Weinberg and linkage equilibrium at each locus using GENEPop. Significance levels were adjusted for multiple comparisons using sequential Bonferroni correction (Rice 1989). We used program CERVUS 3.0.3 (Kalinowski et al. 2007) to estimate the frequency of null alleles.

All but one locus, D106, amplified well and showed sufficient polymorphism to be useful in determining population genetic structure in the pygmy rabbit. The number of alleles per locus ranged from four to 12 (Table 1). Observed heterozygosities ranged from 0.26 to 0.89 and observed and expected heterozygosities were
similar for all loci except D106 (Table 1). Locus D106 deviated strongly from Hardy–Weinberg equilibrium and had twice as many homozygotes as expected. All other loci were in Hardy–Weinberg equilibrium except for D103, for which results were inconclusive ($P = 0.0054$, Bonferroni corrected $P = 0.0056$). All pairs of loci were also in linkage equilibrium. The estimated frequency of null alleles was below 10% for all loci except D106 (Table 1). Loci D106 and D118 have both even and odd alleles that were sequenced, and insertions and deletions were confirmed. These newly developed loci in combination with existing loci isolated from the European rabbit will increase the power to detect population genetic structure and examine patterns of gene flow in the pygmy rabbit. The new loci also will contribute to genetic management in the captive breeding programme for the endangered Columbia Basin pygmy rabbits.

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