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METHODOLOGY

Microsatellite markers for 24 loci developed for genotyping eastern woodrats, *Neotoma floridana*

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Abstract

Population declines have been documented in many species within the genus *Neotoma*. Eastern woodrat, *Neotoma floridana*, recovery efforts in the Shawnee National Forest (SNF), Illinois USA provided an opportunity to study the long-term population-level genetic changes following an augmentation and reintroduction. We developed 24 microsatellite markers using QDD and genotyped 32 eastern woodrats from a single population. Number of alleles per locus ranged from 3 to 14 (mean = 7). Observed heterozygosity ranged from 0.375 to 0.969 per locus and expected heterozygosity from 0.485 to 0.854. Two loci showed significant deviation from Hardy–Weinberg equilibrium following Bonferroni sequential corrections. These markers will provide valuable information useful for studying population dynamics of eastern woodrats and closely-related species.

Keywords Augmentation · Eastern woodrat · Microsatellite · Primer · Reintroduction

The development of microsatellite markers helps to identify and develop appropriate management actions to aid in population recovery (Abdul-Muneer 2014), such as in woodrat recovery programs. Musser and Carleton (2005) reclassified the woodrat genus, *Neotoma*, into 22 distinct species. At least four of these species were endangered or possibly extinct, with many more declining throughout their range (Feldhamer and Poole 2008). Illinois' subspecies of the eastern woodrat (*Neotoma floridana illinoensis*) was placed on the Illinois Endangered Species list during 1977–2020 due to restricted habitats and small populations (Mankowski 2012). A historical metapopulation stretching across five counties and the entire east–west extent of southern Illinois collapsed into several isolated populations due to habitat fragmentation (Monty et al. 2003). By the 1960s, Illinois' subspecies of the eastern woodrat was restricted to three populations located in Union (Pine Hills) and Jackson (Fountain Bluff and Horseshoe Bluff) Counties in southwest Illinois (Crim 1961). By 1974 the Fountain Bluff population was extirpated and the population at Horseshoe Bluff had severely declined (Nawrot and Klimstra 1976). Although woodrat numbers were very low in Jackson County, genetic analyses by Monty et al. (2003) showed significant genetic differences between these geographically-proximate populations. The Illinois Department of Natural Resources implemented genetic augmentations and reintroductions across the SNF during 2003–2014. Microsatellite markers provide a means to assess the genetic structure of these populations.

Tissue samples of eastern woodrats were collected from reintroduced populations in the eastern SNF at Garden of the Gods. Capture and handling activities were conducted in accordance with Institutional Animal Care and Use protocol # 19-003 at Southern Illinois University. Genomic libraries were constructed and sequenced by University of Missouri DNA Core Facility (Columbia, MO) using Illumina sequencing. PCR primers were designed using QDD (Meglecz et al. 2010). We tested 24 primer sets on the 32 individuals from Garden of the Gods (Table 1). DNA was extracted using a section of a 3 mm ear biopsy and the Qiagen DNeasy DNA Micro kit (Qiagen Inc., Valencia, California) and concentrations were quantified using Qubit dsDNA HS Assay Kit

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Table 1 Primer sequences and properties of 24 loci developed for *Neotoma floridana*

Locus	Primer sequence (5′–3′)	Repeat motif	Size range	Sample size	Number of alleles	H_O	H_E	HWE p-value	Annealing Temp. °C
NFL001	F: GCAAGACTGCCAACATGTTC	(AC)17	277–297	32	6	0.813	0.794	0.9396	58
	R: GCCCAAGTCCCTTCCATAGG								
NFL002	F: GGGCACAAAGAAGGTGACATT	(AGAT)10	230–261	32	9	0.865	0.837	0.8052	56
	R: TGAGACTGCCAGGGTTGAAA								
NFL003	F: ACCTCTGACAAATGCACTGA	(AC)14	213–235	32	6	0.688	0.795	0.2077	58
	R: TCACTCCATTGTATACCC ATGCA								
NFL004	F: AGGTGACTCACAACCAACTGT	(AGAT)12	206–218	32	4	0.500	0.485	0.9147	58
	R: ACCATTGAGCTACATCTC CAATG								
NFL005	F: ACCCACTGGTGTGTTCTTCT	(AC)12	281–307	32	5	0.656	0.629	0.7528	56
	R: TGGCCGTGTTATGAGCACTT								
NFL006	F: GCTCATTTAAGCTTGGCTCTGA	(AC)14	140–170	32	11	0.813	0.805	0.2455	54
	R: GTCGTTGGTACTTAGGAG GAAGG								
NFL007	F: ACACCCAATACCACCTTGCT	(AC)15	296–314	32	8	0.625	0.738	0.0800	56
	R: GGTCCAGCAGGTAAAGGCTC								
NFL008	F: AGCAAAGAGTTTCCAGTCCCTT	(AG)10	201–205	32	3	0.484	0.528	0.2583	56
	R: TGGAGGTCAGAGGACAACTC								
NFL011	F: AAAGGAGGAGGGAAGGAAGA	(AAGG)9	190–237	32	9	0.844	0.854	0.0800	54
	R: AGGCAAAGAACCCATACACA								
NFL012	F: GTGGAGAGGTTGAGAGGAGT	(AC)17	155–182	32	11	0.875	0.836	0.3570	56
	R: AGGCAGAGGCAGATCAGTTT								
NFL014	F: ACCTGAGTTCAGTTCCCAGT	(AC)13	274–284	32	5	0.750	0.712	0.2987	56
	R: GACCCTGGTCATTTCTGT TAATC								
NFL015	F: GTGTGTATGTGCGAGTGTGC	(AC)17	291–307	32	5	0.375	0.736	0.0005*	56
	R: GCTAGCCTACTTGGGTATTTGC								
NFL016	F: AGTGTGGGAGCACCTCTGA	(AGAT)11	170–186	32	5	0.750	0.740	0.7203	55
	R:ACAATAGTGGTTCATGAGCCC								
NFL017	F: GCAATCCACATCAATGTTCTGA	(AAC)14	175–205	32	6	0.688	0.689	0.9649	56
	R: TGTTGCCATGGCTCTGTAGG								
NFL018	F: GGCATGAGAGAAAGGAAG AATTC	(AT)18	272–292	32	8	0.625	0.798	0.0002*	54
	R: CAGCTTGTTACTTAAGACCAA GAC								
NFL019	F: TCATTGGCTTTGGTGCTTGC	(AC)18	144–160	32	8	0.813	0.842	0.7568	56
	R: ACCCAAGTAACCCAAGTGTCC								
NFL020	F: CTCTTGAAACCAACGGCAAGA	(AC)16	243–253	32	6	0.813	0.762	0.2197	56
	R: GTGCACACATACACACACGC								
NFL021	F: AGTATGGAAAGCAGGATC AGGG	(AC)15	267–283	32	6	0.875	0.742	0.1036	56
	R: GGTTGTCAGAATCAATGA TGGCC								
NFL022	F: TCTATCTTTCCTTCTCTTCCT TCC	(AAAG)13	267–283	32	6	0.656	0.830	0.0243	54
	R: TGACTGCTCATAGTAGGT GTTCA								
NFL024	F: GCACGAGAGATCTACTGGGAC	(AAAG)12	131–181	32	7	0.781	0.774	0.4176	56
	R: TGTAATGAGATCTGGCGCCC								

Table 1 (continued)

Locus	Primer sequence (5′–3′)	Repeat motif	Size range	Sample size	Number of alleles	H_O	H_E	HWE p-value	Annealing Temp. °C
NFL026	F: CTCCTCTGTACAACTTCTAAGGGA	(AC)20	214–244	32	7	0.848	0.838	0.8533	50
	R: GAGCTTGGATTTGAATCAACTGAC								
NFL028	F: CTGAGGAAGTGATCACAAGGGA	(AC)25	173–199	32	10	0.969	0.848	0.5720	56
	R: TTACTGCTTGTGTACCGGCC								
NFL029	F: TCCCTTCCCTAACTCCTTCCA	(AG)22	181–223	32	14	0.906	0.848	0.5360	46
	R: TGCAAGGCCATATACCCAGG								
NFL032	F: TGACAGGGTCCCTACCTCTG	(AC)19	260–282	32	5	0.719	0.776	0.7465	48
	R: ACAATGTATCTGCAGGTTCCA								

H_O observed heterozygosity, H_E expected heterozygosity. Asterisks (*) denote significant deviations from Hardy Weinberg Equilibrium following Bonferroni sequential correction

(ThermoFisher Scientific, USA). Microsatellites were amplified using PCR in 10 µl reactions using 8–40 ng genomic DNA, 5 µl DreamTaq PCR Master Mix (2X) (ThermoFisher Scientific, USA), 0.5–0.9 µM each of a fluorescently-tagged forward primer and an untagged reverse primer. Microsatellite PCR parameters were as follows: initial denature at 94 °C for 4 min, 32 cycles at 94 °C for 45 s, annealing temperature(s) for 30 s, a 4-min extension at 72 °C, and then a 20-min extension at 70 °C (Castleberry et al. 2002; Matocq 2002; Sousa et al. 2007; Kanine 2013). Products were denatured with HiDi formamide. Reactions were resolved on an ABI 3130XL Gene Analyzer (Applied Biosystems Inc., Warrington, UK) against a 70–400 bp standard (Gel Company, San Francisco) and genotyped using GeneMapper® *ID-X* software version 4.0 (ThermoFisher Scientific, USA). GENEPOP 4.7.5 (Raymond and Rousset 1995) was utilized to calculate Expected (H_E) and observed (H_O) heterozygosity and estimate deviations from Hardy–Weinberg equilibrium (Table 1).

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Declarations

Competing interest The authors declare no competing interest.

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