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MICROSATELLITE LETTERS

Twenty-one novel microsatellite loci for the endangered Florida salt marsh vole (*Microtus pennsylvanicus dukecampbelli*)

James D. Austin · Emily V. Saarinen · Alberto Arias-Pérez · Robert A. McCleery · Robert H. Lyons

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Abstract We present 21 microsatellite loci developed for Florida salt marsh voles (*Microtus pennsylvanicus dukecampbelli*). Microsatellites were identified from single molecule real time sequencing (Pacific Biosciences). We screened 30 loci and identified 21 loci as suitable for genotyping. We screened 17 individuals from Long Cabbage Key, and 3 individuals from an unnamed island. There was no significant departure from Hardy–Weinberg equilibrium or linkage equilibrium. Fifteen of the 21 loci were variable, with overall observed heterozygosity averaging 0.39, and a mean number of alleles of 3.14. Linkage disequilibrium estimate of N_e was 10.7 (95 % CI 6.1–20.1). These markers will be useful for conservation genetics studies of this endangered species.

J. D. Austin (✉) · R. A. McCleery
Department of Wildlife Ecology and Conservation, University of Florida, 110 Newins-Ziegler Hall, Gainesville, FL 32611, USA
e-mail: austinj@ufl.edu

E. V. Saarinen
Department of Natural Sciences, University of Michigan – Dearborn, 4901 Evergreen Road, Dearborn, MI 48128, USA

A. Arias-Pérez
Department of Biological Sciences, University of Southern California, 3616 Trousdale Pkwy, Los Angeles, CA 90089, USA

A. Arias-Pérez
Departamento de Biologia Celular y Molecular, Universidade da Coruña, A Zapateira s/n, 15071 A Coruña, Spain

R. H. Lyons
DNA Sequencing Core, University of Michigan-Ann Arbor, 2800 Plymouth Road, Ann Arbor, MI 48109, USA

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The Florida salt marsh vole (*Microtus pennsylvanicus dukecampbelli*) is an insular form of the meadow vole (*M. p. pennsylvanicus*) species complex. Discovered in 1979, the Florida salt marsh vole was confirmed as *M. pennsylvanicus* based on chromosomal counts and their close genetic identity to *M. pennsylvanicus* (Woods et al. 1982). It can be distinguished from other forms of meadow voles by its larger size, tail length, and darker fur (Woods et al. 1982). The vole has been detected at three locations near the southern-most area of the Lower Suwannee and Cedar Keys National Wildlfie Refuge, Levy County, FL (Hotaling et al. 2010). The closest extant population of *M. pennsylvanicus* is approximately 500 km north, and paleoclimate and fossil records suggest *M. p. dukecampbelli* has likely been isolated for over 5000 years. Ongoing research into the status and distribution of salt marsh voles will benefit from the development of highly variable microsatellite markers for studying, among other objectives, effective population size and metapopulation connectivity.

Genomic DNA for sequencing was extracted from muscle (Florida Museum of Natural History, voucher #32239) using the Qiagen DNeasy tissue protocol (Qiagen, Valencia, California, USA). We used single molecule real-time sequencing (SMRT) cell technology on the PacBio RS II platform (Pacific Biosciences, California). We sheared DNA (140 ng/μL), and annealed primers in accordance with PacBio protocols, generating fragment libraries with average fragment size of 2 Kb. We sequenced on a single SMRT cell using P4/XL chemistry. The ≥2× circular reads gave 46,023 and 54,900 unfiltered reads, and average raw read lengths of

Table 1 Characterization of 21 microsatellite loci developed for Florida salt marsh vole (*Microtus pennsylvanicus dukecampbelli*) from 20 voles sampled at Long Cabbage Key and E-140, FL

Locus	Repeat motif	Primer sequence (5′–3′)[a]	Size	A	A_E	H_O/H_E	F_{IS}
Mp2573	$[AAG]_{41}$	TGGCCCTGAATTGTTATTTGA	220–244	7 (8)	4.38	0.824/0.772	−0.067
		CAGACATACAGATACAGACACATGC					
Mp15592	$[GT]_{33}$	TGGCCCTGAATTGTTATTTGA	196–202	3 (4)	1.83	0.353/0.455	0.224
		CAGACATACAGATACAGACACATGC					
Mp17138	$[GA]_{37}$	AAATGCTTATGGTGGGCAAC	160–170	3	1.35	0.294/0.258	−0.141
		CTCTCTCCCCACACCCACTA					
Mp23837	$[AG]_{37}$	GAACAAGAACGAAAGAACAAATGA	223–231	4	2.17	0.647/0.538	−0.203
		CTGGTGAGTGCTCCCAAGAT					
Mp38784	$[GT]_{28}$	GCTCTCGGTTGGTGTACCTC	263–267	2	2	0.647/0.500	−0.294
		TTGTGGCCCATTGAATTCTT					
Mp54234	$[CCT]_{17}[GCT]_8$	CGCAGTTAGGAATGATGTGC	190	1			
		TCCTCCAGTGGTCCTCTGTC					
Mp54953	$[AC]_{16}$	AGCCAGGAAACAAAAGAGCA	201	1			
		GGCAAAACAGGGACATTAACA					
Mp57264	$[CTAT]_{16}$	GACCCACTGCTATGAACACG	234–250	3 (4)	1.57	0.438/0.361	−0.211
		GGGCACAGGTGAAGGTGTAG					
Mp58282	$[GT]_{15}[GA]_{30}$	GGCATGGGAGTAAACTCAGC	246–254	4	2.65	0.625/0.623	−0.003
		CACTAAGCCCAGACAATGGAA					
Mp59727	$[CA]_{17}$	CCATGGCTCAATCTGGAAAT	251	1			
		CCTGGTCTCCTCTGGTGGTA					
Mp65596	$[GT]_{11}[GA]_{31}$	GTTCAGTCAGGCACCCTCTC	250–262	5 (6)	2.28	0.563/0.561	−0.003
		GGCAGGGCAGAGAGAGAATA					
Mp70418	$[GA]_{35}$	TTCAACATCCAGCTGTCCTG	222–234	3	1.92	0.529/0.479	−0.105
		CCAGGCCAGTATTCCTCTGT					
Mp79656	$[CTTT]_{20}$	TGACTTCCTCCTTGGCAAAT	207–231	3 (4)	2.04	0.588/0.510	−0.153
		GAAGCATTTGTTTAAGCTGGCTA					
Mp80523	$[CTTT]_{18}$	TGAAGACATCCAGGTTTGGAC	247–267	5 (6)	3.18	0.706/0.685	0.030
		CACAGCAAACCCTGTCTTGA					
Mp84995	$[GGAA]_{19}$	ACTGGGGATCAGGTGTTCAG	243–251	3	2.93	0.688/0.658	−0.045
		GGGAAGAGGTGGGGAGATAG					
Mp108309	$[TG]_{16}$	GGCCACTGCAACCAATACTT	251–257	2 (3)	1.13	0.118/0.111	−0.063
		ATCAGAGAGTGCCTGCCCTA					
Mp108477	$[CA]_{18}$	AAGTTGTCTGGCCTCCACAT	265–269	2	1.49	0.412/0.327	−0.259
		AGAGGAGGAAGCAAGGAGGT					
Mp134208	$[AAG]_{28}[CAG]_9$	GCAGGTGGATCGCTGTAAGT	223–247	4	3.93	0.765/0.746	−0.026
		GGCTTCTTGGAAATGGATGA					
Mp146156	$[AC]_{17}$	GCAAGTGGGAATTGGAGAGA	268	1			
		TCTGCGTATGTGCTTGCTTC					
Mp154780	$[GCT]_{16}$	CTTTGCCTGCAGAACAAGGT	227	1			
		AACCTCTGAGCCACCTCTCC					
Mp158180	$[AC]_{17}$	CAAGCCCGACAATCTGAGTT	244	1			
		CATGGCTGCTGAATCCTACA					
Long Cabbage Key loci combined				2.81	1.94	0.390/0.361	−0.092
E140 loci combined				1.81	1.59	0.373/0.264	−0.399
Total sample loci combined				2.31	1.77	0.382/0.313	−0.228

[a] Note that forward primers have a 5′ tag CACGACGTTGTAAAACGAC. Size refers to the length of the amplified alleles. A = number of alleles detected, number in parentheses include alleles found in 3 samples from E140; A_E = effective number of alleles; H_O/H_E = observed/expected heterozygosity; F_{IS} = inbreeding index

10,687 and 10,342 bp, respectively. All analyses were performed on the consensus FASTA files. Low-quality ($Q < 20$) sequence reads were trimmed prior to microsatellite identification. Sequence reads were searched for repeats using Msatcommander 0.8.2 (Faircloth 2008). We limited our search of 8798 consensus reads for repeats to di-tri- and tetra-nucleotides with $\geq$14 repeats, and found 1250 putative microsatellite motifs, and primers were developed for 30 of these. We restricted our loci for primer design to longer repeats due to the lack of polymorphism at protein loci (Woods et al. 1982), which may reflect genetic-level reduced polymorphism.

Twenty-one of thirty microsatellite loci (Table 1) were characterized via polymerase chain reaction (PCR) for 17 Florida salt marsh voles sampled from Long Cabbage Key (UTM 297537 E 3234656N) and three samples caught at an unnamed island (E-140) 7 kms south (UTM 300001.94 E 3228143.31N). Nine loci failed to amplify on initial screens or displayed problematic peak morphology. Primer design and PCR followed Saarinen and Austin (2010). We used GENEPOP on the Web to test for linkage disequilibrium (LD) and deviations from Hardy–Weinberg equilibrium (HWE), applying Bonferroni corrections for multiple tests ($\alpha = 0.05$). We calculate allelic diversity and heterozygosity statistics using GenAlEx 6.5 (Peakall and Smouse 2006). We used LDNe 1.31 (Waples and Do 2008) to estimate the unbiased contemporary effective population size (N_e) of Long Cabbage Key voles.

The Long Cabbage vole population was in HWE overall ($\chi^2 = 16.95$, $df = 30$, $P = 0.97$), with no deviations from HWE. The population was in LE, with no significant locus-by-locus pairwise comparisons after Bonferroni correction. The number of alleles (Long Cabbage Key) averaged 2.81; H_O averaged 0.390 and HE averaged 0.361. We observed five private alleles at five loci (Table 1) in the three voles sampled from E-140. Given the relatively large size of the Long Cabbage sample, the private alleles detected at E-140 suggest that these loci will useful in detected differentiation at scales less than 7 kms. The N_e estimate was 10.7 (95 % CI 6.1–20.1). This estimates is within the expected range for N_e given the demographic estimate of number of individuals of 25 (unpublished data) as the N_e/N ratio is expected to be well less than 0.5 in most endangered species (Kalinowski and Waples 2002).

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