Microsatellite DNA loci suitable for parentage analysis in the yellow-pine chipmunk (*Tamias amoenus*)

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The yellow-pine chipmunk (*Tamias amoenus*) exhibits female-biased sexual size dimorphism (Schulte-Hostedde & Millar 2000), and an understanding of the evolution and/or maintenance of this dimorphism requires the determination of individual reproductive success. DNA-based genetic markers are necessary for assigning parentage to quantify reproductive success in promiscuous mating systems, such as chipmunks (Callahan 1981). Here, we: (i) characterize primers for 11 microsatellite loci suitable for parentage studies of yellow-pine chipmunks; and (ii) assess variation in loci derived from Columbian ground squirrels (*Spermophilus columbianus*) which produce amplification products in the least chipmunk (*Tamias minimus*) (Stevens *et al*. 1997).

We extracted DNA from the kidney of a yellow-pine chipmunk taken from the Kananaskis Valley, Alberta and constructed a plasmid library consisting of 250–400 bp fragments using the method described by Dawson *et al*. (1997). Briefly, approximately 10 µg of DNA was digested and fragments containing 250–400 bp were purified from an agarose gel and cloned into a plasmid vector. The library was transformed into XL1-Blue (Stratagene) competent cells and plate lifts made using Hybond-N (Amersham-Pharmacia) nylon membranes. Approximately 50 000 colonies were screened using two dinucleotide polymers [$(TG)_n$ and $(TC)_n$ (Amersham-Pharmacia) labelled with ^{32}P-dCTP] and 165 positive clones were identified. Twenty-five clones, each containing a single insert, were sequenced by MOBIX Central Facility, McMaster University, using dye-terminator chemistry on an ABI 373 A Stretch DNA sequencer. Primers to amplify regions containing repeats were designed from 17 clones using PRIMER (version 0.5; Lincoln *et al*. 1991); however, only 11 of these primer pairs were sufficiently variable for parentage studies (i.e. ≥3 alleles). To assess variability of these 11 loci, we used DNA from ear tissue collected from 76 chipmunks (43 adults, 33 juveniles) in the Kananaskis Valley in 1999. DNA was extracted using QIAGEN® QIAmp tissue kits. Polymerase chain reaction (PCR) was performed on the samples generally following Dawson *et al*. (1997) on a 480 Perkin-Elmer DNA Thermal Cycler, with the following changes: after the

Table 1 Primer sequences, repeat motif, PCR product size for clone, annealing temperature (T_a), number of alleles among 43 adults surveyd, observed (H_O) and expected (H_E) heterozygosity, and GenBank accession nos for microsatellite loci of the yellow-pine chipmunk. F and R are the forward and reverse primer, respectively

Primer	Primer sequence	Repeat	Clone size	T_a	No. of Alleles	H_O	H_E	GenBank
EuAmMS 26	**F** 5′ ACA GGA ACA GCA GAT TGT TGT 3′ **R** 5′ CAC TGT TTG CCT GTG AAG AG 3′	$(CA)_{20}$	181 bp	55 °C	4	0.605	0.551	AF255957
EuAmMS 35	**F** 5′ ATC CGT TTA GTC TGT TAT GTC TCA 3′ **R** 5′ TTT AAT CTA AAG GAC AAC AAT TGC 3′	$(TG)_{12}$	139 bp	55 °C	5	0.674	0.657	AF255958
EuAmMS 37	**F** 5′ CCT GGG AGA AAA TAC TTG GAT G 3′ **R** 5′ AGA AAT GAG GGC AGG GAT AAT T 3′	$(GA)_{17}$	134 bp	55 °C	3	0.488	0.506	AF255959
EuAmMS 41	**F** 5′ ATT CAG GCT CCA GAA AAA CAA A 3′ **R** 5′ TCT GCC CCA GAG ATA TTG ATC T 3′	$(GT)_{16}$	143 bp	54 °C	5	0.721	0.715	AF255960
EuAmMS 86	**F** 5′ AAA GAA TGT GCA GCA AAC CTG 3′ **R** 5′ TTC AAT CCT TTC TAG TGC TCT TCC 5′	$(AC)_{21}$	159 bp	55 °C	5	0.465	0.533	AF255961
EuAmMS 94	**F** 5′ TGG CTC AGT TTT TCA GTT TTT 3′ **R** 5′ ATC TCA AAG CCA TCA AGA GTT T 5′	$(GT)_{14}$	104 bp	51 °C	4	0.279	0.282	AF255962
EuAmMS 108	**F** 5′ TCC CAA CAA CCT CTC TTG ATG 3′ **R** 3′ AAC TTG AAA ATT TTC TTC TGG GC 3′	$(GT)_{10}$	182 bp	53 °C	4	0.651	0.634	AF255963
EuAmMS 114	**F** 5′ CTC AGT CTC CCC AAA CAT TG 3′ **R** 5′ TAG TTC AGT GGT AGG GCA TTC 3′	$(CT)_{21}$	159 bp	53 °C	8	0.860	0.745*	AF255964
EuAmMS 138	**F** 5′ AAT GTA TGC TAG AGT GCC CAC C 3′ **R** 5′ TTT TCT AGA GAC ACA AAA ATT TAG CA 3′	$(AC)_{19}$	128 bp	54 °C	5	0.581	0.694	AF255965
EuAmMS 142	**F** 5′ CTG TGG CGG TCT TAT CTG TAT G 3′ **R** 5′ CCA GTT ACA GCC AGA ACC ACT T 3′	$(CT)_{14}(CA)_{14}$	120 bp	53 °C	4	0.814	0.698	AF255966
EuAmMS 163	**F** 5′ GCC CAT CAA TAG TTG AAT GGA TA 3′ **R** 5′ CCT GGA AAT GCC ATA ATT TTA TTC 3′	$(TC)_6G(TC)_5G(TC)_9(AC)_{20}$	169 bp	60 °C	9	0.710	0.642	AF255967

*indicates a significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$).

initial denaturing step at 94 °C for 3 min, 32 PCR cycles were performed consisting of 45 s at 94 °C, 45 s at the appropriate annealing temperature, and 45 s at 72 °C. Amplification products were resolved on polyacrylamide gels, as described in Dawson *et al*. (1997) except gels were run at 70 W. PCR reactions (1 µL volume) consisted of the following reagents; 2.5 mM of $MgCl_2$ (MBI Fermentas), PCR buffer [75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.01% Tween (MBI Fermentas)] 1 µg/µL BSA (Amersham-Pharmacia), 200 µM dNTP's, 0.25 U *Taq* DNA polymerase (MBI Fermentas), 0.2 pmol of the forward primer labelled with [γ-^{33}P]-ATP (Amersham-Pharmacia), 0.3 pmol of the unlabelled forward primer, and 0.5 pmol unlabelled reverse primer. Table 1 describes the primer sequence, size of clone product, annealing temperature, and number of observed alleles for each locus. We determined whether there were deviations from Hardy–Weinberg equilibrium for each locus from adult chipmunks using GENEPOP (Raymond & Rousset 1995). Only EuAmMS 114 was found to deviate from Hardy–Weinberg expectation due to heterozygote excess (Table 1).

To assess the utility of these microsatellite loci for parentage analysis, we used the likelihood-based approach and simulation procedures of CERVUS 1.0 (Marshall *et al*. 1998). Using this program, we were able to assign maternity to all 33 juveniles (100%) with 80% confidence, 18 (54.5%) of these with 95% confidence. Using known maternity data, we were able to assign paternity to 30 juveniles (90.9%) with 80% confidence, 20 (60.6%) of these with 95% confidence. The microsatellite loci presented here provide adequate information to assess parentage in yellow-pine chipmunks.

We also attempted to amplify samples of yellow-pine chipmunk DNA using four primers which amplify DNA from Columbian ground squirrels and least chipmunks (Loci: GS3, GS17, GS20, and GS34) (Stevens *et al*. 1997). Only two alleles were observed among samples from 22 chipmunks for GS22. At a low-stringency annealing temperature (50 °C) we found only GS20 to amplify, producing one allele. These primers were not considered to be appropriate for further parentage analysis.

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Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae*

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The study of cetaceans by genetic methods is moving increasingly towards the estimation of kinship among individuals using genetic data. Microsatellite loci are ideal for this kind of study given their high mutation rates and co-dominant inheritance. However, a large number of loci need to be genotyped in order to ensure reliable estimation of kinship. Even for relatively small sample sizes, reliable identification of parent–offspring pairs is likely to require more than 17 loci genotyped in each individual (Palsbøll 1999). Towards this end, we isolated an additional nine polymorphic microsatellite loci from genomic DNA of the humpback whale, *Megaptera novaeangliae*, which are presented here.

The loci originate from the same partial genomic library from which we previously presented tri- and tetra-nucleotide microsatellite loci (Palsbøll *et al*. 1997). In this paper, we present additional di-nucleotide loci identified among the positive clones in the above-mentioned genomic library. The isolation and sequencing of clones containing inserts has been described previously (Palsbøll *et al*. 1997). The data presented here are based upon genotypes obtained from up to 353 individual humpback whales, 65 individual fin whales (*Balaenoptera physalus*), 169 individual minke whales (*B. acutorostrata*) and 92 individual blue whales (*B. musculus*).

Total-cell DNA was extracted from skin biopsies by standard phenol and chloroform extractions (Sambrook *et al*. 1989) and the DNA re-suspended in 1 × TE (Sambrook *et al*. 1989). The nucleotide sequence at each locus was amplified by polymerase chain reaction (PCR) (Mullis & Faloona 1987) using 10 µL reaction volumes, each containing 10 ng of genomic DNA, 67 mM Tris–HCl, pH 8.8, 2 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 0.2 mM dNTPs, 1 mM unlabelled oligo-nucleotide primer, 40 µM end-labelled oligo-nucleotide