PRIMER NOTE

Cloning and characterization of 29 tetranucleotide and two dinucleotide polymorphic microsatellite loci from the endangered marbled murrelet (Brachyramphus marmoratus)

MARY BETH REW,* M. ZACHARIAH PEERY,* STEVEN R. BEISSINGER,* MARTINE BÉRUBÉ,* JEFFREY D. LOZIER,* EMILY M. RUBIDGE* and PER J. PALSBØLL†

*Department of Environmental Science, Policy and Management, University of California, Berkeley, 137 Mulford Hall 3114, Berkeley, California 94720-3114, USA, †Museum of Vertebrate Zoology, University of California, Berkeley, 3101 Valley Life Sciences Building, Berkeley, California 94720-3160, USA

Abstract

We developed 31 novel, polymorphic microsatellite loci in the marbled murrelet (Brachyramphus marmoratus), a critically endangered seabird. Variability was tested on 15 individuals from the Santa Cruz, California population, with each locus characterized by two to 12 alleles. Observed levels of heterozygosity ranged from 0.13 to 0.93. These loci provide a valuable means of assessing the population structure and demographic parameters of this species, which may be critical to its conservation across a fragmented habitat.

Keywords: Brachyramphus marmoratus, marbled murrelet, microsatellite, parentage

Received 25 August 2005; revision accepted 5 October 2005

The marbled murrelet (Brachyramphus marmoratus) is an endangered seabird found throughout coastal areas in the Pacific Northwest, Canada and Alaska, associated with old growth forests (USFWS 1997). Understanding the population structure and demographic parameters (e.g. by parentage analysis) of this species is critical to its continued conservation. One approach to gaining inference about fine-scale structuring is from multiple variable STR (short tandem repeat) loci. To this end, we have developed and characterized 31 STR loci from a marbled murrelet genomic library enriched for di-, tri- and tetranucleotide STRs.

A genomic library of marbled murrelet DNA was generated following the protocol of Glenn & Schable (2005). Blood samples were collected from individual marbled murrelets and stored in either 95% ethanol or Longmire’s solution at −20 °C. Genomic DNA was extracted from blood using DNEasy Extraction Kit (QIAGEN) following the manufacturers instructions. Four replicates of genomic DNA were digested with Rsal restriction enzyme (New England Biolabs). Digested DNA was ligated to universal SNX linkers SuperSNX24F and SuperSNX24+4P. The four restriction ligation reactions were each double enriched by hybridization with a cocktail of the following biotinylated oligonucleotide probes: replicate 1: (AACC)5, (AACG)5, (AAGC)5, (AAGG)5, (ATCC)5, (AC)13; replicate 2: (TG)12, (AG)12, (AAG)8, (ATC)8, (AAC)8, (ACT)12; replicate 3: (AAAC)6, (AAAG)6, (AATC)6, (AATG)6, (ACCT)6, (ACTC)6, (ACTG)6; and replicate 4: (AAAT)8, (AACT)8, (AAGT)8, (ACAT)8. The hybridized DNA was captured by isolation with Dynabeads (Dynal) (Glenn & Schable 2005).

We compiled a library of 372 serially enriched clones and generated sequences for 224 of these. Individual bacterial colonies were lysed by boiling, and the cloned inserts were amplified by polymerase chain reaction (PCR, Mullis & Faloona 1987) using T7 and M13 oligonucleotide primers, under the following reaction conditions. Approximately 10 ng of genomic DNA was amplified in a 20-µL reaction containing 67 mM Tris-HCl (pH 8.8), 2 mM MgCl2, 16.6 mM (NH4)2SO4, 10 mM β-mercaptoethanol, 0.2 mM of each dNTP, 0.4 U Tag DNA polymerase (New England Biolabs) and 1 µM of each primer. The PCR consisted of a denaturing step for 2 min at 94 °C; 28 cycles of 1 min at 94 °C, 1 min at 54 °C and 4 min at 72 °C. PCR amplifications took place on a Dyad thermocycler (MJ Research). PCR products were sequenced with T7 and M13 oligonucleotide primers using standard dNTPs-based cycle sequencing (BigDye...
version 3.1, Applied Biosystems) according to the manufacturer’s instructions. Cycle sequence products were resolved on an ABI 3730 automated sequencer (Applied Biosystems). Out of 224 clones, 151 sequences contained a microsatellite. We therefore obtained 67% enrichment of inserts containing marbled murrelet microsatellite DNA.

Of the 151 sequences containing tandem repeats, 94 were simple repeats of dinucleotide \( (n = 40) \), trinucleotide \( (n = 1) \) or tetranucleotide \( (n = 53) \) motifs. The other 57 loci were comprised of either compound or interrupted repeats. Locus names signify the repeat motif (the dominant motif when two or more were present). The number of tandem repeats among the clones for which primers were designed ranged from five (BmaGACA340) to 53 (BmaGATA465).

Locus-specific oligonucleotide primers were designed for 50 loci using PRIMER 3 software (Rozen & Skaletsky 2000), and to the 5’-end of each forward oligonucleotide primer, a universal M13 \( (5’-\text{TGTAAGACGACGCA}GT-3’) \) tail was added. Oligonucleotide primers were obtained (Integrated DNA Technologies), and experimental conditions optimized. Genotyping was carried out in a single reaction cocktail including 67 mM Tris-HCl (pH 8.8), 2 mM MgCl₂, 16.6 mM \( \text{NH}_4\text{SO}_4 \), 10 mM \( \beta \)-mercaptoethanol, 0.2 mM of each dNTP, 0.4 U Taq DNA polymerase (New England Biolabs), 800 nM of reverse primer, 800 nM of forward primer. The PCR consisted of a denaturing step for 2 min at 94 °C; 22–24 cycles of 30 s at 94 °C, 30 s at 59 °C (see exceptions in Table 1) and 30 s at 72 °C (step 1); 8–12 cycles of 30 s at 94 °C, 30 s at 53 °C and 30 s at 72 °C (step 2); and a final extension for 10 min at 72 °C. Extension times were increased to 60 s for BmaGACA56 and BmaGATA464. Amplifications took place on a Dyad thermocycler (MJ Research), and PCR products were visualized on 1.7% agarose gels stained in ethidium bromide.

Of the 50 loci tested, 37 produced distinct bands on agarose, and the amplification products were sized by electrophoresis on an ABI 3730 sequencer using LIZ 500 size standard (Applied Biosystems) to screen for variability. Variability was tested using 15 individual marbled murrelet samples from the Santa Cruz, California population. Alleles were scored using GENOTYPER 3.7 software (Applied Biosystems).

Out of the 37 loci screened, 2 were monomorphic and 35 were polymorphic (2–12 alleles), but 4 polymorphic loci were unable to be scored and were excluded from the analyses. For the 31 remaining loci, the mean number of alleles was 6.16.

Observed \( (H_O) \) and expected \( (H_E) \) heterozygosities were estimated using an unpublished ANSI-C program written by PJP. \( H_E \) was estimated as the expected proportion of heterozygote individuals given the observed allele frequencies for the target locus. \( H_O \) was calculated as the observed number of heterozygote individuals at the target locus. The probability of obtaining \( H_O \) by chance, given the observed population allele frequencies, was estimated by randomizing alleles among individuals and estimating \( H_O \). The probability of \( H_O \) was estimated as the fraction of 10,000 simulations that yielded a similar or more extreme value of \( H_O \). After correcting for multiple tests (Rice 1989), three loci still exhibited statistically lower \( H_O \) than expected under random mating (Table 1). \( H_O \) ranged from 0.13 to 0.93, and the mean \( H_O \) was estimated at 0.60 for all loci and at 0.77 for the 20 most variable loci. The probability of identity (Paetkau et al. 1995) for each locus is listed in Table 1; values of \( I \) ranged from 0.028 to 0.62, with the mean estimated at 0.19. For all loci combined, \( I \) was estimated at 4.6 × 10⁻²⁰. We tested for linkage disequilibrium using GENEPOP (Raymond & Rousset 1995) and found no evidence of significant linkage among the 31 loci tested.

The 31 STR loci presented here yield unambiguous data with high levels of variation, making them suitable to estimate genetic divergence among populations as well as individuals.

Here is the Table 1:

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequences</th>
<th>( T_a )</th>
<th>Cycles</th>
<th>( N )</th>
<th>Size range (bp)</th>
<th>( H_O )</th>
<th>( H_E )</th>
<th>( P ) value</th>
<th>( I )</th>
<th>GenBank Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BmaAAAC336</td>
<td>F: CCCCCCTTCTGTGTTGGTTTT</td>
<td>59/53</td>
<td>22/8</td>
<td>15</td>
<td>176–196</td>
<td>0.13</td>
<td>0.62</td>
<td>&lt; 0.0001</td>
<td>0.20</td>
<td>DQ173162</td>
</tr>
<tr>
<td>BmaAAAG043</td>
<td>F: GGGGAATTTCATTTATTTTACCA</td>
<td>59/53</td>
<td>22/8</td>
<td>15</td>
<td>209–213</td>
<td>0.33</td>
<td>0.28</td>
<td>&lt; 0.9999</td>
<td>0.56</td>
<td>DQ173163</td>
</tr>
<tr>
<td>BmaAAAG433</td>
<td>F: TCAGGAGATTTTCTTTTTTTCTCA</td>
<td>59/53</td>
<td>22/8</td>
<td>15</td>
<td>165–203</td>
<td>0.87</td>
<td>0.79</td>
<td>&lt; 0.8109</td>
<td>0.077</td>
<td>DQ173164</td>
</tr>
<tr>
<td>BmaACCT555</td>
<td>F: GAGCAGTATGTAATGGAGAGATGG</td>
<td>59/53</td>
<td>22/8</td>
<td>15</td>
<td>132–194</td>
<td>0.93</td>
<td>0.87</td>
<td>&lt; 0.8153</td>
<td>0.032</td>
<td>DQ173165</td>
</tr>
</tbody>
</table>
P values in boldface indicate loci with significantly lower $H_O$ than expected, assuming random mating. Annealing temperatures ($T_a$) for nested PCR steps 1 and 2 (as referred to in the text) are given in °C, and numbers of PCR cycles likewise correspond to steps 1 and 2.

© 2006 Blackwell Publishing Ltd, Molecular Ecology Notes, 6, 241–244
Acknowledgements

The authors acknowledge support for the laboratory work from the Washington Department of Fish and Wildlife’s Nestucca Restoration Fund, facilitated by Dr Ken Warheit and the US Fish and Wildlife Service (Sacramento and Arcata Field Offices). The authors also thank the California Department of Fish and Game, the Oiled Wildlife Care Network, Pacific Lumber Company, Big Creek Lumber Company, California State Parks, the University of California at Berkeley and the Environmental Protection Agency for funding sample collection. Veronica Morris offered technical assistance.

References


