Characterization of seven polymorphic microsatellite loci in the Common Loon (Gavia immer)

AMY M. McMillan,* MARK J. Bagley† and DAVID C. Evers‡
*Biology Department, SUNY College at Buffalo, 1300 Elmwood Ave., Buffalo, NY 14222, USA, †U.S. Environmental Protection Agency, Cincinnati, OH 45268, USA, ‡BioDiversity Research Institute, 19 Flaggy Meadow Road, Gorham, ME 04038, USA

Abstract

We describe polymerase chain reaction (PCR) primers and conditions to amplify seven microsatellite DNA loci isolated from the Common Loon (Gavia immer). The PCR primers were tested on 83 individuals from 10 locations in North America, including breeding, migration stopover, and wintering areas. Between two and seven alleles were observed to segregate at the seven microsatellite loci, with observed heterozygosities ranging from 0.048 to 0.695.

Keywords: Aves, Gaviidae, loons, microsatellite, PCR, primer

Received 13 January 2004; revision received 25 February 2004; accepted 25 February 2004

The Common Loon (Gavia immer) is a large obligate piscivore that breeds in lakes of North America and Iceland. Extensive demographic research suggests that loons are highly philopatric and territorial in breeding areas (Evers 2001), whereas wintering loon populations represent individuals originating from many geographical areas. In North America, wintering populations are found primarily in nearshore coastal environments. Winter populations are susceptible to oil spills while summer populations are susceptible to mercury poisoning (Evers et al. 1998, 2003), lake acidification (Doka et al. 2003), and many other threats. Migratory populations are susceptible to botulism outbreaks (Sea Grant 2003). Despite significant demographic data, little is known about the population genetic structure of Common Loons. Polymorphic genetic markers for loons will assist in understanding the population genetic structure and dispersal of summering loons, help to establish the origins of loons on their migration route and wintering grounds, and assist in developing strategies for their conservation.

DNA was extracted from blood samples stored in phosphate buffer solution (PBS) taken from live-caught Common Loons using Qiagen DNeasy kits. Extracted DNA was enriched for (CA)_{12}, (AG)_{12}, (AAG)_{9}, (AAC)_{9}, (AATG)_{6}, (TAGA)_{6}, (AAGG)_{6}, (GATC)_{6}, and (CCAT)_{6} following a protocol by Glenn et al. (2000) that was slightly modified. DNA was digested in three separate digests with HaeIII, RsaI, and AluI and ligated to 5′ reverse phosphorylated linkers (forward 5′-GTTTGTAAGGCCTAGCTAGCAGAATC-3′, reverse 5′-GATTCTGCTAGCTAGGCCTTACAAACAAA-3′). The resulting segments were hybridized to biotinylated microsatellite oligonucleotides and captured on streptavidin beads. Captured DNA was amplified by polymerase chain reaction (PCR) using a primer complementary to the forward linkers. The product was ligated into pGEM®-T Easy Vector (Promega), and transformed into Epicurian Coli® SoloPack® Gold competent cells (Stratagene). Colonies with inserts were amplified using M13 forward and reverse primers and the presence of microsatellites was confirmed by probing dot blots with biotin-labelled microsatellite motifs and chemiluminescent detection using streptavidin coupled to alkaline phosphatase (Roche). Colonies positive for microsatellites were sequenced using ABI Prism® BigDye™ Terminator v3.0 chemistry on an ABI 3100 Genetic Analyser. Primer pairs for each microsatellite-containing sequence were chosen with primer 3 software (Rozen & Skaletsky 1997).

Initial screening for microsatellite amplification and polymorphism was performed in 20 µL PCR reactions with an MJ Research Dyad Thermal Cycler. The PCR included 10 pmol each unlabelled forward and reverse primers, 200 µm each dNTP, 1.5 mM MgCl2, 1 U Taq DNA polymerase (Gibco BRL) and 25 ng template DNA in a buffer containing 20 mM Tris-HCl and 50 mM KCl (pH 8.0). The thermal cycling regime utilized a touchdown protocol encompassing...
a 10 °C drop in annealing temperature from the beginning to the end. Cycling parameters were: 1 min at 95 °C; 12 cycles of 30 s at 95 °C, 30 s at 64 °C, dropping 0.8 °C for each cycle after the first, 45 s at 72 °C; 23 cycles of 30 s at 95 °C, 30 s at 54 °C, 45 s at 72 °C; followed by a 5 min extension at 72 °C and a 10 °C hold. PCR products were sequenced for polymorphism on 5% acrylamide gels stained with a Vistra Green® (Amersham) agarose overlay (Rodzen et al. 1998) on a Fluorimager 595 (Molecular Dynamics).

The forward primer of each polymorphic primer pair was fluorescently labelled (see Table 1) and multiplex sets were developed for further analysis. Two multiplex primer sets were developed containing three primer sets each (see Table 1). Loon DNA samples from Maine (n = 2), Nevada (n = 9), New Hampshire (n = 17), New York (n = 10), Vermont (n = 5), and Virginia (n = 4) in the USA and New Brunswick (n = 2), Nova Scotia (n = 1), and Quebec (n = 2) in Canada were amplified using the multiplex sets with a PCR thermal regime as described above. The 15 µL PCR reactions included 3 pmol each labelled forward and unlabelled reverse primer, 250 µM each dNTP, 1.5 mm MgCl₂, 0.6 U Taq DNA polymerase (Gibco BRL) and 15 ng template DNA in the previously described PCR buffer. PCR products were diluted tenfold in formamide, mixed with ROX 500 size standard (ABI), and electophoresed on a BaseStation genetic analyser (MJ Bioworks). Microsatellite alleles were sized using Capilograph (ver. 1.2.4sg) software (MJ Bioworks).

A summary characterization of each microsatellite locus is presented in Table 1. Expected heterozygosity was calculated using Nei’s unbiased estimator (Nei 1987). Deviations from Hardy–Weinberg equilibrium were evaluated using genepop (Raymond & Rousset 1995). A heterozygote deficiency was noted for locus GimD12EPA when all sites were pooled. No Hardy–Weinberg disequilibrium was noted in the analysis of individual locations, which may suggest that the observed heterozygote deficiency was due to the presence of population substructure.

This initial screening revealed a small degree of substructure in North America. We are presently following up this analysis by increasing sample sizes from these locations and collecting more Common Loon samples across the species range. The polymorphic markers obtained from this study should prove useful for evaluating genetic relationships among birds from different geographical areas, which should allow us to connect breeding and wintering populations of these birds and estimate environmental vulnerabilities to different population segments.

Acknowledgements

We thank the staff at BioDiversity Research Institute, the New York Department of Environmental Conservation, and Environment Canada for tissue collection. Suzanne Christ and Eric Waits, USEPA Cincinnati, OH provided invaluable technical expertise in completing the laboratory portion of this project. This work was performed while AMM held a National Research Council Research Associateship. The United States Environmental Protection Agency through its Office of Research and Development partially funded and collaborated in the research described here. It has been subjected to Agency review and approved for publication.

© 2004 Blackwell Publishing Ltd, Molecular Ecology Notes, 4, 297–299

Table 1 Characteristics of seven polymorphic microsatellite loci in Gavia immer. Primer sequences are listed with the fluorescent dye label indicated. Allele size refers to the predicted size (bp) of the cloned allele with the observed allelic size distribution for each locus in parentheses. Two multiplex sets with a PCR thermal regime as described (GimA12EPA was not multiplexed). Expected heterozygosity (Hₑ), observed heterozygosity (Hₒ), repeat sequence, observed number of alleles, and accession numbers are listed.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequence (5’–3’)</th>
<th>Repeat sequence</th>
<th>No. alleles</th>
<th>Allele size (range)</th>
<th>Hₑ</th>
<th>Hₒ</th>
<th>Multiplex set</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GimA12EPA</td>
<td>HEX-TCACTTGGTGTATATATATGACCA</td>
<td>(CCAN)₈</td>
<td>7</td>
<td>138 (129–153)</td>
<td>0.728</td>
<td>0.695</td>
<td>single</td>
<td>AY509039</td>
</tr>
<tr>
<td>GimC5EPA</td>
<td>FAM-AGTATCTGAGAGAGAGGGTG</td>
<td>(GGT)₉</td>
<td>4</td>
<td>108 (102–112)</td>
<td>0.425</td>
<td>0.494</td>
<td>1</td>
<td>AY509040</td>
</tr>
<tr>
<td>GimD12EPA</td>
<td>FAM-TCTTCTTGTTGTGTGAGAGATTC</td>
<td>(GGT)₇</td>
<td>4</td>
<td>195 (188–198)</td>
<td>0.379</td>
<td>0.305</td>
<td>1</td>
<td>AY509037</td>
</tr>
<tr>
<td>GimE11EPA</td>
<td>TET-GGATATTAAACACGCAACC</td>
<td>(CA)₅₋₃</td>
<td>2</td>
<td>146 (146–148)</td>
<td>0.053</td>
<td>0.048</td>
<td>1</td>
<td>AY509043</td>
</tr>
<tr>
<td>GimD9EPA</td>
<td>FAM-TCAGTGAGAAGCTCTGGG</td>
<td>(GGT)₉</td>
<td>4</td>
<td>169 (173–181)</td>
<td>0.473</td>
<td>0.542</td>
<td>2</td>
<td>AY509042</td>
</tr>
<tr>
<td>GimC11EPA</td>
<td>HEX-CTACAGCTTAAAATTTGGAATCA</td>
<td>(GGT)₁₀</td>
<td>4</td>
<td>177 (174–180)</td>
<td>0.588</td>
<td>0.561</td>
<td>2</td>
<td>AY509043</td>
</tr>
<tr>
<td>GimA9EPA</td>
<td>TET-CACAGCAACAAGCAGACAC</td>
<td>(GGT)₇</td>
<td>3</td>
<td>147 (143–147)</td>
<td>0.554</td>
<td>0.530</td>
<td>2</td>
<td>AY509038</td>
</tr>
</tbody>
</table>

*A Alleles 188, 196, and 198 are clearly defined. The 192 allele may be a composite of a 191 bp and 192 bp allele but could not be reliably differentiated.
References


