PRIMER NOTE

Characterization of microsatellite loci in Humboldt penguin (
*Spheniscus humboldti*) and cross-amplification in other penguin species

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Abstract

The Humboldt penguin (*Spheniscus humboldti*) was once very common throughout its range along the coast of Peru and Chile. Today, listed as endangered, it is crucial to gain an understanding of gene flow and levels of genetic variation between breeding colonies to protect this species effectively. We developed seven microsatellite primers to investigate gene flow and population structure among four colonies. Locus-specific allelic diversity ranges from five to 11 alleles and observed heterozygosity ranges from 0.50 to 0.88. These markers cross-amplify in eight penguin species over five genera.

Keywords: cross-amplification, microsatellite, Penguin, *Spheniscus humboldti*

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The Humboldt penguin, *Spheniscus humboldti*, is endemic to the west coast of South America and is one of the most endangered penguin species in the world (Wallace *et al.* 1999). Little is known of this penguin’s dispersal behaviour and population structure, although it is thought to be sedentary (Williams 1995). The use of DNA microsatellites provides an alternative to behavioural and tracking studies in a species that spends much of its life off shore. In this note, we report seven microsatellite primers developed from *S. humboldti* that will be used to study genetic diversity and population structure in this species. Cross-species amplification of these primers in eight additional penguin species is also described.

Primers were developed following the protocols of Garner *et al.* (2000) and Gautschi *et al.* (2000). Extracted DNA was digested with Tsp509I (New England Biolabs), 500–1000 bp size fragments were isolated and ligated to TSPADSHORT/TSPADLONG linkers (Tenzer *et al.* 1999) and polymerase chain reaction (PCR) was used to enrich the fragment pool. PCR products were hybridized to biotinylated CA repeat probes attached to Dynabeads M-280 Streptavidin (DYNAL, France). After a second PCR, these enriched fragments were cloned using the Invitrogen TA® cloning kit. After plating cells on a selective (X-gal) agar media, white colonies were replica-plated and subsequently dot-blotted onto Hybond N+ membranes (Amersham Pharmacia). Dot blots were screened for inserts with CA repeats following the ECL oligolabelling kit protocol (Amersham Pharmacia). M13 primers and the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, version 2.0 (PE Biosystems) and the ABI 377 automated sequencing system (PE Biosystems) were used to sequence plasmids isolated from positive clones. We used primer 3 software (Rozen & Skaletsky 1998) to design primers.

Genomic DNA was extracted from blood following a modified protocol of Sambrook *et al.* (1989). All primers were first optimized using a temperature and MgCl2 gradient on a BIORAD iCycler. PCR amplification was performed in a 15-μL reaction containing 1× reaction buffer (Promega), 2 mM MgCl2 (1.5 mM for Sh1Ca9 and Sh2Ca21), 2.25 μM dNTP, 0.6 μM of each primer, 0.75 U of Taq polymerase (Promega), and 30–40 ng DNA. Thermal cycling was performed using a BIORAD iCycler and the following conditions: 3 min at 95 °C, 35 cycles at 95 °C for 30 s, 40 s at 53–60 °C (see Table 1), and 40 s at 72 °C, followed by a final step at 72 °C for 5 min PCR products were combined with the Beckman Coulter CEQ DNA Size Standard 400 and formamide and run on a Beckman Coulter CEQ 2000XL

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capillary genotyping system. We used DNA ANALYSIS SYSTEM Software, version 4.3.9 (Beckman Coulter, Inc.) to visualize and characterize the microsatellites.

Polymorphism and heterozygosity for Humboldt penguin microsatellites were assessed using 24 samples from three Chilean and one Peruvian breeding colony. Characteristics of seven successfully amplifying loci are provided in Table 1. Observed and expected heterozygosity and deviations from Hardy–Weinberg equilibrium (HWE) were calculated using genepop (Raymond & Rousset 1995). All loci were in HWE, except locus Sh2Ca22 which showed significant heterozygote deficiency (P = 0.0217). This deviation is more likely due to scoring error rather than null alleles, since no null homozygotes were observed.

Eight additional penguin species were also tested: Gentoo penguin (Pygoscelis papua), Chinstrap penguin (P. antarctica), Rockhopper (Eudyptes chrysocome), Maccaroni penguin (E. chrysolophus), King penguin (Aptenodytes patagonicus), and Little Blue penguin (Eudyptula minor). In these cases, samples from captive individuals from North American zoos were amplified and genotyped as described above (primers were not optimized for these species). Results for each locus and species are summarized in Table 2. The ability of these primers to amplify microsatellites across a range of species and genera suggests their usefulness for a variety of population genetics studies.

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