Usefulness of avian buccal cells for molecular sexing

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Recently non-invasive sampling has become important for DNA analysis in avian ecology. In the last decade, blood samples, feathers or urine samples have been widely used as non-invasive samples. Recently, Seki (2003) proposed buccal cells as a non-invasive sample for molecular studies in ornithology because sampling buccal cells is an easily performed and safe method taking less time than sampling blood. However, he investigated only one species (Ryukyu Robin Erithacus komadori) and found lower yields of DNA from buccal cells than from blood samples (Seki 2003). In the present study, we performed sex determination based on DNA extracted from the buccal cells (buccal DNA) of 12 wild bird species and argue for the efficiency of avian buccal cells as a non-invasive sample for molecular studies.

MATERIALS AND METHODS

Sampling was performed between February and June in 2004 and 2005 in six areas in Japan: in forest of the Kamigamo Experimental Station, Field Science Education and Research Center, Kyoto University, Kyoto Prefecture (35°04′N, 135°45′E); Mt. Daimonji state forest, Kyoto Prefecture (35°01′N, 135°48′E), Tanba town, Kyoto Prefecture (35°09′N, 135°24′E), Kanmuri Island, Kyoto Prefecture (35°40′N, 135°25′E), Yasu River, Moriyama City, Shiga Prefecture (35°02′N, 136°01′E), and Lake Koyaike Park, Itami City, Hyogo Prefecture (34°47′N, 135°23′E). Bird species exhibiting morphological sexual dimorphism, were attracted by song playback and captured in mistnets (under license), and were sexed based on morphological characteristics. In addition, nestlings of the Great Cormorant Phalacrocorax carbo were captured by hand from the colony in Lake Koyaike Park, and 10 μl of blood samples were collected (see Kajita 1999). The sex of each Great Cormorant was determined by PCR using DNA extracted from blood (blood DNA; see Fridolfsson & Ellegren 1999) using the same molecular method as that for the buccal cell samples described below.

Buccal cells were collected from each captured bird by rolling a cotton swab against the inside of the mouth and throat of each bird for approximately five seconds. Each swab was placed into a microtube containing 1 ml of 99.5% ethanol, rotated for c. 10 s, and the swab was discarded. The above was repeated once more using another swab, which was put into the same microtube. The microtube was kept at −20°C for about three months.

Prior to extraction of DNA, each microtube was centrifuged at 4°C at 6000×g (14,000 rpm) for 15 min, the ethanol was discarded without disturbing the cell pellet, and the pellet was allowed to dry. Each of the samples was then digested in 400 μl of STE buffer (150 mM NaCl, 10 mM Tris-HCl pH 8.0, 10 mM EDTA) with 4 μl of 10% SDS and 80 μg proteinase K at 37°C overnight. DNA purification was performed by the conventional phenol/chloroform method (Maniatis et al. 1982).

We used one primer pair 2550F (5′-GTTACTGGATTCGTCTACGAGA-3′) and 2718R (5′-ATTGAAATGATCCAGTGCTTG-3′) to amplify the Z-linked avian CHD gene (CHD1Z gene) and the W-linked copy of the CHD gene (CHD1W gene; Fridolfsson & Ellegren 1999). All PCRs were performed in 10 μl volumes on a Perkin Elmer 9600 Thermal Cycler. We performed three methods of PCR for all samples. In the first place we performed one-step PCR using Ampli Taq Gold (Applied Biosystems) as Taq DNA polymerase (single PCR). Each reaction mixture of the single PCR contained 0.1 units of Taq, 200 μM dNTPs, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 3 mM MgCl2, 2 μM of each primer, and c. 100 ng of DNA template. The thermal profile com-

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