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Use of Buccal Swabs for Sampling DNA from Nestling and Adult Birds

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Abstract

*We evaluated the feasibility and efficiency of using swabs to collect buccal epithelial cells from small (2- to 13-g) birds as a source of DNA for genetic studies. We used commercially available buccal swab kits to collect samples from 42 adult and 39 nestling (4- to 8-day-old) black-capped chickadees (*Poecile atricapillus*) and from 6 4-day-old nestling boreal chickadees (*P. hudsonica*). We compared DNA from buccal epithelial samples to that from blood samples from the same individuals. We extracted sufficient quantities of DNA for analysis from all buccal samples, and samples remained viable even after being stored in original plastic sampling tubes at room temperature for up to 18 months. Yields were equivalent whether extracted using the proprietary quick-extraction solution provided with buccal swab kits or using a salt-extraction process with inexpensive reagents. Yields of DNA from buccal samples were consistently lower than those from blood samples, but quantities were sufficient for all analyses. Assignment of sex, based on DNA extracted from paired buccal and blood samples, was identical for all 87 birds. We found no difference in the genotypes obtained from buccal and blood samples for 12 individuals tested using 5 microsatellite loci and found perfect concordance in sequencing of an 823-base-pair segment within the control region of mitochondrial DNA for 7 individuals tested. Use of buccal swabs is highly recommended as a rapid, noninvasive technique for sampling avian genomic DNA, especially for extremely young altricial nestlings or small-bodied adults, or for any birds for which blood sampling may be impossible or stressful.*
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Key words

black-capped chickadee, boreal chickadee, buccal swab, DNA, microsatellites, noninvasive sampling, Poecile atricapillus*,* Poecile hudsonica*, polymerase chain reaction, population genetics, sexing.*

Analysis of DNA has become an essential tool in avian ecology, providing critical information for studies of phylogeny and systematics, population genetics, and mating systems. For example, gender can be determined for many sexually monomorphic species using the chromodomain helicase DNA-binding (CHD) gene (Griffiths et al. 1998), the occurrence of extra-pair paternity can be assessed using DNA-microsatellite genotyping (Leech et al. 2001), and the degree of population structuring can be evaluated by sequencing mitochondrial DNA (mtDNA) and genotyping microsatellites (Lanctot et al. 1999, Pearce et al. 2004).

Genetic studies involving live birds most often rely on extraction of DNA from samples of nucleated red blood cells, but DNA also has been successfully extracted from feathers (Ellegren 1992, Pearce et al. 1997), skin (Groombridge et al. 2000), claws (Drummond et al. 1997), vascularized eggshell membranes (Pearce et al. 1997, Kimwele et al. 1998), and eggshells (Strausberger and Ashley 2001). Drawing blood is an invasive procedure that requires several minutes of handling time and may increase the stress level of the birds (Wingfield 1999), although it has been shown to have minor impact on their behavior and survival (Bigler et al. 1977, Stangel 1986, Hoysak and Weatherhead 1991, Lanctot 1994). Obtaining blood by clipping a toenail is a less invasive technique that has been used for sampling DNA from adult endangered songbirds (Busch et al. 2000) but not yet demonstrated for young nestlings. Minimizing investigator-caused disturbance is critical for assessing true effects of natural factors in studies of breeding success, behavior, and survival of adults and young. Blood sampling can be particularly problematic for small-bodied adults and young nestlings of altricial species because of their small veins and low blood volume (Wingfield 1999). Altricial nestlings are particularly susceptible to investigator disturbance because of their limited ability to thermoregulate. Drawing blood also potentially exposes researchers to blood-borne pathogens and requires proper disposal of biohazardous materials.

Sampling buccal epithelial cells with either buccal swabs or mouthwash has recently gained acceptance as a reliable, noninvasive technique for acquiring human genomic DNA for forensic and epidemiological studies (Rudbeck and Dissing 1998, Heath et al. 2001, Neuhaus et al. 2004). More recently, the buccal-swab technique has been used successfully to sample nonhuman mammalian DNA for veterinary and laboratory studies (Brooks et al. 2003, Oberbauer et al. 2003, Meldgaard et al. 2004) and amphibian DNA for field studies (Pidancier et al. 2003). The purpose of our study was to test the feasibility and efficiency of using swabs to sample buccal epithelial cells from small altricial birds for extraction and analysis of genomic DNA. Through a comparison with standard blood

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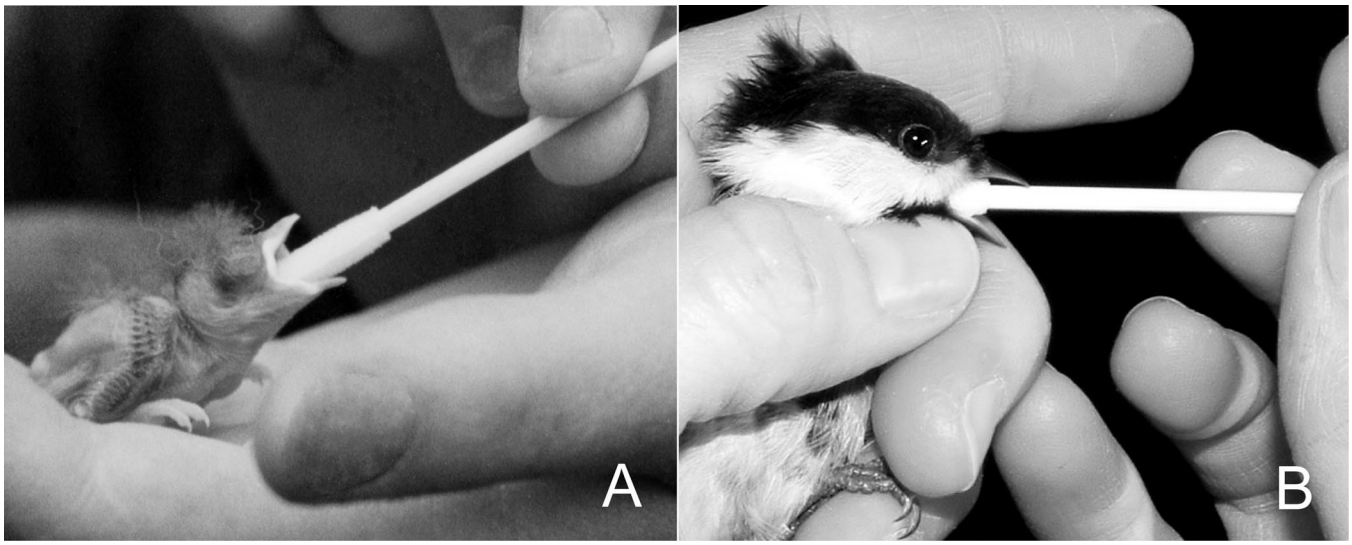

Figure 1. Buccal epithelial cells were sampled from (A) a 4-day-old, 2.1-g nestling and (B) an adult black-capped chickadee by gently rotating a foam-tipped buccal swab against the inside of the cheeks and across the tongue.

sampling, we sought to determine whether buccal sampling would yield adequate amounts of genomic DNA for accurate molecular sexing, microsatellite genotyping, and mtDNA sequencing in black-capped (*Poecile atricapillus*) and boreal (*P. hudsonica*) chickadees.

Methods

Collection of Samples

From 2001 to 2003 we collected blood and buccal epithelial cells from 42 adult and 39 nestling (4- to 8-day-old) black-capped chickadees (42 M, 39 F) and 6 nestling boreal chickadees (4 M, 2 F) as part of a study of beak deformities among birds in Alaska, USA. We captured nestlings and some adults in experimental nest boxes and other adults in winter traps at various field sites in south-central Alaska. We trapped breeding adults by placing a hoop of net attached to a long handle over the entrance hole while the adult was inside the nest box incubating eggs (female) or provisioning young (male). We flushed the adults into the net by gently tapping on the boxes and removed nestlings by hand through the removable top. Rectangular winter traps were constructed of plastic-coated wire mesh and designed as per Senar et al. (1997), except that the funnel opening was made smaller (2-cm diam) to prevent red squirrels (*Tamiasciurus hudsonicus*) from entering. We suspended traps on pulleys in trees and baited them with sunflower seeds and peanut butter, on which trapped birds could feed ad libitum. During the summer we banded, measured, and sampled tissues from adults and nestlings as rapidly as possible with a portable kit adjacent to each nest box. During the winter we checked traps every 40–60 minutes, removed birds from traps by hand, and transported them in individual laundered and bleached cotton bags to a nearby indoor laboratory for processing. We captured birds under United States Geological Survey Banding Permit 20022. We followed recommendations by the Ornithological Council for handling wild birds and sampling tissues (Gaunt and Oring 1999), and committees from the United States Geological Survey and Fish and Wildlife Service approved our protocols (Protocol #1130-7F22).

We collected buccal epithelial cells from each chickadee by holding the bird in one hand and gently rotating a sterile foam-tipped buccal swab (Epicentre® Catch-All™ Sample Collection Swabs, Madison, Wisconsin) with the other hand 3–5 times against the inner cheeks and across the tongue (Fig. 1). Each sample required only 5–10 seconds to collect. We washed our hands with an alcohol-based disinfectant before handling birds but did not use gloves. We sampled most nestlings at 4 days old and nestlings were naked except for a few downy plumes. Mass of black-capped chickadee nestlings averaged 4.1 g (SE = 0.16, range 2.3–7.6) and mass of adults averaged 11.2 g (SE = 0.11, range 9.8–12.5). Mass of 4-day-old boreal chickadees averaged 3.7 g (SE = 0.44, range 2.1–5.0). Each buccal swab had a 150-mm-long plastic shaft tipped with a foam "bud" (4-mm diam, 15-mm length) that was slightly longer than the buccal cavity of an adult chickadee; several brands of similar swabs are available commercially in various sizes. We easily inserted swabs into the buccal cavity because adults aggressively bit the swabs and nestlings readily gaped when a swab was presented. After collecting buccal samples, we allowed swabs to air-dry in the field (summer) or laboratory (winter) for 10–15 minutes before replacing them in their individual plastic collection tubes and then stored them in the central laboratory at room temperature for 1–18 months until extraction.

From each of these birds, we also collected 5–50 μL of blood in 70-μL nonheparinized microhematocrit capillary tubes by basilic venipuncture with a sterilized 27.5-gauge needle after disinfecting the skin with 70% isopropyl alcohol. We applied pressure to the puncture wound with a sterile cotton ball for 1 minute or until bleeding stopped. We collected blood at the same time as buccal samples for adults but at later ages (12–14 days) for nestlings, when body mass and blood volume were greater and bodies were

fully feathered. We immediately transferred blood samples to 400 μL of Longmire buffer solution (Longmire et al. 1988) in 1.7-mL microcentrifuge tubes and stored them at room temperature for 1–8 months until we extracted DNA.

DNA Extraction

For buccal samples from all birds except 6 black-capped and 6 boreal chickadee nestlings, we followed DNA extraction protocols provided with the buccal swab kits (BuccalAmp™ DNA Extraction Kit, Epicentre Technologies, Madison, Wisconsin). We rotated each swab 15–20 times in a 1.7-mL microcentrifuge tube with 500 μL of QuickExtract™ (Epicentre Technologies) DNA extraction solution. We pressed and rotated the foam bud against the side of the tube while removing it to ensure most of the liquid remained in the tube. We vortexed tubes for 10 seconds, then incubated them for 1 30-minute period at 65°C and 2 8-minute periods at 98°C, alternating and ending with vortex bouts of 15 seconds.

We extracted genomic DNA from all blood samples following the salt-extraction protocol as described in Medrano et al. (1990) and modified by Sonsthagen et al. (2004). To determine if we could extract more DNA from buccal swabs than what was yielded by using the commercial QuickExtract solution, we also tested the salt-extraction protocol on 12 nestling buccal samples. We rotated each buccal swab 15–20 times in a tube of lysis buffer (50 mM Tris-HCl [pH 8.0], 50 mM ethylenediaminetetraacetic acid [EDTA; pH 8.0], 1% sodium dodecyl sulfate, 100 mM NaCl, with 1% beta-mercaptoethanol added to lysis buffer just prior to use). We then followed the salt-extraction procedure (Medrano et al. 1990, Sonsthagen et al. 2004) with the addition of 0.5 μL of molecular-grade glycogen (20 mg/mL; Roche Diagnostics 901393, Indianapolis, Indiana) during the isopropanol precipitation stage. To measure the amount of DNA extracted, we added 2 μL of DNA extract to fluorometry solution for a final volume of 2,000 μL in a 1-cm cuvette. We quantified DNA extractions using a DyNA Quant™ 200 Fluorometer (Amersham Biosciences, GE Healthcare, Piscataway, New Jersey), with which original concentrations could be measured accurately down to 10 ng/μL. We diluted the DNA extract, if necessary, to working solutions of <50 ng/μL. We stored all processed swabs and extracted DNA at −20°C in 1.7-mL microcentrifuge tubes.

Molecular Sexing

We amplified DNA from both buccal and blood samples of all 87 individuals under standard polymerase chain reaction (PCR) conditions using the P8/P2 primer set to determine gender of each bird based on the CHD gene (Griffiths et al. 1998) that has been used successfully for black-capped chickadees (Ramsay et al. 2003). In this species the reaction yields a 324-base-pair (bp) product from the *Z*-chromosome (both males and females) and a 380-bp product from the *W*-chromosome (females only). Among boreal chickadees, the reaction yields a 324-bp product from the *Z*- and a 386-bp product from the *W*-chromosome.

We performed PCR reactions on a RoboCycler® Gradient 96 (Stratagene Corporation, La Jolla, California). We carried out PCR amplifications of DNA in a final volume of 10 μL containing 1 μL of DNA extract, 10.0 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50.0 mM KCl, 0.01% gelatin, 0.01% NP40, 0.01% Triton-X 100, 0.2 mM deoxyribonucleotide triphosphate (dNTP), 3.6 pmoles unlabeled forward P8 primer, 4.0 pmoles unlabeled reverse P2 primer, 0.4 pmoles labeled P8 primer, 0.1 μg/μL bovine serum albumin, and 0.75 units *Taq* polymerase (United States Biochemical, Cleveland, Ohio). The PCR reactions began at 94°C for 90 seconds; continued with 40 cycles each of 48°C for 45 seconds, 72°C for 45 seconds, and 94°C for 30 seconds; and concluded with a final annealing and extension step of 48°C for 60 seconds and 72°C for 5 minutes. We electrophoresed PCR reaction products on a 48-well 18-cm 6% polyacrylamide gel on a LI-COR® 4200LR automated sequencer (LI-COR, Inc., Lincoln, Nebraska). We assigned sex based on the absence (male: *ZZ*) or presence (female: *ZW*) of the band for the *W*-chromosome.

Microsatellite Genotyping

We used both buccal (QuickExtract) and blood samples to genotype 9 individual black-capped chickadees and 3 boreal chickadees at each of 5 microsatellite loci (PmaGAn11, PmaGAn28, PmaTAGAn71 [Saladin et al. 2003]; *Esc*μ6 [Hanotte et al. 1994]; PAT MP 2–14 [Otter et al. 1998]) known to be polymorphic for populations in Alaska (L. M. Pajot, United States Geological Survey, unpublished data). Four of the 5 loci contain dinucleotide repeats; PmaTAGAn71 contains a tetranucleotide repeat. We followed protocols described in Sonsthagen et al. (2004) for PCR amplifications but the 5 loci were multiplexed into 2 PCR reactions (PmaGAn11, PmaGAn28, and PmaTAGAn71; *Esc*μ6 and PAT MP 2–14). In the first multiplex, the forward primer for each primer pair was synthesized with a modified 19- to 20-bp tail added to the 5′ end of the oligonucleotide (Oetting et al. 1995). We used a complementary fluorescently labeled (IRD700 or IRD800) primer to detect alleles at these 3 loci. The multiplex containing *Esc*μ6 and PAT MP 2–14 employed custom IRD-labeled primers, 800 and 700, respectively. The IRD800 is a heptamethine cyanine dye absorbing and fluorescing in the near-infrared region of the spectrum (approx. 795 nm). The IRD700 is a pentamethine carbocyanine dye fluorescing at approximately 685 nm (LI-COR 1999).

We carried out amplifications in a final volume of 10 μL that contained 1 μL DNA extract, 0.2 mM dNTPs, 0.1 μg BSA, 1× PCR buffer (Perkin Elmer Cetus I; PE Biosystems, Forest City, California), and 0.2 units *Taq* polymerase. Primer concentrations for the first multiplex were 10.0 pmoles unlabeled primers and 1.0 pmole fluorescently labeled primer; concentrations for the second multiplex were 3.6 pmoles unlabeled forward primer, 4.0 pmoles unlabeled reverse primer, and 0.4 pmoles fluorescently labeled forward primer for each primer pair in the reaction. The PCR reactions began at 94°C for 90 seconds and continued with 40 cycles each of 94°C for 30 seconds, 50–56°C for 30 seconds, and 72°C for 60 seconds. We

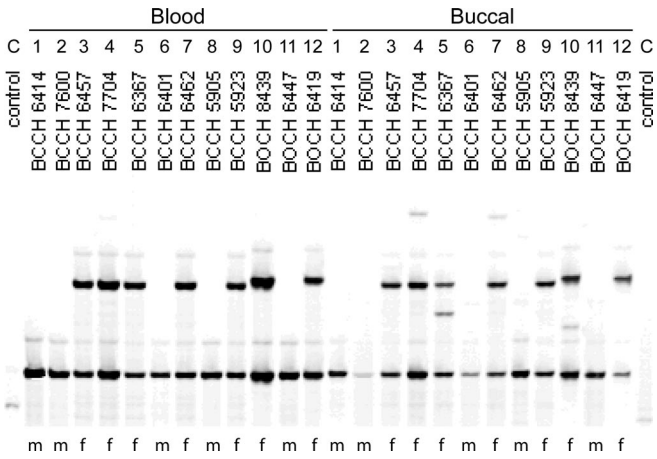

Figure 2. Patterns of amplified DNA after polymerase chain reaction for molecular sexing of 9 black-capped chickadees (BCCH) and 3 boreal chickadees (BOCH) from paired blood and buccal samples. The first and last lanes (C) show negative controls. Individuals 1, 2, 6, 8, and 11 are males (m), with a single 324-base-pair (bp) product; other individuals with 2 bands are females (f). Female boreal chickadees (individuals 10 and 12) have a slightly higher second band (386 bp) than female black-capped chickadees (380 bp). Concentrations of DNA amplified from buccal epithelial cells were consistently lower than those from blood, sometimes resulting in bands of lower intensity from buccal DNA.

concluded each reaction with a final extension at 72°C for 30 minutes. We electrophoresed PCR products on a 48-well 25-cm 6% polyacrylamide gel on a LI-COR 4200LR automated sequencer (LI-COR, Inc.). We designated allele sizes by referencing an M13 DNA sequence ladder. We used samples of scored individuals on subsequent gels to size new genotypes using Gene ImagIR™ 4.05 software (Scanalytics, Inc., Fairfax, Virginia).

Mitochondrial DNA Sequencing

We amplified and sequenced an 823-bp fragment within the control region of the mtDNA using primers LBCCHCR (5′-CATGCTTTAYAGGGTATGC-3′) and the heavy-strand primer HBCCHCR (5′-AATAGCGCGGTT-TAACG-3′) from DNA from each of 7 paired samples of buccal swabs and blood from the same black-capped chickadee individuals. Primers were synthesized with added universal sequences (BluescriptT7P; GTAATACGACT-CACTATAGGGC; and M13Rev; GGATAACAATTT-CACACAGG) on the light- and heavy-strand primers, respectively, to allow for subsequent simultaneous bidirectional sequencing (SBS™; LI-COR, Inc. 1999) using universally tailed primers (Oetting et al. 1995). The PCR products were electrophoresed in TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA) against a 100-bp DNA ladder on a 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light. We purified PCR products using a PEG precipitation (30% PEG 3350/1.5M NaCl) protocol modified by S. L. Talbot (unpublished data) from Kusukawa et al. (1990). Purified products were cycle-sequenced via sequencing by synthesis (SBS) using a commercial kit (Sequitherm LCII 2.0®; Epicentre Technologies, Madison, Wisconsin). We used fluorescently labeled universal primers (LI-COR; BluescriptT7P and M13Rev) to prime the SBS reaction. We electrophoresed SBS products on a 64-lane 41-cm 5.5% polyacrylamide gel on a LI-COR 4200L automated sequencer (LI-COR, Inc.). We analyzed MtDNA sequences using LI-COR eSeq™ imaging software and aligned using AlignIR 2.0™.

We strictly observed sterile techniques when handling DNA. We performed all procedures using positive controls (to provide replication) and negative controls (to provide evidence of contamination). For sexing and genotyping, we performed 2 replications of all samples and up to 7 replications for weak individual samples (mostly buccal). At times the results of one of the multiplex reactions (usually *Esc*μ6 and PAT MP 2–14) were weak, so we re-amplified samples for the loci separately. We performed 2 full replications for sequencing, and 3 samples were re-extracted from the original swab. The 12 duplicate extractions of buccal swabs (salt vs. Epicentre solution) served as additional replications for those samples. In all procedures we used molecular-grade reagents, which are available from many suppliers.

Results

We obtained fluorometer readings of 4.3 ± 4.9 (SD) ng/μL (range 0–30, $n = 75$) of DNA from buccal samples extracted with the rapid process using the QuickExtract solution. These were marginally higher (Mann–Whitney $U = 292.5$, $P = 0.05$) than readings from buccal samples extracted with the salt-extraction protocol (2.7 ± 3.9 ng/μL, range 0–13, $n = 12$), although most buccal samples fell below the level at which our fluorometer could measure concentrations precisely (i.e., <10 ng/μL). Nonetheless, quantities of DNA from all samples were sufficient for sexing, genotyping, and sequencing of mtDNA. By comparison, yields from blood samples using salt extraction were 257 ± 202 ng/μL (range 5–924, $n = 87$), significantly higher than those from buccal samples from the same individuals ($t_{86} = 11.64$, $P < 0.0001$). The total time to extract genomic DNA was much less using the QuickExtract solution (1.5 hr) compared with the salt-extraction procedure, in which we allowed DNA to incubate for a minimum of 12 hours, although hands-on processing time was only slightly greater for salt extraction. Quick extraction required labeling only 1 tube per sample whereas the salt-extraction protocol required 2 tubes, or 3 if dilution was required, adding time for processing samples and increasing the possibility of errors through mislabeling.

We found 100% agreement in molecular sexing of 81 black-capped chickadees and 6 boreal chickadees from the comparative analysis of genomic DNA from buccal and blood samples (Fig. 2). All 12 microsatellite-genotyping results from buccal samples corresponded to results from the blood samples from the same individuals (Fig. 3). We observed no allelic dropout, but band intensity for the molecular sexing and genotyping of the buccal samples was weaker than for the blood samples. Results from sequencing of mtDNA also showed complete correspondence between buccal and blood samples for all 7 birds tested. Band

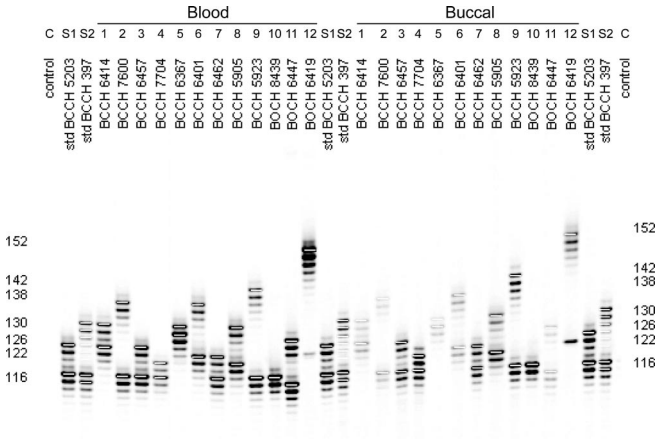

Figure 3. Comparison of microsatellite alleles at the *Esc*μ6 locus (Hanotte et al. 1994) from polymerase chain reaction (PCR)–amplified DNA extracted from paired samples of blood and buccal epithelial cells from 9 black-capped chickadees (BCCH) and 3 boreal chickadees (BOCH). This locus was 1 of 5 microsatellite loci run in a multiplex PCR reaction. The first and last lanes (C) show negative controls. S1 and S2 were individuals with known fragment lengths used as size standards; numbers show size of products in base pairs. Horizontal white bars indicate allele fragments (except for S2, where they also indicate stutter bands for sizing purposes). Band intensity generally was lower for buccal samples, but all birds could be genotyped. Individuals 8 and 9 were adults; the others were 4- to 6-day-old nestlings.

intensity was stronger for buccal samples than for blood in 2 cases but otherwise fairly equivalent (Fig. 4). No contamination was observed in negative controls for any of the procedures. We also observed no other evidence of contamination (e.g., more than 2 alleles at microsatellite loci, identical genotypes for different individuals, or comigration of bands in sequencing reactions).

Discussion

We demonstrated that buccal epithelial cells from birds can be used as a reliable source of genomic DNA for a broad array of molecular genetic studies. Quantities of DNA from individual samples were sufficient for multiple analyses, including molecular sexing, microsatellite genotyping, and mtDNA sequencing in black-capped and boreal chickadees, although the yield from buccal cells was consistently lower than that from blood, as found in other vertebrates (Brooks et al. 2003, Neuhaus et al. 2004). The perfect concordance in results from analyses of chickadee buccal and blood samples suggests that the buccal-swab technique is as robust and reliable for birds as it is for humans (Walker et al. 1999, Bennett et al. 2000), other mammals (Oberbauer et al. 2003, Meldgaard et al. 2004), and amphibians (Pidancier et al. 2003).

The ease and rapidity of using buccal swabs can minimize time spent handling birds not only during nesting but also at other times when birds may be susceptible to stress, such as during cold or inclement weather or periods of molt. Individual plastic containers provided with the buccal swab kits were easy to label and could be stored for at least 18 months at room temperature. Having to air-dry swabs for 10–15 minutes before closing the tubes might be inconvenient, but a silica gel desiccant could be used to accelerate

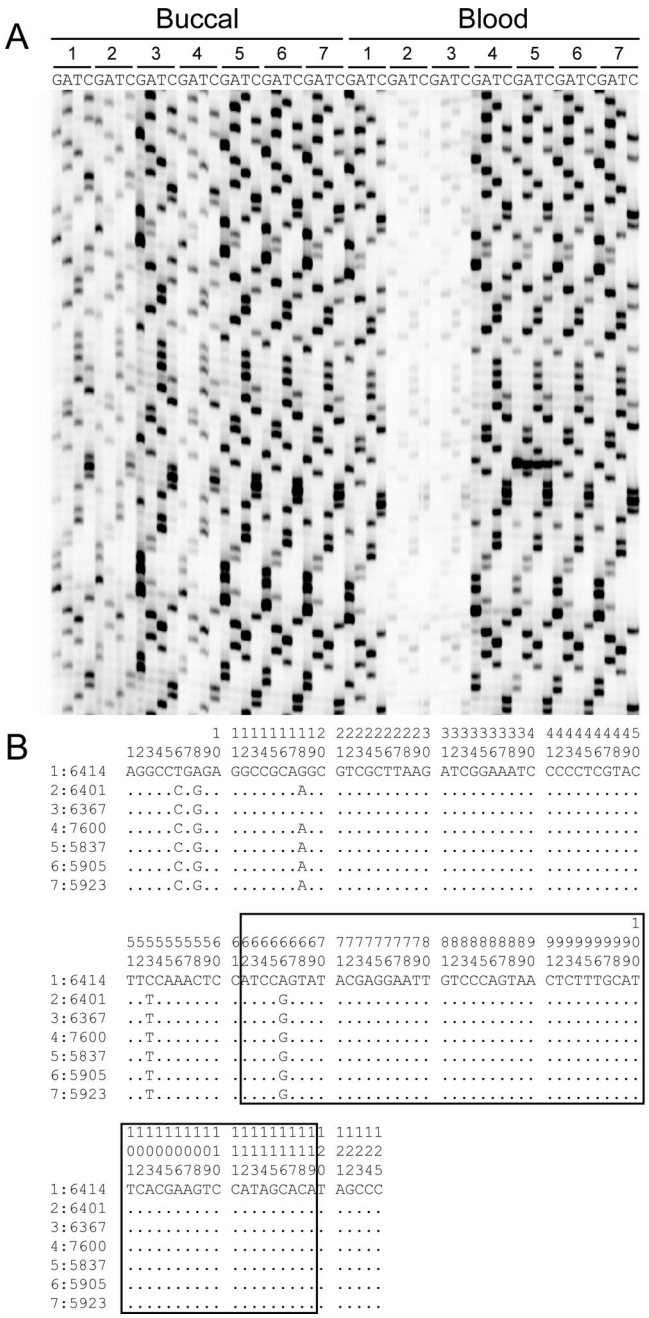

Figure 4. (A) Image from polyacrylamide gel showing 58 base pairs (bp) from an 823-bp segment of the mitochondrial DNA (mtDNA) control region of 7 black-capped chickadees from buccal epithelial and blood cells. Band intensity was variable among nestlings (1–4) because of the difficulty sometimes encountered in drawing blood from small veins or obtaining enough buccal cells to extract adequate DNA. Band intensity was consistently strong among adults (5–7) for both types of samples. (B) Observed nucleotide sequence data from 125 bp of the mtDNA control region for these 7 individuals, with sample number following colon. The 58 bp illustrated in panel (A) are shown inside boxes. Numbered nucleotide position (read vertically) refers to the location of each variable site in the observed sequences, which are counted from the bottom to the top in panel (A). Dots (.) indicate similarity with the first sequence (sample 6414).

the drying process. Failing to dry samples before storage could lead to extremely low DNA yields, likely through decay of the sampled cells and their DNA content under humid conditions (Meldgaard et al. 2004). Buccal samples from amphibians that had been stored at room temperature

for 9 weeks yielded less DNA than samples that were fresh or had been stored frozen at −18°C, likely due to nucleic acid degradation (Pidancier et al. 2003). As an alternative to dry storage, foam buds from the swabs could be snipped off directly after sampling into microcentrifuge tubes filled with Longmire buffer solution and stored until salt extraction of the DNA. After extraction DNA from mammalian buccal epithelial cells has remained stable for at least 5 years when stored at −20°C (Oberbauer et al. 2003).

The major drawback of sampling buccal rather than blood cells was the much lower yield of DNA, regardless of the extraction method used. During optimization experiments for molecular sexing, which required higher concentrations of DNA than other genetic analyses, we found that adding more *Taq* polymerase to the PCR amplification master mix produced higher yields of DNA from the buccal cells. Doubling the final PCR volume as well as the volume of DNA extract also increased the yield for reliable testing, as it did in testing of mammalian buccal cells (Meldgaard et al. 2004). Another alternative for increasing the amount of DNA available for extraction would be to collect multiple buccal samples per individual (Oberbauer et al. 2003). Because contamination can greatly affect DNA extractions with low yields, we recommend strict adherence to sterile techniques such as those outlined for studies involving hair or feathers (Paetkau 2003). For developing new loci, testing protocols, or training new laboratory technicians, we recommend that researchers also obtain samples of blood or other tissues from a subsample of individuals to ensure extraction of high quantities of DNA.

Although using the commercial, proprietary quick-extraction solution enabled more rapid results than standard salt extraction, the actual hands-on processing time was about equivalent for the 2 protocols. In most wildlife studies, accommodating longer incubation periods would be a relatively minor inconvenience given that the salt-extraction procedure uses much less expensive, commonly available reagents and yields equivalent amounts of DNA.

Buccal swabs may be the ideal tool for sampling DNA of altricial nestlings, precocial shorebird young, and small adult birds. Studies examining paternity or sex ratios among small passerines (e.g., Martins 2004, Yamaguchi et al. 2004) typically wait to sample blood from nestlings until they are $\geq$6 days old because of the difficulty or negative impacts of drawing blood from smaller nestlings. Mortality of nestlings before they are old enough to be sampled may bias the results of such studies, so investigators generally either limit analyses to broods with no mortality (which does not preclude bias) or settle for the uncertain assignment of nestlings that disappeared (Grindstaff et al. 2001, Leech et al. 2001). Although DNA has been successfully extracted from posthatch egg membranes for genotyping offspring of some waterfowl and other large birds (Pearce et al. 1997, Kimwele et al. 1998), songbirds and shorebirds typically remove eggshells immediately after hatch, negating this technique as a sampling alternative for many species. Because of their natural gaping response, even newly hatched songbird nestlings can be sampled readily with buccal swabs, thereby minimizing potential bias from early nestling mortality. Buccal swabs also may prove useful for sampling DNA from live adult hummingbirds (Trochilidae), newly hatched shorebirds, and other species whose very small body size or protected status necessitates alternative techniques to traditional blood sampling (Wingfield 1999, Busch et al. 2000, Hiebert et al. 2000, Pidancier et al. 2003).

Acknowledgments

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Literature Cited

Bennett, L. C., R. Kraemer, and S. Liechti-Gallati. 2000. Buccal cell DNA analysis in premature and term neonates: screening for mutations of the complete coding region for the cystic fibrosis transmembrane conductance regulator. European Journal of Pediatrics 159:99–102.

Bigler, W. J., G. L. Hoff, and L. A. Scribner. 1977. Survival of mourning doves unaffected by withdrawing blood samples. Bird-Banding 48: 168.

Brooks, R., J. Williamson, A. Hensley, E. Butler, G. Touchton, and E. Smith. 2003. Buccal cells as a source of DNA for comparative animal genomic analysis. Biotechnology Letters 25:451–454.

Busch, J. D., M. P. Miller, E. H. Paxton, M. K. Sogge, and P. Keim. 2000. Genetic variation in the endangered southwestern willow flycatcher. Auk 117:586–595.

Drummond, P. B., Y. Song, D. Vaughn, A. Gbadamosi, K. Butler, and E. J. Smith. 1997. Isolation of genomic DNA from claw clippings for genetic analysis in chickens. BioTechniques 22:874–876.

Ellegren, H. 1992. Polymerase-chain-reaction (PCR) analysis of microsatellites—a new approach to studies of genetic relationships in birds. Auk 109:886–895.

Gaunt, A. S., and L. W. Oring, editors. 1999. Guidelines to the use of wild birds in research. Second edition. The Ornithological Council, Washington, D.C., USA.

Griffiths, R., M. C. Double, K. Orr, and R. J. G. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071–1075.

Grindstaff, J. L., C. A. Buerkle, J. M. Casto, V. Nolan Jr., and E. D. Ketterson. 2001. Offspring sex ratio is unrelated to male attractiveness in dark-eyed juncos (*Junco hyemalis*). Behavioral Ecology and Sociobiology 50:312–316.

Groombridge, J. J., C. G. Jones, M. W. Bruford, and R. A. Nichols. 2000. 'Ghost' alleles of the Mauritius kestrel. Nature 403:616.

Hanotte, O., C. Zanon, A. Pugh, C. Greig, A. Dixon, and T. Burke. 1994. Isolation and characterization of microsatellite loci in a passerine bird: the reed bunting *Emberiza schoeniclus*. Molecular Ecology 3:529–530.

Heath, E. M., N. W. Morken, K. A. Campbell, D. Tkach, E. A. Boyd, and D. A. Strom. 2001. Use of buccal cells collected in mouthwash as a source of DNA for clinical testing. Archives of Pathology and Laboratory Medicine 125:127–133.

Hiebert, S. M., M. Ramenofsky, K. Salvante, J. C. Wingfield, and C. L. Gass. 2000. Noninvasive methods for measuring and manipulating

corticosterone in hummingbirds. General and Comparative Endocrinology 120:235–247.
Hoysak, D. J., and P. J. Weatherhead. 1991. Sampling blood from birds: a technique and an assessment of its effect. Condor 93:746–752.
Kimwele, C. N., J. A. Graves, T. Burke, and O. Hanotte. 1998. Development of microsatellite markers for parentage typing of chicks in the ostrich *Struthio camelus*. Molecular Ecology 7:249–251.
Kusukawa, N., T. Uemori, K. Asada, and I. Kato. 1990. Rapid and reliable protocol for direct sequencing of material amplified by the polymerase chain reaction. BioTechniques 9:66–72.
Lanctot, R. B. 1994. Blood sampling in juvenile buff-breasted sandpipers: movement, mass change and survival. Journal of Field Ornithology 65:534–542.
Lanctot, R., B. Goatcher, K. Scribner, S. Talbot, B. Pierson, D. Esler, and D. Zwiefelhofer. 1999. Harlequin duck recovery from the *Exxon Valdez* oil spill: a population genetics perspective. Auk 116:781–791.
Leech, D. I., I. R. Hartley, I. R. K. Stewart, S. C. Griffith, and T. Burke. 2001. No effect of parental quality or extrapair paternity on brood sex ratio in the blue tit (*Parus caeruleus*). Behavioral Ecology 12:674–680.
LI-COR, Inc. 1999. DNA sequencing manual. Global edition, IR^2 system. Publication number 9910-150, LI-COR, Inc., Lincoln, Nebraska, USA.
Longmire, J. L., A. K. Lewis, N. C. Brown, J. M. Buckingham, L. M. Clark, M. D. Jones, L. J. Meincke, J. Meyne, R. L. Ratliff, F. A. Ray, R. P. Wagner, and R. K. Moyzis. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. Genomics 2:14–24.
Martins, T. L. F. 2004. Sex-specific growth rates in zebra finch nestlings: a possible mechanism for sex ratio adjustment. Behavioral Ecology 15:174–180.
Medrano, J. F., E. Aasen, and L. Sharrow. 1990. DNA extraction from nucleated red blood cells. BioTechniques 8:43.
Meldgaard, M., P. J. A. Bollen, and B. Finsen. 2004. Non-invasive method for sampling and extraction of mouse DNA for PCR. Laboratory Animals 38:413–417.
Neuhaus, T., G. Geisen, H. M. Bolt, V. Janzen, A. Kraemer, H. Vetter, and Y. Ko. 2004. Reliability of non-invasively acquired human genomic DNA as a substrate for real-time PCR-assisted analysis of genetic polymorphisms. Archives of Toxicology 78:390–396.
Oberbauer, A. M., D. I. Grossman, M. L. Eggleston, D. N. Irion, A. L. Schaffer, N. C. Pedersen, and J. M. Belanger. 2003. Alternatives to blood as a source of DNA for large-scale scanning studies of canine genome linkages. Veterinary Research Communications 27:27–38.
Oetting, W. S., H. K. Lee, D. J. Flanders, G. L. Wiesner, T. A. Sellers, and R. A. King. 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. Genomics 30:450–458.
Otter, K., L. Ratcliffe, D. Michaud, and P. T. Boag. 1998. Do female black-capped chickadees prefer high-ranking males as extra-pair partners? Behavioral Ecology and Sociobiology 43:25–36.
Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology 12:1375–1387.
Pearce, J. M., R. L. Fields, and K. T. Scribner. 1997. Nest materials as a source of genetic data for avian ecological studies. Journal of Field Ornithology 68:471–481.
Pearce, J. M., S. L. Talbot, B. J. Pierson, M. R. Petersen, K. T. Scribner, D. L. Dickson, and A. Mosbech. 2004. Lack of spatial genetic structure among nesting and wintering king eiders. Condor 106:229–240.
Pidancier, N., C. Miquel, and C. Miaud. 2003. Buccal swabs as a nondestructive tissue sampling method for DNA analysis in amphibians. Herpetological Journal 13:175–178.
Ramsay, S. M., D. J. Mennill, K. A. Otter, L. M. Ratcliffe, and P. T. Boag. 2003. Sex allocation in black-capped chickadees *Poecile atricapilla*. Journal of Avian Biology 34:134–139.
Rudbek, L., and J. Dissing. 1998. Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR. BioTechniques 25:588–590,592.
Saladin, V., D. Bonfils, T. Binz, and H. Richner. 2003. Isolation and characterization of 16 microsatellite loci in the great tit *Parus major*. Molecular Ecology Notes 3:520–522.
Senar, J. C., J. Domènech, L. M. Carrascal, and E. Moreno. 1997. A funnel trap for the capture of tits. Butlletí del Grup Català d'Anellament 14:17–24.
Sonsthagen, S. A., S. L. Talbot, and C. M. White. 2004. Gene flow and genetic characterization of northern goshawks breeding in Utah. Condor 106:826–836.
Stangel, P. W. 1986. Lack of effects from sampling blood from small birds. Condor 88:244–245.
Strausberger, B. M., and M. V. Ashley. 2001. Eggs yield nuclear DNA from egg-laying female cowbirds, their embryos, and offspring. Conservation Genetics 2:385–390.
Walker, A. H., D. Najarian, D. L. White, J. M. Jaffe, P. A. Kanetsky, and T. R. Rebbeck. 1999. Collection of genomic DNA by buccal swabs for polymerase chain reaction-based biomarker assays. Environmental Health Perspectives 107:517–520.
Wingfield, J. C. 1999. Minor manipulative procedures. Pages 29–33 *in* A. S. Gaunt and L. W. Oring, editors. Guidelines to the use of wild birds in research. Second edition. The Ornithological Council, Washington, D.C., USA.
Yamaguchi, N., K. K. Kawano, K. Eguchi, and T. Yahara. 2004. Facultative sex ratio adjustment in response to male tarsus length in the varied tit *Parus varius*. Ibis 146:108–113.

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