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SHORT NOTE

Buccal swabs as a reliable source of DNA for sexing young and adult Common Swifts (*Apus apus*)

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Abstract We investigated the reliability of buccal swabs for molecular sex determination in very young nestlings (0–14 days old) and adults of the Common Swift (*Apus apus*) compared to the results from blood samples of the same individuals. Sex determination based on buccal swabs matched the result of sex determination based on blood samples in 46 out of 47 nestlings (98 %) and in all 10 adults (100 %). Therefore, we consider that buccal swab sampling is a reliable noninvasive method to obtain DNA for sex determination in swifts. We recommend buccal swabbing as an alternative to blood sampling in future genetic studies in birds.

Keywords Noninvasive sampling · Sex determination · Nonpasserine bird · Chelex

Zusammenfassung

Speichelproben als zuverlässige DNS-Quelle für molekulare Geschlechtsbestimmung bei jungen und adulten Mauerseglern (*Apus apus*)

Wir untersuchten die Zuverlässigkeit der molekulargenetischen Geschlechtsbestimmung anhand von Speichelproben bei sehr jungen Nestlingen (bis 14 Tage alt) und Altvögeln des Mauerseglers (*Apus apus*) im Vergleich zu Blutproben derselben Individuen. Die Geschlechtsbestimmung mit Hilfe von Speichelproben stimmte bei 46 von 47 Nestlingen (98 %) und bei allen 10 Altvögeln (100 %) mit den Ergebnissen der Geschlechtsbestimmung anhand von Blutproben überein. Daher sehen wir die Entnahme von Speichelproben als eine zuverlässige, nicht-invasive Methode an, DNS zur Geschlechtsbestimmung bei Mauerseglern zu gewinnen, und empfehlen den Einsatz von Speichelproben bei zukünftigen genetischen Untersuchungen an Vögeln.

Introduction

Generally, blood or feather samples are taken to get DNA-containing tissue for sex determination in adult and young sexually monomorphic birds. There is an ongoing discussion, however, about whether these two invasive sampling methods are harmful and may have negative effects on breeding success and survival (Sheldon et al. 2008; Voss et al. 2010; McDonald and Griffith 2011). Recently, Brown and Brown (2009) showed that blood sampling led to a 21–33 % reduction in average annual survival in adult Cliff Swallows (*Petrochelidon pyrrhonota*). In young nestlings, taking blood samples for sex determination may also adversely affect survival because of their small body size and low mass, and it is often simply not feasible. Furthermore, in altricial birds, young nestlings are unfeathered, so plucking a feather is no alternative to blood sampling. Likewise, DNA extracted from droppings is likely to be too degraded to allow successful genetic analysis, or the residues in such extractions inhibit a polymerase chain reaction (PCR; Seki 2003). Especially in

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experimental studies, it is necessary to determine the sex of a nestling as soon as possible after hatching. Thus, a feasible alternative noninvasive sampling method to get DNA material for sexing birds is to sample buccal swabs. Buccal swabs are commonly used in epidemiological and forensic investigations to acquire human DNA (Handel et al. 2006). They were applied recently in field and laboratory studies of nonhuman mammals (e.g., Brooks et al. 2003; Mitrečić et al. 2008), reptiles and amphibians (e.g., Poschadel and Möller 2004), and even fish (Smalley and Campanella 2005). Although buccal swabs have lately been used in bird studies (e.g., Brubaker et al. 2011; Yannic et al. 2011), only a few studies have evaluated the reliability of buccal swabs for sex determination in birds, especially in very young nestlings (Seki 2003; Arima and Ohnishi 2006; Handel et al. 2006). The aim of our study was to carry out such an evaluation by using buccal swabs of young nestlings (aged between 0 and 14 days) and adults of the Common Swift (*Apus apus*). We validated the accuracy of the method by comparing the results with those of sex determination based on blood samples from the same individuals.

Methods

Study site and sampling

We sampled Common Swifts in a breeding colony situated in a concrete federal highway bridge spanning the Bigge Reservoir near Olpe, North Rhine-Westphalia, Germany (51°02′33″N 07°49′40″E). During breeding season in 2009 and 2010, we collected both buccal swabs and blood samples from 11 adult birds and 53 nestlings. Nestlings were individually marked immediately after hatching. At the time of buccal swab sampling, 26 nestlings were 0–4 days old (body mass: 2.29–10.42 g) and 27 nestlings were 8–14 days old (body mass: 26.24–40.86 g; ±0.01 g accuracy; KERN digital balance). Buccal swabs were taken according to a manual enclosed with a mini sample kit developed by I. Adam, M. Honarmand, and C. Scharff (pers. comm.; Adam's manual). Accordingly, we used a strip of chromatography paper (strip size: about 10 cm in length × 4 mm in width) to sample DNA-containing epithelial cells with saliva. Each insalivated tip of the paper strip was cut off and placed in 200 µl of 5 % (w/w) Chelex100™ (Bio-Rad Laboratories, München, Germany). We took blood samples from the same individuals by brachial venipuncture (stored in 1 ml PBS/EDTA buffer). Nestlings were sampled at the age of 18–21 days.

Genetic analysis

DNA of buccal swabs was extracted according to the protocol of Adam's manual. Each sample was incubated at 56 °C for 15 min in a water bath and vortexed for 10 s afterwards. The samples were then boiled for 8 min in a water bath and vortexed for 10 s again. Finally, the samples were centrifuged at 13,000×*g* for 3 min. DNA of blood samples was extracted either with a NucleoSpin™ blood purification kit (Macherey-Nagel, Düren, Germany) or using a standard proteinase K/chloroform-isoamyl alcohol method.

We amplified sequences of the chromodomain helicase DNA-binding (*CHD*) genes on the *W* and *Z* sex chromosomes using the P8/P2 primer set (Griffiths et al. 1998). PCR amplifications of DNA were performed in a final volume of 25 µl on a TProfessional thermocycler (Biometra, Göttingen, Germany). Each reaction volume contained 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.0 mM $MgCl_2$ [10× *Taq* buffer advanced and $MgCl_2$ (25 mM); VWR International, Darmstadt, Germany], 200 µM of each dNTP (Carl Roth, Karlsruhe, Germany), and 1.5 units of 5 PRIME *Taq* polymerase (VWR). We used 5 pmol of each primer in reactions for buccal swabs and 4 pmol in reactions for blood samples, respectively. Finally, we added 11–13 µl of DNA solution from buccal swabs or 1–2 µl of DNA extract from blood samples to the respective reaction mixture. PCR with DNA from buccal swabs started at 94 °C for 5 min, continued for 46 cycles of 94 °C for 30 s, 47 °C for 30 s, and 72 °C for 45 s, and finished with 72 °C for 5 min (Adam's manual). Reaction volumes with DNA from blood underwent a program starting with 95 °C for 2 min, continuing with 31 cycles of 94 °C for 30 s, 47 °C for 1 min, and 72 °C for 45 s, and ending with 72 °C for 7 min. PCR was performed only once per individual. In swifts, PCR with the primer set P8/P2 yields a product of 370 base pairs (bp) from the *Z* chromosome and a product of 380 bp from the *W* chromosome. Reverse primer P2 was FAM-labeled to separate out sex-specific signals using an automated ABI DNA sequencer (ABI 3130 or 3730). The fragment length was determined with the Peak Scanner™ software package, version 1.0 (Applied Biosystems, Life Technology Corp., CA, USA). We assigned sex based on the absence (male) or presence (female) of the 380 bp signal from the *W* chromosome (Fig. 1).

Results

In nestlings, PCR amplification and sex determination based on blood samples were successful in all samples ($n = 53$). We determined 26 males and 27 females. Using DNA from buccal swabs, a PCR product could be amplified in 47 out of 53 nestlings (89 %). In 46 out of these 47 samples (98 %), sex determination based on DNA from buccal swabs was identical to that based on DNA from blood samples.

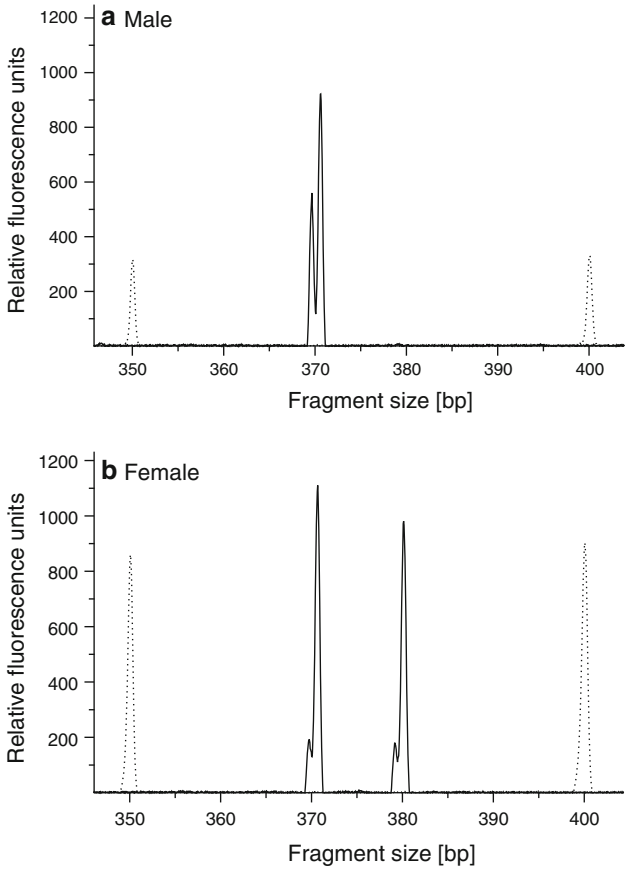

Fig. 1a–b Fluorescence signals (*solid lines*) of the FAM-labeled PCR products amplified with P8/P2 primer set using DNA from buccal swabs of the Common Swift (**a** adult male, **b** female nestling). The 380 bp signal from the *W* chromosome is only present in females. *Dotted signals* come from a size standard

In adult swifts, a PCR product was obtained from all blood samples and the sex of each bird was determined ($n = 11$). In 10 out of 11 adults, buccal swabs could be used for sex determination (91 % PCR success rate). We got a 100 % match between sex determination using DNA from buccal swabs and DNA from blood samples in adult birds.

Discussion

Our study provides a good indication of the reliability of buccal swabs as an alternative source of DNA for sexing adults and very young nestlings in the Common Swift. The amount of DNA was sufficient to successfully perform a PCR, even in buccal swabs taken from nestlings immediately or within the first days after hatching. Although PCR amplification was carried out only once for each individual bird, we received a PCR product in a high percentage of buccal swabs (success rate: 89 % in nestlings, 91 % in adults). Arima and Ohnishi (2006) achieved an overall PCR success rate of 82.2 % (88 out of 107 individual samples) in sex determination via buccal swabs in 12 wild bird species (adults of 11 passerine species and nestlings of the Great Cormorant *Phalacrocorax carbo*). In other studies concerning bird sexing, PCR amplification failure either did not occur or was not mentioned (Seki 2003; Handel et al. 2006; Brubaker et al. 2011). Likewise, we achieved high consistency in results of sex determination based on DNA from buccal swabs and based on DNA from blood samples (98 % matching in nestlings, 100 % in adults). As far as we know, this is the first study evaluating the reliability of buccal swabs for sexing in a small non-passerine bird.

Moreover, we were able to show that taking buccal swabs is feasible for sensitive bird species like the Common Swift, for which it is important to minimize any manipulation and duration of handling the birds. Animal welfare considerations (e.g., the 3R principle as published by Russell and Burch 1959) and issues regarding the protection of endangered bird species also strongly indicate the implementation of sampling methods that do not impose unnecessary harm and distress on the animals. Therefore, we strongly recommend buccal swabbing as a noninvasive sampling method in birds, and especially in nestlings which are too small for blood sampling.

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