



UNIVERSITY OF ARIZONA
SCHOOL OF NATURAL
RESOURCES

Buccal Swabs Yield PCR-Amplifiable DNA in Harris's Hawks

James F. Dwyer¹, and Melanie Culver²

¹ 106 Cheatham Hall, Department Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24062

² 325 Biological Sciences East, School of Natural Resources, University of Arizona, Tucson, AZ, 85721



Abstract

Assessing sex ratio in avian populations can be critical to maximizing conservation strategies. Historically, behavioral or morphometric analyses distinguished sex in birds, but these methods fail to resolve sex in some species and age classes. Primary sex ratio determination in nestlings is particularly difficult with traditional methods, and has contributed to the rise of molecular techniques. Molecular analyses of feathers and blood are widely used but can be difficult to implement, particularly in endangered species, if permits are disallowed.

We examined whether buccal swabs might offer a feasible alternative for collecting PCR-amplifiable genetic material from birds. In Tucson, Arizona, USA, we collected paired breast feather and buccal samples from 19 wild-caught Harris's Hawks (*Parabuteo unicinctus*). We compared quality and quantity of DNA in each pair of samples, and found buccal swabs do yield amplifiable DNA. Harris's hawks have relatively large mouths, but we sampled only one cheek. In smaller species, sampling could include more of the mouth. Thus, with appropriately sized swabs, the effectiveness of buccal sampling should not be limited to large birds, and may be especially useful in endangered species, and in nestlings whose large gapes should facilitate the method.

Study Area and Methods

Our study occurred in Tucson, Pima County, Arizona, USA, in the northeastern corner of the Sonoran desert (Brown et al. 1979). All study animals were captured with a bal-chatri trap (Bloom 1987) between 1 February 2003, and 31 May 2004. Feather and buccal samples were collected in pairs so feather DNA could be used to evaluate the quality and accuracy of buccal DNA. With tweezers, we plucked a single contour feather from the breast or belly of each sampled hawk. A growing (pin) feather was selected when available. The proximal 3 cm of each feather calamus (quill) was placed in a 2 mL microcentrifuge tube containing 500 μ L of TES buffer.

Buccal swabs (Omni Swab, Whatman International Limited, UK) were inserted into the mouth and rotated in a circular motion along the inside of one cheek for 5 seconds. Care was taken to brush as much of the inside of the cheek as possible without entering the throat. Swab tips were ejected immediately into a 2 mL microcentrifuge tube containing 500 μ L TES buffer.

DNA was extracted from feathers and swabs using a QIAamp DNA mini kit following the "tissue protocol" for feathers and the "buccal swab spin" protocol for swab samples (Qiagen Inc., Valencia, CA). Four μ L of each sample were subjected to electrophoresis on a 1% agarose gel in 1X TBE buffer using an OWL D2 gel apparatus (OWL separation systems, Portsmouth, NH) and compared to standards of 50 ng, 100 ng, and 250 ng of Lambda DNA. We diluted DNA extracts to approximately 10-25 ng / μ L for use in PCR amplification with primers for the CHD-1 gene under PCR conditions as in Fridolfsson and Ellegren (1999).

PCR reactions were performed using AmpliTaq Gold PCR Master Mix at 1X concentration, 0.1 μ M of each primer (2550F and 2718R), and 1 to 8 μ L of genomic DNA, and cycled in an Eppendorf Mastercycler. We used a touchdown thermocycling procedure with 1°C increments starting at 60°C and ending at 50°C. PCR amplification products were mixed with bromophenol blue dye, and electrophoresis was conducted in a 1% agarose gel at 60 V for 120 minutes.

Results

Individual	Mass	Sex by Mass	Sex by Feather DNA	Pre-PCR DNA (feather)	Sex by Swab	Pre-PCR DNA (swab)
1	595.6	M	M	100	M	10
2	606.7	M	M	1	M	10
3	674.9	M	M	100	M	25
4	710.5	M	M	100	M	1
5	710.5	M	M	10	U	40
6	735.5	M	M	100	M	25
7	744.4	M	M	25	M	25
8	814.4	U	F	10	F	10
9	830.4	U	F	10	F	10
10	934.7	F	F	25	F	25
11	952.0	F	F	100	F	25
12	990.8	F	F	100	F	40
13	1019.9	F	F	40	F	25
14	1029.8	F	F	100	F	25
15	1039.2	F	F	100	F	100
16	1040.3	F	F	40	F	10
17	1053.9	F	F	1	F	10
18	1111.3	F	F	25	F	10
19	1152.3	F	F	1	F	10

We collected genetic samples from 19 Harris's Hawks (n=38 samples). By weight, 10 were female, 7 male, and 2 undetermined (Dawson 1988). All 38 molecular samples (feathers and buccal swabs) yielded DNA which was amplifiable using CHD-1 primers (Table 1). As suggested by Fridolfsson and Ellegren (1999), males produced one band of 500 bp, and females produced one band of 700 bp.

In 16 hawks, weight, feather, and swab analyses all concurred on the sex of birds. In 2 individuals (4 genetic samples, feather and buccal swab from each individual) DNA analysis enabled sexing of birds whose weight was inconclusive, and in both of these cases, the results from feather and buccal analyses agreed. One hawk, a male by weight and feather DNA, produced a swab sample with both a 500 bp and a 700 bp band. Though females of some avian families do exhibit two bands (Fridolfsson and Ellegren 1999), this is not expected in raptors, and we believe the sample was either contaminated, or that the two bands were a PCR artifact of the relatively high number of cycles in our touchdown procedure.

Acknowledgements

We thank Bill Mannan for assistance in trapping birds, Ruth Tingay for aid in interpretation of male and female band sizes, and Chris Kirkpatrick for editorial comments. This study was partially funded by the Arizona Game and Fish Department, and the Tucson Electric Power Company.

Discussion and Conclusions

Molecular sexing of Harris' Hawks is possible using existing primers, and buccal swabs can be used to collect amplifiable genetic material suitable for molecular analyses. Harris's Hawks have relatively large mouths, but we sampled from only one cheek. In smaller birds, sampling could include both cheeks, the roof of the mouth, and under the tongue. Thus, with appropriately sized buccal swabs, the effectiveness of buccal sampling should not be limited to large birds. This method may be especially useful in endangered species, and in identifying the primary sex ratios of nestlings with large gapes.

INSERT PHOTO OF GEL HERE

Literature Cited

Bloom, P. H. 1987. Capturing and handling raptors. Pages 101-104 in Raptor Management Techniques Manual. National Wildlife Federation. Washington D.C.

Brown, D. E., C. H. Lowe, and C. E. Pase. 1979. A digitized classification system for the biotic communities of North America, with community (series) and association examples for the Southwest. Journal of the Arizona-Nevada Academy of Science 14 (1, Supplement).

Dawson, J.W. 1988. The cooperative breeding system of the Harris' Hawk in Arizona. Master's thesis. University of Arizona. Tucson, AZ.

Fridolfsson, A. K., and H. Ellegren. 1999. A simple and universal method for molecular sexing of non-ratite birds. Journal of Avian Biology 30:116-121.