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SEX IDENTIFICATION IN FOUR OWL SPECIES FROM IDAHO: DNA AND MORPHOMETRICS

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Accurate sex identification is necessary for a broad spectrum of bird studies, including brood sex ratios, sex differences in migration timing, sex-biased behavior, and sex differences in physiological responses. However, for sexually monomorphic species, neither structural measurements nor external plumage characteristics can be used to assign sex reliably (Morrison and Maltbie 1999, D'Aloia 2002). In those cases, DNA sexing has proven to be a reliable, efficient method for sexing birds (Kahn et al. 1998, Fridolfsson and Ellegren 1999, Oddie and Griffiths 2002) including some raptor species (Norris-Caneda and Elliott 1998, Balbontin et al. 2001, Shepard et al. 2004).

Many raptor species are sexually dimorphic, with females being larger than males. A morphometric chart, using body mass and a structural measurement, wing chord, has been developed to identify the sex of Northern Saw-whet Owls (*Aegolius acadicus*) along migration routes in the eastern United States (Cannings 1993, Brinker et al. 1997). However, there is an area of overlap in which small females and large males cannot be distinguished by measurements and must be reported as unknown sex. In contrast, Flammulated Owls (*Otus flammeolus*) are not sufficiently dimorphic to determine sex from morphological measurements (McCallum 1994). Burrowing Owls (*Athene cunicularia*) and Western Screech-Owls (*Megascops kennicottii*) fall in between, being somewhat sexually dimorphic at some life stages and monomorphic at earlier stages (Haug et al. 1993, Cannings and Angell 2001).

Strigiformes can be sexed using DNA methods; however, only a small number of individuals from two to five common species have been tested (Fleming et al. 1996, Griffiths et al. 1998, Brommer et al. 2003). In this study, we broadened the taxonomic scope of species sexed using DNA methods and sampled many individuals from each species. In addition, we evaluated a morphological field sexing chart created from migrating Northern Saw-whet Owls in the eastern United States for use in the western United States and against DNA sexing methods.

METHODS

Northern Saw-whet Owls and Flammulated Owls were captured using audio lures during fall migration between August–November 1999 and 2000 at the Idaho Bird Observatory on Lucky Peak, Boise County, Idaho (43°33.76′N, 116°3.75′W). Each station consisted of five mist-nets positioned around two speakers broadcasting male calls of each species from 2000–0800 H MST nightly. Captured birds were bled within 30 min of removal from the net. Approximately 40 µl of blood was collected from the brachial vein into a 200 µl heparin-coated Caraway tube and transferred to an EDTA-coated microcentrifuge tube. All blood sample processing was done within 24 hr of collection. The red blood cells from each sample were frozen in separate tubes at −20°C.

Each owl was banded with a unique, numbered aluminum United States Geological Survey leg band; wing chord and weight measurements were taken. In the field, we assigned sex to Northern Saw-whet Owls using a morphometric table developed from a discriminant function calculation on wing chord and weight measurements of surgically-sexed birds (Brinker et al. 1997).

To verify the accuracy of the DNA methods, we collected blood from nine Western Screech-Owls in a nest box study along the Snake River near CJ Strike Reservoir, Elmore County, Idaho. These owls had been monitored for several years and their sexes had been determined by both physical characteristics and morphological measurements. These samples were used for the initial screening of each

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Table 1. Summary of cloning and sequencing of the amplified products from four owl species using methods of Griffiths et al. 1998 (1) or Fridolfsson and Ellegren 1999 (2).

SPECIES	USGS BAND NO.	SEX	METHOD	NO. BANDS AMPLIFIED/ INDIVIDUAL	NO. CLONES	INSERT SIZE (BP)	GENBANK NUMBER
WESO	1705–41320	M	1	1	6	362	DQ985233-DQ985238
WESO	1705–41320	M	2	1	2	642	DQ985239-DQ985240
BUOW	804–10154	M	1	1	6	377	DQ985241-DQ985246
BUOW	804–10154	M	2	1	5	652	DQ985274-DQ985251
BUOW	804–10074	M	1	1	2	377	DQ985252-DQ985253
BUOW	804–10074	M	2	1	4	652	DQ985254-DQ985257
NSWO	1353–15054	F	1	1	4	362	DQ985258-DQ985261
NSWO	1353–15227	F	1	4	2	367, 362	DQ985262-DQ985263
FLOW	1043–98961	M	1	1	3	362	DQ985264-DQ985266
FLOW	1043–98941	F	2	1	2	642	DQ985267-DQ985268
FLOW	1043–98955	F	2	1	4	642	DQ985269-DQ985272

sexing method to verify proper technique and see the band results for both sexes. We included one known-sex Western Screech-Owl sample in every gel throughout the study to provide control comparisons for the technique. For broader taxonomic scope, we included in this study samples from 365 Burrowing Owl nestlings bled for another project in southwest Idaho.

DNA was extracted from red blood cells using the Promega Wizard DNA Purification Kit (Promega, Madison, WI) following the manufacturer's instructions for 300 µl of blood, with minor modifications. Although blood was collected in heparinized capillary tubes, occasionally clots interfered with the extraction process. Therefore, 200 µl of cell lysis solution were added directly to the blood sample, mixed with a pipette, and incubated at room temperature for 10 min. The resulting mixture of 200 µl of cell lysis solution and blood cells was then added to 700 µl of cell lysis solution in a new tube. DNA amplification was performed in a thermal cycler (PTC-100, MJ Research, Watertown, MA) using one of two methods.

We followed the protocol of Griffiths et al. (1998) which used primers P2 and P8 (Method 1). The amplified products were subjected to restriction digest because the W- and Z-CHD (chromo-helicase-DNA binding) bands were indistinguishable on 3% agarose gel in owls (Griffiths et al. 1998). Restriction digest samples were incubated overnight at 37°C mainly for convenience and due to scheduling constraints. Bands were viewed under ultraviolet light after electrophoresis. Females, having the *Hae III* restriction site, had two bands and males had one; all bands were between 360–380 bp.

Following Fridolfsson and Ellegren (1999), we used primers 2550 and 2718 (Method 2). After electrophoresis, males were identified by the presence of a single CHD-Z band at 640–660 bp. The presence of both CHD-Z and CHD-W or the single CHD-W band designated females at 1.0–1.2 bp. The amplification of only the CHD-W gene is discussed in Fridolfsson and Ellegren (1999).

With the Fridolfsson and Ellegren (1999) technique, multiple bands were detected in some samples. To resolve whether these bands were additional alleles or were simply due to nonspecific binding, some were cloned and sequenced. Amplified products were separated on 1.0% agarose and all detected bands for each individual were removed; the amplified product was purified using Millipore Ultra Free DA tubes. Each band was ligated and cloned using the pGEM T-easy kit from Promega. Cloned products were subsequently sequenced using a Li-Cor Long ReadIR 4200. Sequences were identified by conducting a BLAST (Altschul et al., 1997) search to find the closest match to these sequences in Genbank.

RESULTS AND DISCUSSION

We sexed 701 owls of four species: 365 Burrowing Owls, 78 Flammulated Owls, 249 Northern Saw-whet Owls, and nine Western Screech-Owls. All samples with products amplified produced a sexing result. Both DNA methods produced results that could be interpreted when assigning sex to an individual bird; however, there was variation in the time required to analyze and interpret results.

The Northern Saw-whet Owl field chart result disagreed with the DNA result in 10 of 249 birds; therefore, the chart-assigned sex accurately identified large females and small males 96% of the time, assuming 100% accuracy of the DNA methods. By using DNA sexing techniques, we were able to further identify the sex of 128 Northern Saw-whet Owls previously categorized as unknown sex by the chart.

Table 1 presents the results of the sequenced ''extra'' bands, which matched the CHD region. One to four bands were visible on the agarose gels although the results of cloning clearly indicated that only one of these bands successfully yielded product matching the targeted CHD insert.

With Griffiths et al. (1998), PCR competition and additional bands due to nonspecific primer binding did not interfere with sex determination. Because restriction digest was required with the Griffith et al. (1998) protocol, more laboratory time was required than with the Fridolfsson and Ellegren (1999) technique. The Fridolfsson and

Table 2. Comparison of Method 1 (Griffiths et al. 1998) and Method 2 (Fridolfsson and Ellegren 1999).

	METHOD 1	METHOD 2
DNA amount	1 µl	1 µl
Primers used	P2, P8	P2550, P2718
Primer amount	100 ng	4 pg
Taq amount	0.25 u	0.5 u
PCR (hours)	3.5	2
Restriction digest	Yes	No
Gel type	3% agarose	2% agarose
Electrophoresis conditions (hr)	1 @ 60V	1.5 @ 60V
Ambiguous results for unknown birds	2%	6%
Number of runs (*N*)	75	465
Cost per sample	$1.96	$1.61

Ellegren (1999) method was the most cost-effective; however, PCR competition sometimes resulted in the amplification of only the W band. In such cases, an individual might be misinterpreted as male instead of female. We recommend preliminary work using known-sex samples to optimize PCR reaction conditions and decrease PCR competition. Table 2 summarizes the pertinent method differences and costs.

Although many of the cloned inserts resulted in a clear match to CHD genes, other inserts did not match the CHD gene during BLAST searches despite having the primers in the sequence. In a few cases, these sequences did not have good matches at all; instead they corresponded to short sequences of 50–100 base pairs in the human genome and had low scores. In a few cases, the amplified products were less clearly matched to nontarget CHD genes. These inserts had a partial match to CHD, generally either the first or second part of the gene, and the remainder of the product aligned poorly or not at all with other CHD genes from other species, individuals, or sometimes even from the same individual. BLAST searches of parts of these inserts verified that certain portions were incomplete matches to CHD genes whereas the other parts matched various avian genes. The most likely explanation for these was nonspecific primer binding and PCR recombinants. Alternatively, these bands may have been CHD genes but due to sequence divergence of the intron did not match other CHD sequences currently in Genbank.

Northern Saw-whet Owls were sexed in the field using a morphometric chart created by Brinker et al. (1997) with data obtained from migration routes along eastern United States flyways. In general, the morphometric chart accurately sexed large females and small males along a western United States flyway. This chart could be used reliably throughout the United States to morphologically sex migrating Northern Saw-whet Owls in the field.

Using DNA sexing techniques, we were able to sex another 128 Northern Saw-whet Owls previously categorized as unknown by the chart. We sexed 78 Flammulated Owls and 365 Burrowing Owl nestlings that could not have been sexed using body measurements because both are considered monomorphic species. Therefore, we recommend DNA sexing for all birds identified as unknown sex by field sexing charts and for all monomorphic species.

IDENTIFICACIÓN DEL SEXO EN CUATRO ESPECIES DE LECHUZAS DE IDAHO: ADN Y MORFOMETRÍA

RESUMEN.—Para determinar el sexo de individuos de algunas especies dimórficas de rapaces en el campo se emplean cuadros morfométricos. Sin embargo, usualmente existe un rango en el que las medidas de los dos sexos se sobrelapan, por lo que es imposible determinar el sexo de algunas aves. Además, algunas especies son monomórficas, por lo que el sexo de los individuos no puede determinarse con base en su morfología externa. Las técnicas basadas en ADN que se enfocan en los genes conservados CHD para determinar el sexo, han sido empleadas en rapaces. Utilizamos dos de estas técnicas para determinar el sexo de 365 individuos de la especie *Athene cunicularia*, 78 de *Otus flammeolus*, 249 de *Aegolius acadicus* y nueve de *Megascops kennicottii*. Para *A. acadicus* comparamos los resultados derivados de los cuadros morfométricos con los obtenidos mediante técnicas basadas en ADN. El cuadro morfométrico presentó una exactitud del 96%, suponiendo que la exactitud de los métodos basados en ADN es del 100%. Además, empleando ADN pudimos determinar el sexo de 128 de los individuos para los cuales no pudo determinarse el sexo utilizando el cuadro morfométrico. Se recomienda el uso de técnicas basadas en ADN para especies monomórficas y para aves con mediciones que caen en la región de sexo desconocido en las tablas morfométricas.

[Traducción del equipo editorial]

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