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Sex Determination of Red-Tailed Hawks (*Buteo jamaicensis calurus*) Using DNA Analysis and Morphometrics

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ABSTRACT

Currently the sex of Red-tailed hawks (Buteo jamaicensis) cannot be determined by in-hand methods. Males and females do not differ in plumage and overlap in size. During migration, we collected feather samples and morphological measurements from birds at four sites in the Western United States. Sex was determined for individual birds using sex-specific DNA markers and Polymerase Chain Reaction was used to identify these DNA markers. Through Discriminant Function Analysis, we created equations for determining the sex of Red-tailed hawks using in-hand measurements based on the DNA-determined sexes. We formed two equations, one for adults, which was 98% accurate, and one for hatch-year birds, which was 97% accurate. Our results will aid future studies looking at intra- and intersexual differences in the Western Red-tailed hawk.

Key words: Red-tailed hawk, Buteo jamaicensis, sex determination, Discriminant function analysis, DNA analysis

Determining sex in natural populations is important for studying population dynamics, population structure, habitat use, behavior and mating systems, and for making management decisions (Hughes 1998, Ito et al. 2003). Unfortunately, for many avian species it is difficult to determine sex from morphometrics and plumage (Ito et al. 2003). This is particularly true for several monomorphic raptor species. For example, Red-tailed hawks (Buteo jamaicensis) lack plumage differences between the sexes, but show some sexual size dimorphism (Palmer 1988). However, the size differences between male and female Red-tailed hawks have not been quantified in a manner useful for field situations. Although it is possible to determine the sex of individual birds by observing copulation and courtship behaviors (Catry et al. 1999), or by cloacal examination in some species (Boersma and Davies 1987, Gray and Hamer 2001), these methods are limited to the breeding season.

The Red-tailed hawk has a wide distribution, ranging from central Alaska south to Panama and east to the Virgin Islands and is very common (Figure 1). The ability to determine the sex of individuals in the hand using simple measurements would greatly improve our ability to study sex-specific movements and behaviors in this species effectively. For example, sex determination is important in investigating research questions that address foraging behavior (Kelly and Wood 1996, Gonzalez et al. 2000, Noske 2003), dispersal (Brooke 1978), and migration patterns (Evans and Day 2001). One successful approach in sexing many bird species involves discriminant analysis using morphological measurements (Balbontin et al. 2001, Bertellotti et al. 2002, Quintana et al. 2003, Mizuta et al. 2004, Setiawan et al. 2004). Additionally, because females are heterogametic, sex in birds can be determined using

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a molecular technique, Polymerase Chain Reaction (PCR) amplification of DNA. Red-tailed hawks can be sexed by PCR (Norris-Caneda and Elliott 1998) and a particularly inexpensive technique was developed by Fridolfsson and Ellegren (1999) for use with non-ratite birds. We developed a cost-effective and accurate method of sexing Red-tailed hawks in the hand, based on discriminant analysis of morphometrics, which we verified with molecular techniques using feathers as the source of DNA (Griffiths and Tiwari 1995, Bello et al. 2001, Sacchi et al. 2004).

METHODS

We collected feather samples during fall migration of 2002 and 2003 at four HawkWatch International banding sites. These sites are in the Goshute Mountains, NV, Bonney Butte, OR, Chelan Ridge, WA, and Manzano Mountains, NM (Figure 1, Table 1). All sites are manned by volunteers.

Red-tailed hawks were capture using standard trapping techniques (Bloom, 1987) and banded with United States Geological Survey aluminum bands. In addition, the following morphological measurements were taken (see Hull and Bloom 2001): body mass (g), and natural wing chord, tail, hallux, culmen, and tarsus lengths (all in mm). We measured natural wing chord with a ruler from the wrist of the wing to the tip of the longest flight feather without flattening the wing against the ruler. Tail length was measured with a ruler between the two middle tail feathers, from the base of the feathers to the end of the longest feather. Hallux talon length was measured with calipers from the base of the talon to the tip of the talon. Culmen length was measured with calipers from the base of the culmen to its tip. Finally, tarsus length was measured with calipers from the front of the tarsometatarsal bone at the toe-joint to the end of the bone below the ankle-joint.

For DNA sexing, three breast feathers were plucked from each bird and placed in a coin envelope with the band number, measurements, age, date, and capture site information provided on the label. DNA was extracted from plucked breast feathers using a protocol provided by Dr. I. Lovette of Cornell University (Lovette et al. 2004) using the commercially available Qiagen DNeasy Kit®.

Genetic sex was determined following the method described by Fridolfsson and Ellegren (1999) using primers 2550 and 2718. We prepared a master mix of Promega 1X buffer, 1.5 mM of 10X MgCl2, 200 uM of each dNTP, 4pM of each primer, and 0.5 units of Promega *Taq* polymerase for a total of 10 uL for amplification. Thermal cycling consisted of an initial denaturing step of 120 s at 94˚C, followed by repeated denaturing, annealing, and extension steps for 30 cycles of 30 s at 94˚C, 30 s at 50˚C, and 30 s at 72˚C, with a final extension step of 300 s at 72˚C. Samples were then placed in a 2% agarose gel containing 10 uL ethidium bromide and electrophoresis was run in 0.5X TBE at 70V for approximately 75 min. Gels were visualized under UV light and photographs were taken of all successful runs. Female sex was assigned if both the CHD-Z and CHD-W bands were present, and male sex was assigned if a single CHD-Z band was present.

We employed SAS statistical software (SAS Institute 1999) to perform a MANOVA on both age and sex. Because adult birds might differ from hatch-year birds in measurements, a MANOVA was run on age class. Subsequently, we conducted separate Discriminant Function Analyses (DFA) on adult and hatch-year birds, along with backward elimination to determine the most useful variables for determining sex using in-hand measurements. Various combinations of measurements were run in the DFA to account for differences in the types of measurements taken at other banding sites.

Sex was successfully determined by PCR for 175 Red-tailed hawks, 100 from the Goshute Mountains, 26 from Chelan Ridge, 21 from Bonney Butte and 28 from the Manzano Mountains. Three birds had missing measurements and were eliminated from the analyses. An additional hatch-year male was excluded from analyses. This bird was an extreme outlier in wing chord, hallux and culmen and perhaps was transcribed incorrectly from the data sheet to the feather envelope. The remaining 121 hatch-year birds and 50 adult birds were used to produce a discriminant function with morphometrics.

RESULTS

Adults differed significantly from hatch-year birds on mass, culmen, and tail measurements (Table 2), so adult and hatch-year birds were treated separately in all further analyses. A MANOVA run on sex class demonstrated that females were significantly larger than males in all measurements in both adult and hatch-year birds (Tables 3 and 4).

Backward elimination of variables following discriminant analysis selected wing chord and mass as significant morphological measurements for distinguishing between the sexes in adult birds, and this produced the following equation: 0.166 x wing chord + 0.026 x mass = Z. If $Z > 94.902$, then the bird is female; if $Z \leq 94.902$, then it is male. This equation accurately assigned sex to 98% of the 50 adult Red-tailed hawks whose sex was determined by PCR. The one misclassified bird was a female with an exceptionally small wing chord measurement of 381mm. This bird may have been mismeasured, since the mean (± SE) wing chord measurement for a B. j. calurus female is 412 mm ±14.9 (Preston and Beane 1993). See Figure 2a for the distribution of males and females based on their discriminant scores.

Backward elimination of variables following discriminant analysis selected body mass, wing chord, hallux, and culmen as significant morphological measurements for discriminating between the sexes in hatch-year birds. The following equation was produced: 0.2 x wing chord + 0.011 x mass + 1.302 x hallux + 1.356 x culmen = Z. If $Z > 160.933$, then the bird is female; if $Z \leq 160.933$ then it is male. This equation accurately assigned sex to 97% of the 121 hatch-year Red-tailed hawks of known sex. The four misclassified birds consisted of two females with short wing chords, a female with a short culmen, and a female with measurements close to both male and female sizes. See figure 2b for the distribution of males and females based on their discriminant scores.

Additional equations are given in Tables 5 and 6, along with their accuracy (all > 90%), using only wing chord and mass, which we provide because some banders do not take measurements requiring calipers.

DISCUSSION

The DNA sexing technique unambiguously sexed individual birds for use in discriminant analyses, confirming the usefulness of plucked feathers for extracting DNA, as shown elsewhere (Griffiths and Tiwari 1995, Bello et al. 2001, Sacchi et al. 2004). Plucking feathers does not require special training and the feathers do not require special storage, other than an envelope, making them extremely practical in remote field situations such as those experienced at many migration monitoring sites. The discriminant functions produced through morphometrics provided an inexpensive and highly accurate method of sexing Red-tailed hawks in the hand. These results should greatly aid future studies concerning this species.

The ability to determine the sex of individual Red-tailed hawks in the hand will be valuable in future studies addressing intersexual and intrasexual differences. For example, in-hand sex determination may facilitate investigation of sex differences in dispersal patterns (Brooke 1978), heritability differences in morphology (Jensen et al. 2003), molt intensity and chronology (Craigie and Petrie 2003), foraging niche partitioning (Gokula et al. 1999, Marsden and Sullivan 2000, Pryzbylo and Merila 2000), foraging strategies (Kelly and Wood 1996, Gonzalez et al. 2000, Noske 2003), prey composition and size (Overskaug et al. 2000, Lee and Severinghaus 2004), migration patterns and sex ratios (Evans and Day 2001), winter spacing patterns (Ohsako 2001), parasite load (Freeman et al. 2001), dominance and aggressive behavior (Tarvin and Woolfenden 1997, Jones and Hunter 1999), and vocalizations (Bretagnolle et al. 1998).

A potential problem in using the discriminant function equations for sexing Red-tailed hawks is individual variation among investigators in taking the measurements. There may also be differences in measurement techniques among and within sites. However, given that the data in this study were collected by as many as 30 volunteer banders at four different locations, accuracy rates consistently greater than 90% suggest that the sexing technique is robust.

Due to concerns about consistent measurement techniques and because they are used relatively rarely, many other potentially useful morphometric measurements were not examined in the study. For example, other studies have used forearm length (Ferrer and De Le Court 1992), tarsal width (Shepard et al. 2004), and bill depth (Bortolotti 1984) to successfully determine sex in raptor species. However, these measurements are not commonly taken at migration sites in North America and might have proved difficult to teach to the numerous volunteers at four different sites. Therefore, we chose to use a smaller number of frequently collected measurements. However, other additional measurements may be useful in sex determination of birds not easily sexed by commonly taken measurements

Because all sampling sites were located in the western United States, these equations may only be applicable to studies examining the western Red-tailed hawk subspecies (B.j.calurus). Other subspecies may not be accurately sexed due to differences in morphological characters. For example, the average wing chord measurement for B.j.calurus females is 412 ± 14.9 mm and is 386.8 ± 11.4 mm for males, and the mean tail length measurement is 237.3 ± 11.3 mm for females and 224.2 ± 7.9 mm for males. The eastern subspecies, B.j.borealis, is smaller than B.j.calurus (Preston and Beane 1993). Future research should assess the need to develop in-hand sexing techniques for the other subspecies of Red-tailed hawk.

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Table 1. Collection locations for sampled Red-tailed hawks.

HawkWatch International Site	Location	Coordinates
Goshute Mountains	Northeastern Nevada Bureau of Land Management Land	40º 25.417’ N, 114º 16.276’ W
Bonney Butte	North Central Oregon Mount Hood National Forest	45º 15’ 46.8” N, 121º 35’ 31.2” W
Chelan Ridge	Eastern Cascade Mountains Washington State	48º 01’12.8” N, 120º 05’38.4” W
Manzano Mountains	Central New Mexico Cibola National Forest	34º 42.25’ N, 106º 24.67’ W

Table 2. Results of MANOVA on adult and hatch-year age classes of Red-tailed hawks.

Age (Wilks' Lambda F=27.88, d.f. 6,165, pvalue=<0.0001)	Weight Means (F=32.13, P=<0.0001)	Tail Means (F=42.57, P=<0.0001)	Culmen Means (F=6.56, P=0.0113)
Adult	1095±23	220±2	26.0±0.2
Hatch-year	943±14	233±1	25.3±0.2

Table 3. Results of MANOVA and mean body measurements of male and female adult Red-tailed hawks.

Sex Adults (Wilks' Lambda F=28.07, d.f. 6,43, pvalue=<0.0001)	Weight (F=122.65, P= <0.0001	Hallux (F=71.94, P= <0.0001	Tarsus (F=15.90, P= 0.0002	Tail (F=40.35, P= <0.0001	Wing (F=79.53, P= <0.0001	Culmen (F=16.40, P= 0.0002
Female	1266±134	31.5±1.8	88.9±3.1	227±10	415±15	27.0±2.1
Male	923±78	27.7±1.4	85.7±2.6	211±7	381±12	25.0±1.4

Table 4. Results of MANOVA and mean body measurements of male and female hatch-year Red-tailed hawks.

Sex Hatch-year (Wilks' Lambda F=72.46, d.f. 6,114, P=<0.0001)	Weight (F=80.26, P= <0.0001	Hallux (F=180.99, P= <0.0001	Tarsus (F=23.09, P= <0.0001	Tail (F=42.46, P= <0.0001	Wing (F=201.36, P= <0.0001	Culmen (F=113.35, P= <0.0001
Female	1022±116	30.5±1.2	88.2±3.6	238±12	407±12	26.2±1.2
Male	847±95	27.4±1.3	85.2±3.1	226±8	378±10	24.1±1.0

Table 5. Gender determination based on discriminant analysis, using only using only mass for adult birds and wing chord and mass for hatch-year Red-tailed hawks.

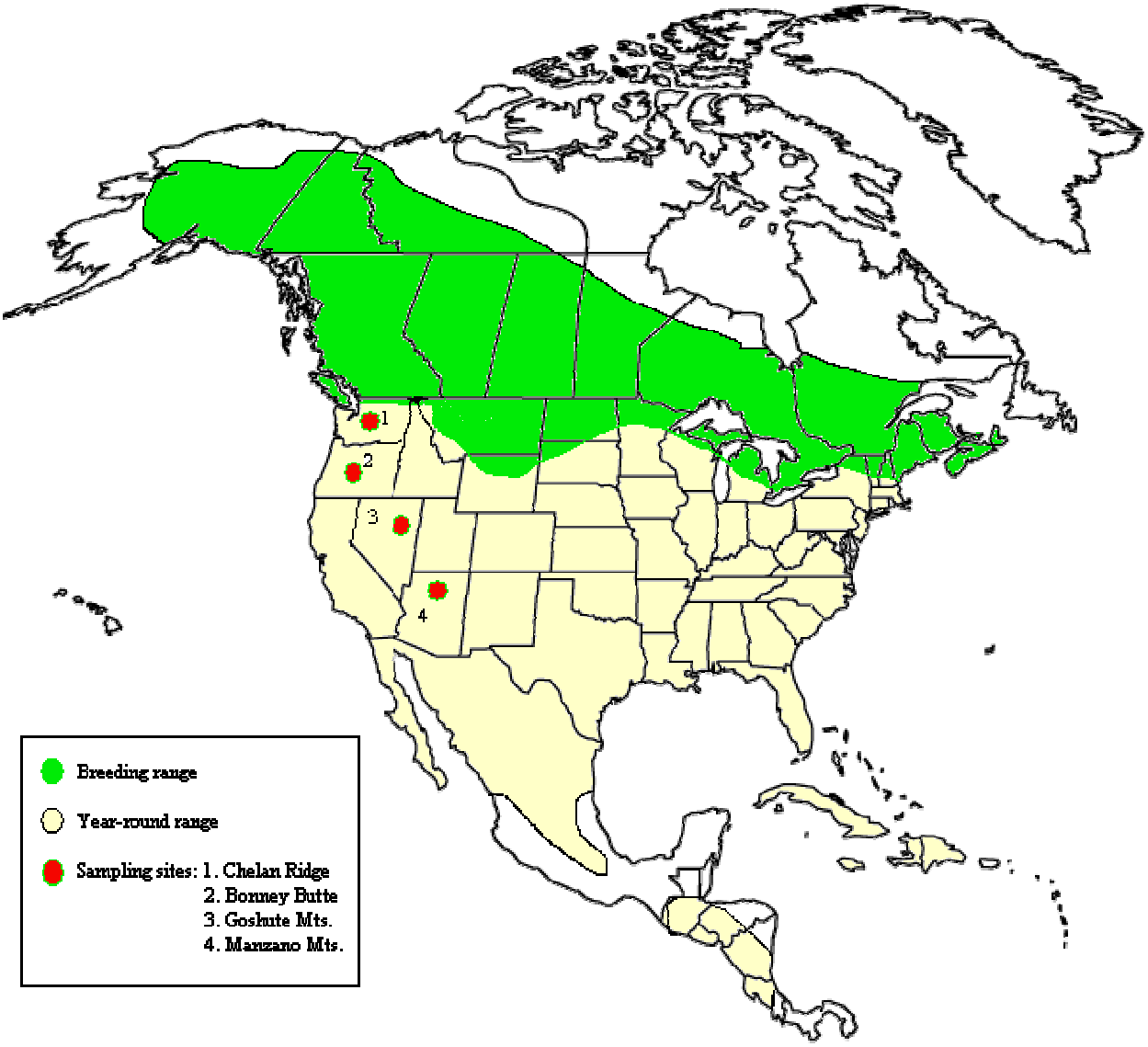
	Equation	Z score – male	Z score – female	Accuracy[a]
Adult	0.029 x mass	≤ 31.359	> 31.359	94%
Hatch-Year	0.227 x wing chord + 0.013x mass	≤ 100.981	>100.981	94.8%

[a] Compared to the results of gender determination based on DNA analysis.

Table 6. Gender determination based on discriminant analysis, using only wing chord for adult and hatch-year Red-tailed hawks.

	Equation	Z score – male	Z score – female	Accuracy[a]
Adult	0.189 x wing chord	≤ 75.345	> 75.345	94%
Hatch-Year	0.24 x wing chord	≤ 94.218	> 94.218	93%

[a] Compared to the results of gender determination based on DNA analysis.



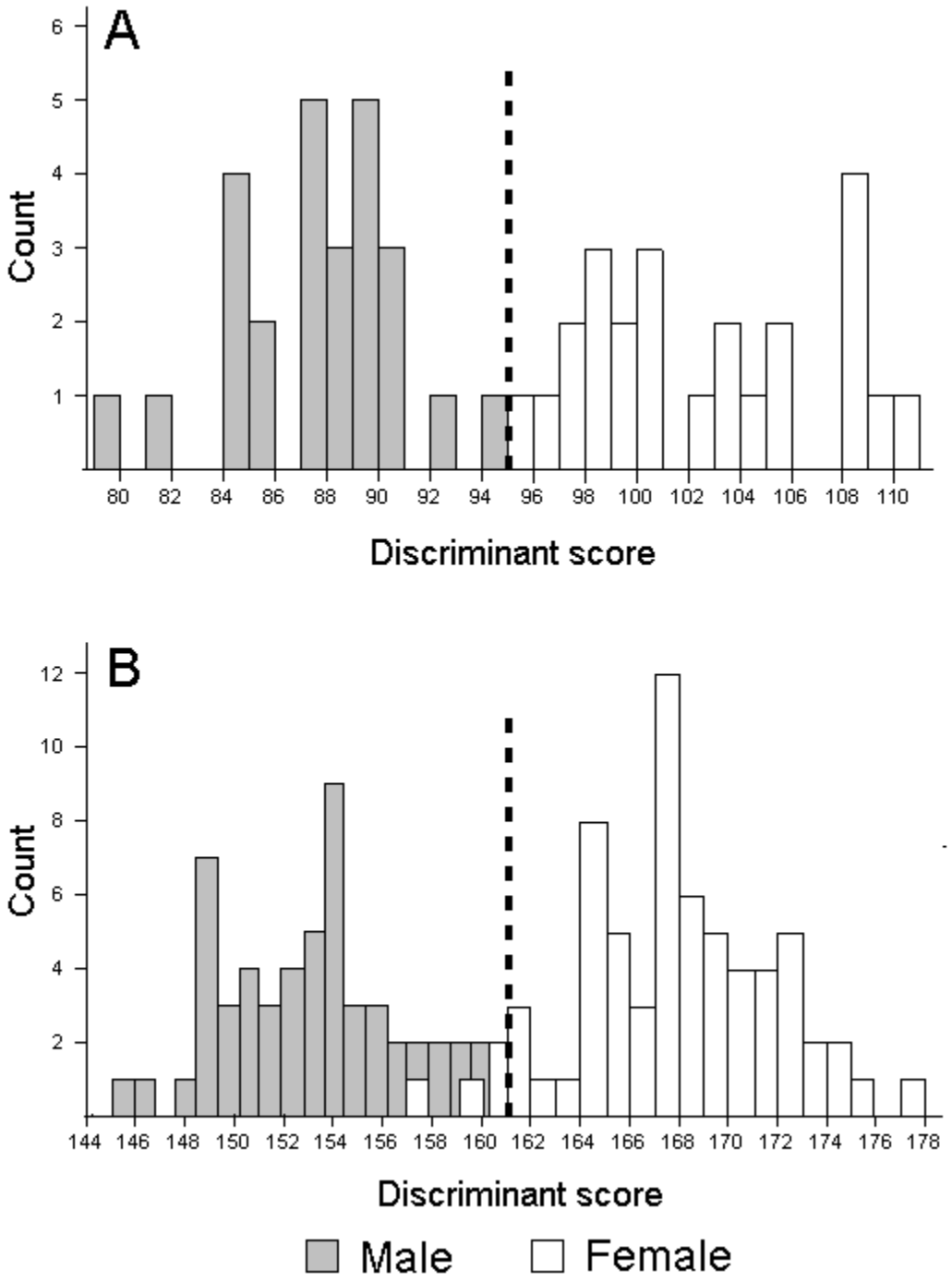


FIGURE LEGEND

Figure 1. Breeding range of the Red-tailed hawk and fall migration sampling sites (adapted from Johnsgard 1990).

Figure 2. Distribution of adult males and females based on discriminant scores. The dotted line represents the cutoff score which males fall to the left of and females to the right. a) Distribution of adult males and females. b) Distribution of hatch-year males and females.