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SEX DETERMINATION IN BOOTED EAGLES (*HIERAAETUS PENNATUS*) USING MOLECULAR PROCEDURES AND DISCRIMINANT FUNCTION ANALYSIS

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ABSTRACT.—We studied a breeding population of Booted Eagles (*Hieraaetus pennatus*) in Doñana National Park (southwestern Spain) to develop a method of determining the sex of an individual based on the use of discriminant functions. Because there are size differences between age classes and sexes of eagles, we developed two different discriminant functions for each age group. Our discriminant function method approached 100% accuracy in correctly aging individuals using forearm length and body mass as predictor variables. Sex of young eagles was also determined with 98.8% accuracy using forearm, tail, bill, and tarsus lengths.

KEY WORDS: *Booted Eagle*; Hieraaetus pennatus; *sex determination*; *morphometrics*; *molecular sexing.*

Determinación del sexo del águila calzada *Hieraaetus pennatus* utilizando tecnicas de sexado molecular y analisis discriminates

RESUMEN.—Una población reproductora de águila calzada ha sido estudiada en el Parque Nacional de Doñana (Sudoeste de España) con el objetivo de obtener un modelo de clasificación de los sexos basados en ánalisis discriminantes apoyados en procedimientos de sexado molecular. Existen diferencias importantes en el tamaño entre águilas adultas y pollos, por lo que se han desarrollado dos funciones discriminantes de sexo diferentes para cada clase de edad. El sexo de los adultos se determina con una función discriminante que clasifica bien el 100% de los individuos, utilizando el antebrazo y el peso como variables predictoras. El sexo de los pollos es determinado también correctamente con una función discriminate que clasifica bien el 98.8% de los individuos, utilizando cuatro variables predictoras: El antebrazo, la cola, el pico y el tarso.

[Traducción de Autores]

Easy and reliable methods to identify the sex of individuals are useful for the study of many aspects of avian biology, including foraging ecology (Anderson and Norberg 1981), behavior, evolutionary ecology and genetics (Clutton-Brock 1986), survivorship (Newton et al. 1983), and dispersion and conservation genetics (Griffith and Tiwari 1995). Sex determination is also important in conservation programs that concern the reintroduction of endangered birds when a fixed sex ratio is preferred. Recently, Ellegren (1996) proposed molecular methods to sex birds based on chromosome differences but few studies have used this information to develop additional methods to sex birds based on biometric data. Field methods to sex raptors have several advantages over molecular techniques that require time and/or money. Despite the fact that the majority of raptors are highly dimorphic in size, which should allow for the development of sexing methods based on morphometric data, only a few species have been utilized (Bortolotti 1984a, 1984b, Garcelon et al. 1985, Edwards and Kochert 1987, Ferrer and De Le Court 1992). The majority of these studies have been based on live individuals and museum skins. In most cases, both adults and immatures have been studied at museums or in private collections and few studies have been based on wild individuals. The objective of this study was to assess the differences between young and adult Booted Eagles (*Hieraaetus pennatus*) and to develop predictive discriminant models to determine the sexes of adults and immatures of the species.

METHODS

We used a sample of the breeding population of Booted Eagles in Doñana National Park. The park is located in southwestern Spain (37°N,6°30'W). It has a Mediterranean climate with an Atlantic Ocean influence. Marshes, Mediterranean scrubland mixed with scattered cork oak (*Quercus suber*) or stone pine (*Pinus pinea*), and costal

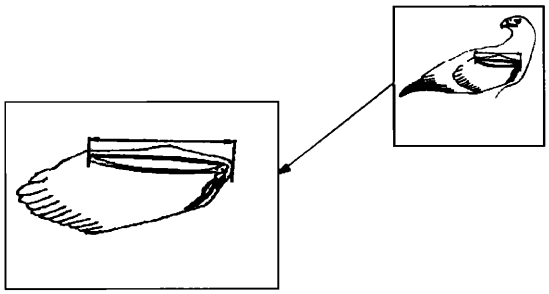

Figure 1. Measurement of forearm length in Booted Eagle.

sand dunes are the main habitats found in the area. A more detailed description of this area is presented in Rogers and Myers (1980).

Six morphometric measurements were taken from wild adult and immature eagles. To obtain measurements, we visited nests when young were 35–45 d old and their skeletons were completely grown but their feathers were still growing. Young leave the nest when they are about 55 d old (Balbontin unpubl. data). A total of 100 young were measured between 1996–98. Adults were trapped using a 2 × 3 m dho gaza net and an unreleasable captive owl (*Bubo bubo*) lure. Forty-two adults were caught using this method, 12 in 1997 and 30 in 1998. We took measurements of wing, tail, bill with cere, and tarsus lengths using a digital caliper to the nearest 0.1 mm and metal rulers to the nearest 1 mm (Bortolotti 1984). We also measured the forearm length, or the length from the front of the folded wrist to the proximal extremity of the ulna using calipers (Fig. 1) (Ferrer and De Le Court 1992). All the individuals were weighed with 1 kg or 2.5 kg Pesola scales with precisions of 5 g and 10 g, respectively.

We extracted 2 ml of blood from the brachial vein of each eagle and stored part of it (50 μl) in buffer and kept it refrigerated for later analysis. The cellular fraction was used to sex the eagles following Ellegren (1996). We used primers 2945F, cfR, and 3224R to amplify the W-chromosome gene following Ellegren's (1996) recommendations. Using this technique, we identified the sexes of 81 immature (41 females, 40 males) and 41 adult eagles (16 males, 25 females) (Fig. 2). This sample of known-sex individuals was used to derive the discriminant function using morphometric data.

Because young often differ in size from adults (Bortolotti 1984b), we used multivariate analysis of variance (MANOVA) to check for differences in size between males and females and young and adult eagles. Six measurements taken from all age and sex classes were compared using univariate analysis of variance (ANOVA) and nonparametric statistics for those variables when homogeneity of variance was not met. We used the SPSS program (Norusis 1992) to do this analysis. We separated young from adults to examine differences between sexes. First, we checked for sexual differences for each of the six morphological characters using *t*-tests. We derived a discriminant function using DISCRIM procedure of the SAS System program (version 6.12). A jackknife procedure was applied to test the efficacy of the estimated discriminant function (Lachenbruch and Mickey 1968). Each individual in the sample was classified using a discriminant function derived from the total sample, excluding the individual being classified (Chardine and Morris 1989, Amat et al. 1993). We chose the function which had the lowest percentage of misclassification based on the molecular determination of gender.

RESULTS AND DISCUSSION

Our analyses of the morphometric data showed that adult Booted Eagles differed significantly in size from young eagles and that males were significantly smaller than females (MANOVA: sex – $F = 72.0$, df = 6, 111, $P < 0.001$; age – $F = 181.85$, df = 6, 111, $P < 0.001$). Tail, wing, and culmen measurement showed the greatest difference between age groups, with the features of adult individuals larger than those of immatures (Table 1). There were no significant age- or sex-specific differences in bone measurements such as tarsus and forearm

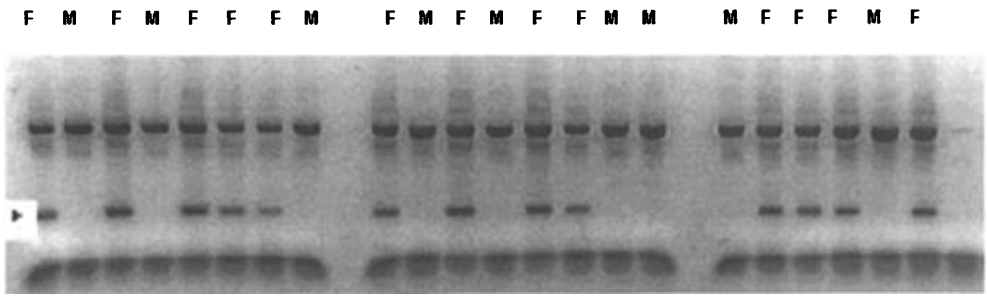

Figure 2. Gender identification using PCR test. A multiple amplification with 2945F and cfR specifically amplify a 210 bp fragment of the W chromosome in females and 2945F + 3224R that amplifies 630 bp fragments in both sexes. Females are indicated by the arrow.

Table 1. Morphometric measurements in mm of young and adult Booted Eagles.

	MALES					FEMALES				
	YOUNG ($N = 40$) ($\bar{x} \pm SD$)	ADULT ($N = 16$) ($\bar{x} \pm SD$)	F	Z	P	YOUNG ($N = 41$) ($\bar{x} \pm SD$)	ADULT ($N = 25$) ($\bar{x} \pm SD$)	F	Z	P
Tarsus	64.4 ± 2.51	64.1 ± 2.77	0.33		0.563	69.3 ± 3.30	69.4 ± 3.2	0.168		>0.05
Forearm	131.5 ± 2.64	132.2 ± 4.72	0.65		0.658	140.0 ± 4.85	143.5 ± 3.2	10.26		<0.01
Culmen	28.8 ± 1.29	31.5 ± 1.14	54.1		<0.001	30.9 ± 1.0	34.8 ± 1.3	164.1		<0.001
Wing	244.4 ± 25.9	363.8 ± 7.99		−5.80	<0.001	244.6 ± 28.8	389.2 ± 9.4		−6.77	<0.001
Tail	121.0 ± 18.6	195.8 ± 8.65		−5.80	<0.001	112.9 ± 21.5	208.7 ± 9.2		−6.74	<0.001
Mass	656.3 ± 68.7	690.9 ± 40.9		−1.76	>0.05	828.7 ± 88.3	973.2 ± 76.9	45.59		<0.001

lengths but forearm length was significantly smaller in young female eagles (Table 1). Booted Eagles show high sexual dimorphism in size and both adults and young differed significantly in the majority of the variables we studied. Adult females were significantly larger than males for all measurements taken, with forearm and body mass the most dimorphic characters (Table 2). Young females are also larger than males and they have also longer forearms and beaks, but similar-sized wings and tails. Our discriminant function analysis classified 100% of the adult female and male eagles correctly using body mass and forearm as predictor variables. The discriminant function equation for adults was:

$$D = -178.885 + 0.05613(\text{MASS}) + 0.95937(\text{FOREARM})$$

Young were classified most accuracy using the four variables forearm, tail, bill, and tarsus as predictors in the model. The discriminant function misclassified only one female. The discriminant function for young was:

$$D = -197 + 0.6761(\text{FOREARM}) - 0.19286(\text{TAIL}) + 2.99438(\text{BILL}) + 0.5858(\text{TARSUS})$$

Values of $D > 0$ represent females and values of $D < 0$ represent males. By deleting tail and wing measurements which are highly variable from the model, young eagles were also classified with 84% accuracy using only tarsus and forearm measurements in the discriminant function:

$$D = -33.815 + 0.147(\text{FOREARM}) + 0.207(\text{TARSUS})$$

The equations we derived for sexing Booted Eagles should be useful for future work on the biology of this species. For immature eagles, measurements of wings and tails should be taken carefully if they are used to discriminate gender because the feathers of young birds keep growing after they first take flight. Adults were correctly classified to gender in 100% of cases examined by using the two variables, body mass and forearm. The latter is an easy measurement to take and repeated measurements taken by different observers showed low variances (Ferrer and De Le Court 1992). Gender discrimination for young eagles is valid at 35–45 d of age when nestlings have almost completed their growth.

Table 2. Differences in morphometric measurements between male and female young and adult Booted Eagles.

	ADULTS				YOUNG			
	MALE (N = 16) ($\bar{x}$ ± SD)	FEMALE (N = 25) ($\bar{x}$ ± SD)	t	P	MALE (N = 40) ($\bar{x}$ ± SD)	FEMALE (N = 41) ($\bar{x}$ ± SD)	t	P
Tarsus	64.1 ± 2.77	69.4 ± 3.23	−5.715	<0.001	64.4 ± 2.51	69.1 ± 3.30	−7.15	<0.001
Forearm	132.2 ± 2.64	143.5 ± 3.20	−12.40	<0.001	131.5 ± 4.72	140.0 ± 4.85	−7.95	<0.001
Culmen	31.5 ± 1.14	34.8 ± 1.32	−8.604	<0.001	28.8 ± 1.29	30.9 ± 1.09	−7.93	<0.001
Wing	355.0 ± 27.8	389.2 ± 9.41	−5.712	<0.001	244.4 ± 25.9	244.6 ± 28.8	−0.03	0.970
Tail	195.6 ± 8.41	208.7 ± 9.24	−4.763	<0.001	121.0 ± 18.6	112.9 ± 21.5	1.78	0.078
Mass	690.3 ± 40.9	973.2 ± 76.9	−13.46	<0.001	656.3 ± 68.7	828.7 ± 88.3	−9.81	<0.001

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