SHORT COMMUNICATIONS

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GENDER DETERMINATION IN THE SWAINSON'S HAWK (*BUTEO SWAINSONI*) USING MOLECULAR PROCEDURES AND DISCRIMINANT FUNCTION ANALYSIS

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Gender identification of individuals is important in many studies of wild animals. However, easy determination of sex is difficult for monomorphic species including most birds of prey. Many raptor species show little plumage dimorphism, and although females are generally larger than males, overlap in morphometric measurements and body mass make gender determination difficult even when birds are captured and handled.

The Swainson's Hawk (*Buteo swainsoni*) breeds in North America and migrates to southern South America during the boreal winter that involves a trip of ca. 10 000 km each way (second longest migration distance among raptors; England et al. 1997, Fuller et al. 1998). Male and female Swainson's Hawks are similar in plumage when adults (Wheeler and Clark 1995, England et al. 1997), and as in most of the genus *Buteo,* plumage polymorphism occurs both in immature and adult birds. Although accurate methods for gender determination using morphometric data have been developed for several raptor species (Bortollotti 1984a, 1984b, Garcelon et al. 1985, Edwards and Kochert 1986, Ferrer and De Le Court 1992, Balbontín et al. 2001, Palma et al. 2001), no reliable criteria for gender determination of Swainson's Hawks using external characteristics have been described.

During the last decade, the development of laboratory techniques involving molecular procedures has provided reliable methods for the accurate gender determination of the majority of avian species (Ellegren and Sheldon 1997). PCR-based methods targeting CHD1-Z and CHD1-W genes are purported to be of universal application to birds, with the exception of ratite species (Ellegren 1996, Fridolfsson and Ellegren 1999). The aim of this study was to develop an accurate method for gender determination of Swainson's Hawks using molecular procedures and morphometric criteria. Our goal was to obtain a general model, derived from discriminant analysis, to determine gender of immature and adult Swainson's Hawks.

METHODS

We captured and sampled free-living Swainson's Hawks during two wintering seasons (austral summers) in three study areas located in central Argentina. We captured 34 hawks in the vicinity of a roost site near Las Varillas, Córdoba province (31°58′S, 62°50′W), from 19–26 January 2003. One hawk was captured in northern La Pampa province (35°14′S, 63°57′W) on 21 November 2002, and 34 hawks from 7–10 December 2003 at the same site. The sample was completed with 35 hawks captured near Santa Rosa (36°33′S, 64°07′W), La Pampa province, from 21–29 January 2004. The habitat where trapping was conducted consisted of agricultural fields of continuous crops, with soybeans as the principal crop. Planted pastures and natural fields comprised the remaining habitat

Hawks were captured in open fields near the roost using bal-chatri traps (Berger and Mueller 1959) in early morning and during the afternoon. Traps were set in front of fence posts usually used by hawks for perching, both when they left roosts in the morning and during late afternoon before returning. Captured hawks were aged as juveniles or adults based on plumage characteristics (Wheeler and Clark 1995), with immature birds grouped with juveniles using the same criteria employed by Goldstein et al. (1999). Hawks were banded and weighed with a 1500 g Pesola scale (Pesola AG, Baar, Switzerland) to the nearest 2 g. Six morphometric measurements were taken from adults and juveniles. We measured the length of wing chord (WING) and tail (TAIL) using a plastic rule to the nearest 1 mm, and length of the exposed culmen (CULMEN), tarsus (TARSUS), and hallux claw (HALLUX) using a caliper to the nearest 0.05 mm. We also measured the forearm length (FOREARM), or the length from the front of the folded wrist to the proximal extremity of the ulna, also using a caliper (Ferrer and De Le Court 1992, Balbontín et al. 2001; Fig 1). For a few birds only some of the body measurements were recorded (Tables 1, 2).

Approximately 2 ml of blood were taken from each bird from the brachial vein. The blood was placed in tubes with 96% ethanol that were kept in coolers until analysis in the laboratory. The cellular fraction of the

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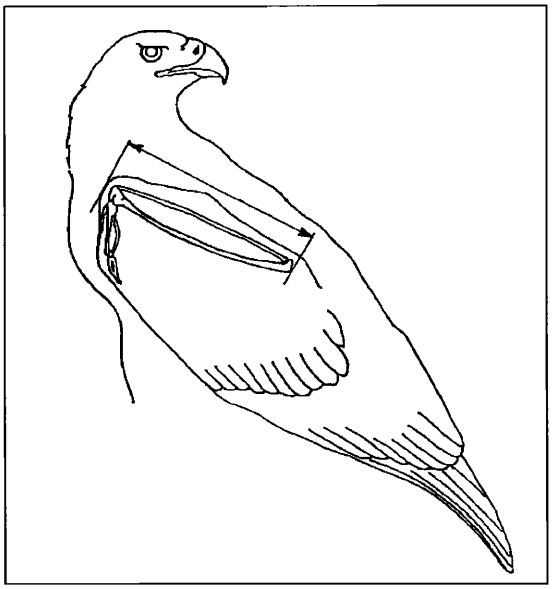

Figure 1. Measurement of forearm length in Swainson's Hawk.

blood sample was used to determine gender for all hawks. For this analysis, we used primers 2550F (5′-GTTACTGATTCGTCTACGAGA-3′) and 2718R (5′-ATTGAAATGATCCAGTGCTTG-3′) to amplify the W chromosome gene following Fridolfsson and Ellegren (1999; Fig. 2).

We performed multivariate analysis of variance (MANOVA) followed by univariate analysis of variance (ANOVA) to check for differences in morphometric measures among age and sex classes (Zar 1996). Sexes were discriminated with a stepwise discriminant analysis. We used a cross-validation (also called Jacknife) procedure to assess the predictive power of the discriminant functions, in which each individual was classified using a function derived from the total sample less the individual being classified (Manly 1986). Cohen's Kappa statistic was also calculated and significance tests were performed for each of the resulting discriminant functions. This statistic estimates the correct classification rate adjusted by chance, considering also the effect of unequal group sizes in the probability of correct classification (Titus and Mosher 1984).

RESULTS

The total sample analyzed included 66 males (32 juveniles and 34 adults) and 38 females (17 juveniles and 21 adults). Juvenile and adult Swainson's Hawks differed significantly in overall size (MANOVA, $F = 3.21$, df = 7, 93, $P < 0.01$). An ANOVA for each morphometric variable indicated that the difference was primarily due to body mass, culmen, and hallux length differences between adult and immature hawks, while there were no significant differences in the remaining measurements (Table 1). MANOVA test also showed differences between males and females ($F = 35.8$, df = 7, 93, $P < 0.001$). Univariate analysis of variances showed males being significantly smaller than females for all measures (Table 2). The ranges for the six variables were overlapping between gender groups in all cases.

Due to age-related differences in some of the morphometric measures, and in order to obtain a general method to individualize male and female hawks independently of age, we excluded body mass, culmen, and hallux length from the discriminant analysis and considered only those morphometric measures that did not differ between age groups. Discriminant function analysis using single measurements showed that most of the variables were good predictors of gender (Table 3), but every variable considered separately failed to classify 100% of the individuals in the sample correctly. Forearm was the best predictor variable considering the percentage of cases correctly classified and the value of Cohen's Kappa, with a resulting standardized linear function equal to $D_1 = 0.49$ FOREARM − 69.85. The function assigned all but 11 individuals to the correct sex after cross-validation (four males and seven females, overall success 89.4%), where values of $D > 0$ identified females and values of

Table 1. Morphometric measurements of juvenile (includes immatures or second year hawks) and adult Swainson's Hawks and analysis of variance (ANOVA) results for age class differences. All measurements, except mass (g) are in mm.

	JUVENILES				ADULTS				ANOVA	
	$\bar{x}$	SD	RANGE	N	$\bar{x}$	SD	RANGE	N	F	P
Wing	390.0	16.0	350.0–420.0	49	395.0	16.0	370.0–430.0	55	3.21	0.07
Tail	201.0	12.0	180.0–230.0	49	202.0	11.0	180.0–220.0	55	0.17	0.68
Culmen	22.5	1.3	20.3–25.6	48	23.3	1.2	20.5–26.1	54	8.55	<0.01
Tarsus	70.8	4.5	60.3–79.4	49	70.5	3.8	64.2–80.1	54	0.08	0.77
Hallux	23.9	1.3	21.4–26.7	49	24.5	1.2	22.3–28.1	55	5.63	<0.05
Forearm	136.9	6.2	124.0–149.0	49	138.4	7.1	127.0–157.0	55	1.47	0.22
Mass	759.7	116.7	540.0–1100.0	48	824.8	110.9	580.0–1110.0	55	8.40	<0.01

Table 2. Morphometric measurements of Swainson's Hawks in wintering grounds and analysis of variance (ANOVA) test results for gender differences. All measurements except mass (g) are in mm.

	FEMALES				MALES				ANOVA	
	$\bar{x}$	SD	RANGE	N	$\bar{x}$	SD	RANGE	N	F	P
Wing	409.0	11.0	390.0–430.0	38	383.0	11.0	350.0–420.0	66	127.74	<0.001
Tail	211.0	9.0	180.0–240.0	38	195.0	8.0	180.0–230.0	66	88.98	<0.001
Culmen	24.1	1.1	21.0–26.1	37	22.3	1.0	20.3–25.6	65	69.53	<0.001
Tarsus	72.6	4.0	64.6–80.1	37	69.5	3.8	60.3–78.4	66	15.68	<0.001
Hallux	25.3	1.1	22.6–28.1	38	23.7	1.0	21.4–26.4	66	55.97	<0.001
Forearm	144.2	4.8	134.5–157.0	38	134.0	4.3	124.0–144.8	66	122.68	<0.001
Mass	895.1	118.3	590.0–1110.0	38	735.6	66.8	540.0–880.0	65	76.64	<0.001

$D < 0$ identified males. The dividing point between genders for forearm length obtained by solving for 0 was 140.2 mm, with values over this point representing females and values under it representing males. Forearm, tail length, and wing chord length were retained in the stepwise discriminant analysis. The resulting linear function (D_2 = 0.36 FOREARM + 1.36 TAIL + 1.04 WING − 120.95) increased our predictive power against D_1 (Table 3) and a lower value of Wilk's lambda indicated that females and males were better separated with this linear combination of variables than using only forearm.

DISCUSSION

Our data indicated that there were significant differences between male and female Swainson's Hawks. However, there was considerable overlap in the ranges of the morphometric measurements, suggesting that the use of relative size as the only criterion to determine gender in this raptor would result in errors.

Bill depth, toe-pad length, and body mass are morphometric measurements frequently used in determining gender of size-dimorphic birds of prey (Bortollotti 1984a, 1984b, Garcelon et al. 1985, Edwards and Kochert 1986) Forearm length is a body feature traditionally not recorded for wild raptors, but its use has become more common as it is a measure easily obtained from museum skins. Furthermore, it has been shown to be a low variance measure with high repeatability among observers (Ferrer and De Le Court 1992). These features make forearm a reliable morphometric measure that should be considered as a general and standard measure in morphometric gender determination of birds of prey.

Ideally, a technique for gender determination would be applicable to individuals of all ages and under different conditions. Our model based on forearm as a single explanatory variable can be used even on birds showing incomplete molt or evidence of loss of corporal mass due to fasting. The alternative model (D_2) can be also applied in cases in which loss or gain of body mass is suspected to occur (see Smith et al. 1986, Goldstein et al. 1999) The second function, which classified the highest percentage of cases after the cross-validation and chance correction tests, produced a better separation of groups (lower value of Wilk's lambda), but needed more vari-

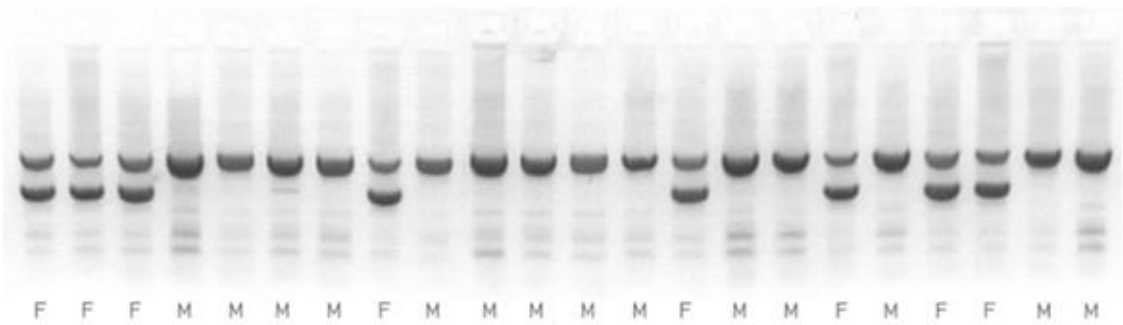

Figure 2. Gender determination using PCR methods. A multiple amplification with 2550F amplify a 420 bp fragment of W chromosome in females and 2550F + 2718R that amplifies 600 bp fragments in both sexes. Males and females are indicated as M and F, respectively.

Table 3. Accuracy of sexing Swainson's Hawks obtained from discriminant analysis using single measurements or combinations of morphometric variables, and assessed by cross-validation procedure and Cohen's Kappa calculation Single variables are ordered from higher to lower values of Cohen's Kappa.

	Cases Correctly Separated						
Variable	Wilk's Lambda	*N*	Percent Females	Percent Males	Percent Overall	Cohen's Kappa	*P*
Forearm	0.454	(104)	81.6	93.9	89.4	0.77	<0.01
Wing	0.444	(104)	81.6	90.9	87.5	0.73	<0.01
Tail	0.534	(104)	81.6	90.9	87.5	0.73	<0.01
Tarsus	0.866	(103)	70.0	87.5	82.4	0.28	>0.05
Forearm + Tail + Wing	0.332	(104)	86.8	97.0	93.3	0.85	<0.01

ables to be applicable. Its use would be better when complete data sets of the variables are available and given that measurements are not biased by external factors (e.g., molting). Furthermore, D_1 discriminant function allows for gender determination of dead birds when natural decomposition and the effect of scavengers after days of exposure in the field makes classification of sex by standard forensic methods impossible.

Insecticide poisoning of hawks in their wintering ground during 1995–96 and 1996–97 austral summers (Woodbridge et al. 1995, Goldstein et al. 1996) has been documented in this species, with ca. 20 000 birds poisoned in 1996–97. Gender determination of Swainson's Hawks would provide a valuable tool for a complete assessment of these mortality incidents, including the gender of affected birds.

RESUMEN.—*Buteo swainsoni* es un ave de presa poco dimórfica, y aunque las hembras suelen ser más grandes que los machos, la determinación del sexo en esta especie puede ser difícil, aún cuando las aves son capturadas y manipuladas. En este artículo presentamos un método de sexado para *B. swainsoni* basado en técnicas moleculares y análisis discriminantes. Los datos empleados corresponden a medidas morfométricas de 104 individuos silvestres capturados en el área de invernada de la especie durante los veranos australes 2002–03 y 2003–04 Encontramos diferencias significativas entre machos y hembras en todas las medidas morfométricas consideradas, mientras que los juveniles se diferenciaron de los adultos sólo en su masa corporal, la longitud del culmen y la longitud del hálux. Usando sólo la longitud del antebrazo como variable de predicción, nuestra función discriminante clasificó correctamente el 89.4% de los machos y el 93.9% de las hembras. Una segunda función que incluía la longitud del antebrazo, de la cola y de la cuerda alar mejoró la separación de los grupos y también el porcentaje de individuos correctamente clasificados (97.0% y 93.3% de los machos y hembras, respectivamente). El uso de medidas relacionadas con el tamaño estructural de las aves como la longitud del antebrazo, de la cola y del ala hacen de éste un método seguro y de amplia aplicación, aún para aves pertenecientes a distintas clases de edad.

[Traducción de los autores]

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PRODUCTIVITY AND FLEDGLING SEX RATIO IN A CINEREOUS VULTURE (*AEGYPIUS MONACHUS*) POPULATION IN SPAIN

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KEY WORDS: *Cinereous Vulture*; Aegypius monachus; *breeding conditions*; *brood size*; *fledgling-sex ratio*; *sexual dimorphism*; *Spain.*

Upon initial review, the mechanism of chromosomal gender determination in birds and mammals seems to be a factor limiting the parents' ability to modify the sex ratio of their progeny (Charnov 1982). However, sex allocation theory (Fisher 1930, Charnov 1982) predicts that the sex ratio can deviate from the expected 1:1, particularly when the costs of rearing the two genders are different. The optimal sex allocation for individuals can be predicted from three basic non-mutually exclusive hypotheses (reviewed by Frank 1990). (1) Fisher (1930) proposed that parental expenditure in the population should be equal for all sons and daughters, which would result in a population sex ratio biased toward the gender that costs less to produce. (2) Trivers and Willard (1973) hypothesized that if the reproductive return differs between genders depending on parental condition at the time of breeding, natural selection would favor facultative adjustments of offspring sex ratios to obtain the maximum fitness from a breeding attempt. (3) Charnov (1982) generalized Trivers and Willard's hypothesis to cover any socio-environmental variable that might pre-

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