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SEX DETERMINATION IN GLOSSY IBIS CHICKS BASED ON MORPHOLOGICAL CHARACTERS

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SUMMARY.—*Sex determination in glossy ibis chicks based on morphological characters*.

Aims: We derived two discriminant equations to sex glossy ibis *Plegadis falcinellus* chicks (2 - 5 weeks old) based on morphological characters.

Location: Doñana, South-West Spain.

Methods: Wing length and tarsus length and width of 198 individuals were measured in the 2002 and 2003 breeding seasons.

Results: Tarsus width was the most discriminant character and in combination with wing length allowed us to sex 86.9 % of individuals correctly. A formula based on wing and tarsus length also sexed 84.9 % of individuals correctly. The reliability of this second equation was also tested with an independent sample of 209 individuals measured in 2004, with error rates similar to those of the original data. Intervals for discriminant scores reducing errors in sexing to 10 % or 5 % were derived, at the cost of reducing the proportion of individuals that can be sexed. These equations should not be applied in other localities without validation, but our analyses suggest they can be used on different cohorts. We also describe a visual sexing technique found to have 95 % accuracy for an experienced observer.

Conclusions: Sex can be reliably determined for many glossy ibis chicks. Given the considerable sexual size dimorphism found in many species of the family Threskionidae, tarsus width is likely to be a good variable to include in future analyses of sexual dimorphism in spoonbills and ibises.

Key words: discriminant function, genetic analyses, glossy ibis, leg morphology, sex determination, sexual size dimorphism, *Plegadis falcinellus*.

RESUMEN.— *Determinación del sexo en pollos de morito común en base a caracteres morfológicos*.

Objetivos: Utilizando caracteres morfológicos derivamos dos funciones discriminantes para determinar el sexo de pollos (2 - 5 semanas de edad) de morito común *Plegadis falcinellus*.

Localidad: Doñana, Suroeste de España.

Métodos: Se midió la longitud del ala, y la longitud y el grosor del tarso en 198 individuos nacidos en las temporadas reproductoras del 2002 y el 2003.

Resultados: El grosor del tarso fue el carácter más dimórfico y en combinación con la longitud del ala permitió determinar el sexo correctamente el 86,9 % de los individuos. Una formula basada en las longitudes del ala y del tarso también permitió determinar el sexo correctamente del 84,9 % de los individuos. La fiabilidad de esta segunda formula discriminante se comprobó con una muestra indepen-

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diente de 209 individuos medidos en el 2004, obteniéndose unos niveles de error parecidos a los de los de la muestra original. Se derivaron los intervalos de confianza de las funciones discriminantes para reducir las tasas de error al 10 % y al 5 %, reduciéndose en estos casos el número de individuos que pueden ser sexados. Estas fórmulas no deben aplicarse a otras localidades sin validación, pero los análisis sugieren que pueden ser utilizadas sin problemas en distintas cohortes. También describimos una técnica visual de identificación del sexo que presento una fiabilidad de hasta el 95 % para un observador experimentado.

Conclusiones: El sexo de los pollos de morito común puede determinarse con fiabilidad. Debido al importante dimorfismo sexual que presentan muchas especies de la familia Threskionidae, el grosor del tarso puede ser una variable a considerar en futuros estudios sobre el dimorfismo sexual de espátulas e ibis.

Palabras clave: análisis genético, determinación del sexo, dimorfismo sexual en tamaño, función discriminante, morfología de la pata, morito común, *Plegadis falcinellus*.

Introduction

Spoonbills and ibis show strong sexual size dimorphism (Threskionidae family). The glossy ibis *Plegadis falcinellus* has a near cosmopolitan distribution across the northern hemisphere, including areas of Africa, America, Asia, Australia and Europe. Like other ibis, it has strong sexual size dimorphism, especially in tarsus length (Blake, 1977; Cramp and Simmons, 1977). While discriminant formulas based on morphological measurements have been developed for many birds, mainly raptors, gulls, terns and other seabirds (e.g., Bosch, 1996; Balbontin *et al.*, 2001; Bertellotti *et al.*, 2002; Devlin *et al.*, 2004), we are not aware of any discriminant function for sex determination in ibises, storks or spoonbills. In the case of glossy ibis, adults are so dimorphic that sexing by tarsus length seems to classify 100 % of birds correctly (Blake, 1977; Cramp and Simmons, 1977). However, this criterion cannot be applied to chicks due to their different growth stages. In this paper, we present different criteria for sex determination in glossy ibis chicks, including visual examination of leg morphology and a discriminant equation based on morphological measurements. We test the reliability of each criterion against a sample of individuals sexed with molecular techniques, and discuss the value of different characters for sex determination in this and other species of the Threskionidae family.

Material and methods

After several years of ringing glossy ibis chicks, one of us (LG) realized that two classes of leg morphologies could be identified based on the relationship between the length and width of the tarsus. We used this difference to sex chicks visually (i.e., without taking measurements). Chicks were ringed with ages ranging from 2 to 5 weeks, but for a given stage of chick development males presented wider tibio-tarsal articulations and greater overall tarsus size. A blood or feather sample was taken for DNA extraction and sex identification using polymerase chain reaction (PCR) amplification of the *CHD* genes (Griffiths *et al.*, 1998).

Given the subjectivity of sex determination based on the visual structure of the leg, initially only one of us (LG) assigned sex to chicks based on morphological characters. Once the reliability of visual sexing was estimated using molecular data, three other observers sexed birds based on leg shape criteria, to calculate differences between observers in reliability of the method.

Additionally, in 2002 and 2003 a total of 99 males and 99 females were measured to derive a discrimination formula based on quantitative morphological measurements. Wing, tarsus length, tarsus width (at the knee) and head length (from the back of the head to the tip of the bill) were measured to the nearest mm. Body mass was measured with a spring balance to the nearest gram. We used a backwards removal procedure to derive discriminant formulas for sex determination based on morphological measurements. The year of measurement was also included in the initial model to estimate the potential for inter-annual differences in chick growth. We also derived a formula excluding tarsus width, since this is not a measure traditionally taken during ringing operations (see Gosler *et al.*, 1998). With this process, we aimed to obtain formulas applicable when a limited number of measurements is available due to time constraints when working in a colony. The reliability of the formula was estimated by a Jacknife (leave one out) statistical procedure (Tabachnick and Fidell, 1996). Each discriminant score (D) has an associated probability that the individual was male or female (Norusis, 1988). The relationship between the probability of being male (or female) and D follows a logistic curve where:

$$\text{Probability} = 1 / 1+ e^{(-k*D-c)}$$

And k and c are constants calculated by fitting the curve to the values obtained from the discriminant analyses (Phillips and Furness, 1997). Individuals with intermediate probabilities of being male or female can be left unsexed to reduce classification error. With this approach, we calculated the discriminant scores interval to reduce errors in sex classification to 5 or 10 %. Individuals with discriminant scores within this interval should be left unsexed.

This increase in accuracy had an associated cost, given that birds with scores in that interval can not be classified according to gender. We also estimated the reliability of the discriminant formulas by scoring the sex of 209 individuals measured in 2004.

Results

Sexual dimorphism

Males and females sexed genetically differed significantly in most of the morphological characters analysed (Table 1). The larger differences occurred in body mass (ratio of mean male to mean female measurements = 1.17). Dimorphism in tarsus length and width was also important (ratio = 1.12 and 1.11, respectively), and was less important but still significant for bill + head length (ratio = 1.05). Interestingly males and females did not differ in wing length (ratio = 1.00).

Sexing visually

The sex of 71 individuals was scored both visually by one of us (LG) and with molecular techniques. In year 2000, 52 individuals were scored, with only 2 erroneous scorings (4 %). In 2001, 19 individuals were analysed with only 1 error in sex assignment (5.3 %).

In 2004, four different observers sexed chicks visually based on the above mentioned leg shape criterion used by LG. The error in sex determination ranged between 0 % and 33.3 % illustrating the main problem of this criterion: inter-observer variability and the need for some time for training in the correct determination of sex (mean error rate = 13.5 %).

Sexing via morphometry

The discriminant formula derived from stepwise backwards removal included wing length (a non dimorphic variable) and tarsus width (a highly dimorphic variable), and classified 86.9 % of individuals correctly (Fig. 1):

Equation 1: $D_1 = 2.861 \times \text{tarsus width} - 0.032 \times \text{wing} - 33.108$

TABLE 1

Mean and SD for genetically sexed males (*n* = 99) and females (*n* = 99) of body measurements used to derive the discriminant equations. Differences between males and females for each variable were tested with a student *t*-test with a df = 196.
*[Medias y desviaciones tipo para machos (*n *= 99) y hembras (*n *= 99) sexados genéticamente de las variables morfométricas utilizadas en el análisis discriminante. Las diferencias entre machos y hembras para cada variable se analizaron con la prueba de la *t *de student con 196 grados de libertad.]*

Measurement ***[Variable]***	Male ***[Machos]***	Female ***[Hembras]***	***t-value***	***P***
Body mass (g) *[Masa corporal]*	588.0 ± 82.9	504.6 ± 63.0	7.96	< 0.001
Tarsus length (mm) *[Longitud del tarso]*	95.35 ± 12.21	85.19 ± 7.65	7.01	< 0.001
Wing length (mm) *[Longitud del ala]*	205.9 ± 38.8	205.8 ± 24.3	0.02	0.99
Bill + head length (mm) *[Longitud de pico + cabeza]*	98.56 ± 12.14	94.22 ± 7.97	2.98	0.003
Tarsus width (mm) *[Grosor del tarso]*	14.66 ± 0.82	13.19 ± 0.79	12.78	< 0.001

Where $D_1 > 0$ for males and $D_1 < 0$ for females

When tarsus width was excluded from the initial model, tarsus length (also a highly dimorphic variable) entered in the final model. This second equation classified correctly a slightly lower proportion of individuals (84.9 %):

Equation 2: $D_2 = 0.3145$ x tarsus length – 0.0821 x wing – 11.4917

Where $D_2 > 0$ for males and $D_2 < 0$ for females

This second equation was cross-validated with a sample of 209 chicks measured in 2004. The range of measurements of the 2004 cohort was larger (including chicks smaller than other years), and ringed in three different colonies. Under these conditions, the reliability of Equation 2 was reduced to 81.3 %. The reliability of the first equation could not be assessed owing to lack of data on tarsus width.

One approach to reduce classification error is to identify the range of discriminant scores more prone to misclassification and leave individuals with scores within this range unsexed. To reduce classification error to less than 10%, individuals with scores between –0.7982 and 0.7982 for the first equation, or –0.5362 and 0.5362 for the second equation, should not be sexed (calculations based on data for 2002 - 2003). However this leaves 10.1 and 13.6 % of the birds unsexed, respectively. To further reduce classification error to less than 5 %, these ranges should be increased to -2.29 – 2.29 for the first equation, and -1.75 – 1.75 for the second equation, with a high increase in the proportion of the sample that cannot be sexed (48.5 and 47.5 %, for equations 1 and 2, respectively).

DISCUSSION

In the glossy ibis, as in many other birds, males are larger than females. However, among ibises and spoonbills, size dimorphism is so extreme that in some species sexes do not overlap in size for some morphometric

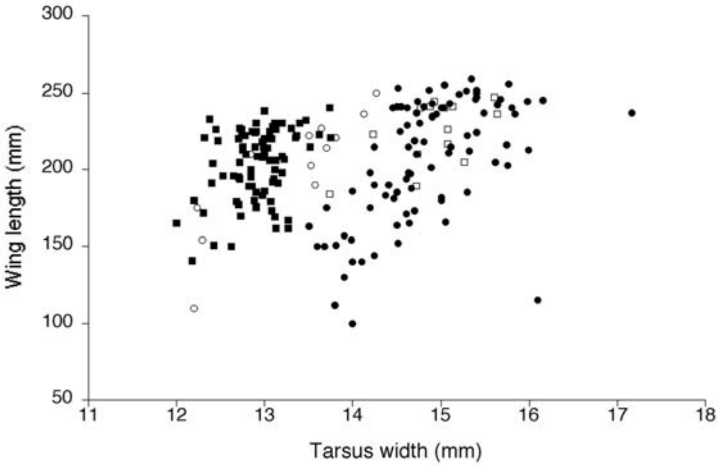

FIG. 1.—Wing length and tarsus width for males (circles) and females (squares) sexed correctly (solid symbols) and wrongly (open symbols) by the equation DS = 2.861 x tarsus width – 0.032 x wing – 33.108. DS > 0 for males and DS < 0 for females.

[Longitud del ala y grosor del tarso para machos (círculos) y hembras (cuadrados) en que el sexo se determinó correcta (símbolos rellenos) o equivocadamente (símbolos vacíos) segun la ecuación DS = 2.861 x grosor del tarso – 0.032 x longitud del ala – 33.108. DS > 0 para machos y DS < 0 para hembras.]

measurements, allowing rapid identification of gender in captured birds. Identification of gender in chicks is more problematic, given that they are not full grown and different stages of chick growth confound differences in size among sexes.

With the application of molecular techniques for sex determination (e.g., Griffiths *et al.*, 1998), it is now possible to identify the sex of immature birds. However, these techniques are still costly, especially for studies that involve a high number of individuals. This is characteristic of many studies of colonial waterbirds (e.g., ca. 2000 glossy ibis chicks were marked in 2004), making the development of cost-free techniques necessary for sex determination in the field. Lack of time available to take both measurements and genetic samples when entering a colony to ring birds is another reason why genetic methods can not always be applied. Our results demonstrate that the sex of glossy ibis chicks can be determined based on bird morphology. Visual sex determination only proved a reliable method for sex determination in glossy ibis for skilled ringers. Unskilled ringers presented high variability in sexing accuracy and error rates that were usually higher than those obtained with the discriminant formulas presented in this paper.

Size differences between sexes even during chick growth were high enough to allow reliable sex determination for more than 85 % of individuals using a combination of only two measurements. The combination of a highly dimorphic variable (tarsus width or length) and a non dimorphic variable (wing length) makes it possible to separate the effects of growth and sexual dimorphism on chick size (Fig. 1). Unfortunately, it is difficult to obtain reliable estimates of chick age that can be directly used in the discriminant formula, because of the high asynchrony of the colony and the mixture of different age birds ringed in each operation. However wing length (a non sexually dimorphic variable) allowed us to control successfully for growth differences when comparing chick tarsus width or length. By restricting the range of scores assigned to males or females, it is possible to further reduce the error in sex determination, with a reduction in the proportion of the sample that can be sexed. Restricting classification only to birds with high absolute discriminant scores also had the effect of biasing the sample of sexed birds towards larger males and smaller females, and this bias should be considered when using the data for analyses of sexual differences in ecology.

The reliability of discriminant formulas can be reduced when applied to different localities (Evans *et al.*, 1993) or years. We validated our results with data obtained in a different season, with less than a 3 % increase in error in sex determination. Furthermore, the discriminant analyses were done with measurements taken by different observers and in two different years to include in our estimates of error the possible effects of lack of consistency between measurements made by different observers (Devlin *et al.*, 2004). However, the use of these equations in different localities must be made with caution, and it is advisable to compare the morphological characteristics of the population of interest with data presented in Table 1.

A skilled observer sexed birds with a higher reliability than the discriminant formulas presented here. This is probably due to the fact that an observer analyses the three dimensional components of leg shape, while the discriminant formula is just based on two measurements. Visual determination of sex based on leg morphology is only recommendable for skilled ringers that have compared their scorings against a sample of birds sexed with molecular techniques. A visual method may be necessary when dealing with a large numbers of chicks, when economic cost precludes molecular sexing of all the chicks, and when limitations of the time that can be spent inside colonies reduces the number of chicks that can be measured. However, whenever possible, the measurement of wing and tarsus width (or length) provides a useful and rapid alternative for sex determination in glossy ibis chicks.

The large size dimorphism characteristic of the Threskionidae family suggests that the dimorphism in chick leg shape reported here probably also occurs in many other species of spoonbills and ibises.

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