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Sexing of white storks *Ciconia ciconia* based on biometric measurements

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ABSTRACT: We examined sexual size dimorphism of the white stork *Ciconia ciconia* in the Poznań Zoological Garden in Poland. From 93 measured storks, 46 were sexed using DNA extracted from blood samples and 12 by autopsy. Body morphometrics recorded included mass, wing chord, tail length, culmen length, depth and width of bill at its base for each bird. Male white storks were generally, on average, larger than females in all measurements. Discriminant analysis based on biometric measurements was applied to sexual identification of white storks. Birds for the study were from those sexed by DNA testing and 89% of birds were correctly identified.

KEY WORDS: *Ciconia ciconia*; sex determination; discriminant analysis, biometry, zoo

Introduction

The identification of the sex of birds is of fundamental importance. Most seabirds are sexually monochromatic and it can be difficult to sex individuals. Relatively easy, non-lethal and non-invasive techniques are useful for aspects of avian biology, where results are divided into sexes, not only for behavioural studies but also in conservation biology, especially for captive breeding of endangered species. Since the possibility of sex determination by DNA analysis appeared it is possible to identify sex without harming the bird. But this method is time consuming and many researchers have decided to still use intensive behavioral observations (copulation) to identify sex (Jodice *et al.* 2000; Ożgo & Bogucki,1999; Meisner &

Bzoma, 2005 after Baker *et al.* 1999) or even dissection (laparotomies or necropsies) in their research (Dorr *et al.*, 2005). Discriminant functions developed from morphological measurements have been useful in identifying the sex of birds.

The first attempt to recognize sexes of the white stork based on biometric measurements (culmen length, bill shape and, unsuccessfully, eye color) was made many years ago by Schierer (1960), Witherby *et al.* (1939) and Beltman & Rueb, (1988). We decided to verify their observations, and find the best way to identify sexes using biometric measurements of the white stork.

Materials and methods

The white storks in Poznań Zoo came mainly from the region of western Poland named Wielkopolska. Measurements were taken on all the white storks in Poznan zoo.

In this investigation we measured 93 white storks. Of these birds 41 had reached maturity and 24 were in their first year. All birds were measured by one of us (P.C.) to eliminate any inter-recorder bias. Six linear measurements were selected that would be repeatable. Measurements were taken on all birds for bill depth (BD) and wide (BW) at the base (by vernier calliper to the nearest 0.1 mm), culmen length (CULM) (straight line down the centre of the bill from the most distal point to the feathered edge at the base), tarsal length (TARS) (metatarsus measured from proximal to distal joint of the right leg). For 56 white storks additional measurements of wing chord (WCH) were taken (wrist joint to the tip of the longest primary of the right wing; the left wing was measured if the right wing was injured). Tail length was measured by slipping a ruler between the central pair of rectrices. Measurements for culmen, tarsus and wing chord were taken to the nearest millimetre using a stopped metric ruler.

Body mass (MASS) for 55 storks was measured to the nearest 0.2 kg. For all storks we made an outline of the bill shape each year. From this contour we calculated a bill index (BI) (difference between upper and lower part of bill, see Fig. 1) and the angle of the curve of the lower mandible (BA) (see Fig. 2).

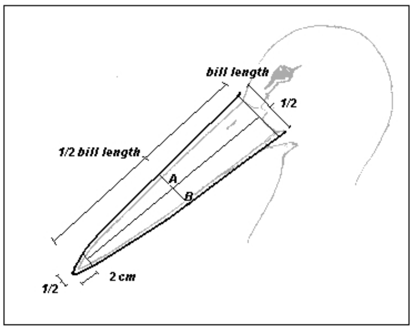

Fig. 1. Method of calculating bill index (BI) (difference from upper and lower part of bill) BI=A–B

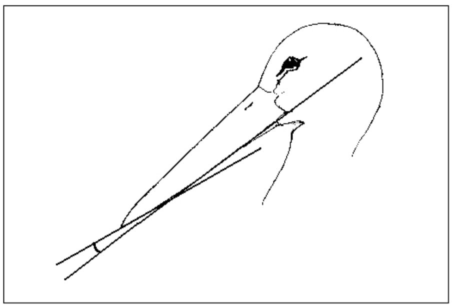

Fig. 2. Method of measuring angle of lower mandible curve (BA) similar to the method used by Kahl (1962)

Age was determined from plumage based on black parts of the bill and metatarsus colour. For 27 storks measurements were repeated after 15 months.

Of the measured birds, six were from before 2002. These birds were sexed by laparoscopy, distinguished visually by gonadal inspection using a speculum. At the end of January 2003 there were 56 white storks at Poznań Zoo (of which 12 birds were examined during autopsy by gonadal inspection). In April 2004 there were 46 birds. Those birds were sexed by DNA analysis of blood samples by a test based on two conserved CHD (chromo-helicase-DNA-binding) genes (Griffiths *et al.* 1998, Fridolfsson & Ellegren 1999). Blood samples were placed in a solution of 10mL buffer (0.1M NaCl, 0.05 M Tris-HCl, 0.01 M Na_2EDTA , pH 8.0), 1mL 10% Tween and 1 mL Proteinase K (20mg/ml; Fisher BP-1700-50). Samples were placed in a 65°C water bath for two hours. To denaturate the proteinase K following the initial incubation, samples were placed in a 95°C water bath for 10 minutes and then allowed to cool to room temperature (Sambrook *et al.* 1989). Sexing of birds followed the procedures described by Fridolfsson and Ellegren (1999). This method uses a pair of PCR primers that consistently amplify different size introns from a gene (CHD1) on each of the W and Z sex chromosomes. PCR products were size-fractionated in 1.6% agarose gels, electrophoresed in TAE buffer stained with ethidium bromide and visualized under UV-light (Sambrook *et al.* 1989). Individuals with a single band were scored as male, those with two bands as female (Fridolfsson & Ellegren 1999).

During 2005 we measured 16 storks (only one sexed post-mortem).

Because one bird sexed by laparoscopy had a different result in its autopsy inspection, we have decided to use in analysis only sexing based on DNA analysis and post mortem autopsy.

Because the data contain gaps, sample size varies slightly between different morphological traits. Throughout the text, values are reported as means ± SD. Calculations were conducted using the SPSS for Windows package and STATISTICA 6.0 PL. All basic statistical analyses were applied according to the recommendations of Zar (1999).

Results

From 93 investigated birds, sex was known for 57 individuals (61.3%), of which 26 were male (45.6%), and 31 female (54.4%). The difference between sexes was not statistically different from an expected 1: 1 ratio (χ^2=0.44; df = 1; p=0.51).

Except two measurements describing bill shape (BI and BA), most of the biometric measurements were highly correlated. Values of Pearson correlation coefficients were highest between culmen length (CULM) and tarsus length (TARS), and between culmen length (CULM) and wing chord (WCH) (Tables 1 & 2).

For discriminant analysis we used measurements which were made on all individuals with known sex (26 males and 31 females)

The discriminant function generated was:

$$D=0.012\ CULM + 0.381\ BD - 0.092\ BW + 0.038\ TARS + 0.032\ TAIL - 27.956$$

Wilks' Lambda = 0.426; $p<0.001$

This function correctly classified 88.9% of white storks of known sex.

Repeated measurements for bill depth (BD) and width (BW), and tarsal length (TARS) didn't show statistical difference. On the other hand measurement for culmen length (CULM) showed statistically significant differences between measurements of storks measured originally as first year birds ($t = -3.67$; $df = 8$; $p= 0.006$). Bills grew by an average 12.1 mm (SD = 0.99).

Table 1. Mean values (± SD) of morphometric variables for male and female white storks. Differences in measurements were tested using t-test. All measurements in mm and grams. Character abbreviations are as follows: (BD) – bill depth, (BW) – bill wide, (CULM) – culmen length, (TARS) – tarsal length, (WCH) – wing chord, (MASS) – body mass, (BI) – bill index, (BA) – lower mandible's curve

Measurement	Males		Females		df	t–value	P
	Mean	Range	Mean	Range			
CULM	168±11	144–192	156±13	134–188	56	–3.91	0.001
BD	34.9±1.6	31.4–38.6	32.3±1.8	27.3–35.1	56	–5.81	0.001
BW	28.6±1.2	26.4–30.4	27.1±2.4	24.5–30.6	56	–3.02	0.004
TARS	223±15	186–245	205±15	176–248	56	–4.54	0.001
TAIL	239±11	218–260	226±12	210–250	35	–3.41	0.002
WCH	575±29	512–620	554±22	510–602	35	–2.37	0.024
MASS	3327±462	2900–4400	3068±514	2100–4000	35	–1.61	0.116
BI	–0.83±0.2	–0.6–0.3	–0.05±0.1	–0.35–0.15	56	0.83	0.408
BA	7.0±2.6	3–12	7.1±2.6	4–15	56	0.14	0.887

Table 2. Pearson correlations among morphometric measurements for white storks at Poznan Zoo. Character abbreviations are as follows: (BD) – bill depth, (BW) – bill wide, (CULM) – culmen length, (TARS) – tarsal length, (WCH) – wing chord, (MASS) – body mass, (BI) – bill index, (BA) – lower mandible's curve

Measurement	CULM	BD	BW	TARS	TAIL	WCH	MASS	BI
BD	0.639**							
BW	0.510**	0.595**						
TARS	0.700**	0.615**	0.460**					
TAIL	0.528**	0.495**	0.212	0.368**				
WCH	0.692**	0.451**	0.291*	0.395**	0.516**			
MASS	0.412**	0.286*	0.431**	0.234	0.237	0.368**		
BI	–0.188	–0.053	–0.095	–0.183	–0.148	–0.223	–0.215	
BA	0.107	–0.079	0.059	0.032	0.035	0,277	0.210	–0.267*

**. Correlation is significant at P=0.01 level (2-tailed)

*. Correlation is significant at P=0.05 level (2-tailed)

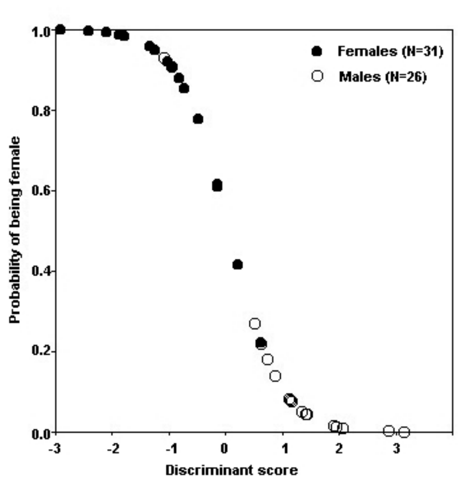

Fig. 3. The probability of being female in relation to the discriminant score

The group of adult storks did not differ significantly in culmen length after a 15-month interval.

Statistical differences between repeat measurements of bill index (BI) were found for both groups of storks (adults and first year birds). For first year birds, BI decreased on average by –0.12 (SD = 0.11; t = 3.19; df =8; p=0.013) and for adult birds this index also decreased by an average of –0.12 (SD= 0.19; t = 2.60; df = 17; p = 0.018).

Discussion

During our investigation we tried to find the best method to sex white storks based on external morphology. At first we used a method proposed by Hornberger (1967) after Schierer (1960), based on the difference in the angle of the lower mandible (Fig. 2). This method was of no value in determining sex. Only slightly better results were obtained using culmen length. According to Schierer (1960) and Cramp & Simmons (1977 after Whitherby *et al.* 1939) males should have bills longer than 170 mm. On the other hand, female bills should be shorter than 150 mm. A similar method of sexing based on bill length (Beltman & Rueb 1988) was successful in 36.6% of 2 year old white storks, and in 46.3% of 7 year old storks. These results are surprising, because in many bird species bill length alone is adequate for sexing e.g. in american white pelican *Pelecanus erythrorhynchos* or arctic tern *Sterna paradisea* (Dorr *et al.* 2005, Devlin *et al.* 2004)

During investigations carried out on oriental white stork (*Ciconia boyciana*), Murata *et al.* (1988) ascertained that there are marked sexual differences in the external measurements of the head region, with male bills being longer than those of females. In addition there were sexual differences in other bill measurements (bill depth and bill width). The difference in bill morphology between the two sexes can account for the sexual dimorphism in the fundamental frequency and repetition rates of sound elements (Eda-Fujiwara *et al.* 2004). Thus, it is possible to sex the oriental white stork non-invasively based on the acoustic structure of the clatter sound (Eda-Fujiwara *et al.* 2004). Differences in morphology from this closely related species of stork suggests that it will be much more difficult or even impossible to sex white stork based on acoustic signals. On the other hand, different acoustic signals can come from different postures during calling, as found by Pavlova & Panov (2005)

In the discriminant analysis, tarsus length had a low influence. This is a pity because it is the only measurement which, on another stork species – wood stork *Mycteria americana*, stopped growing before fledging (Kahl, 1962). If tarsus growth in white stork was similar it could allow sexing during nesting.

According to the observations of Van den Bossche *et al.* (2002), it is impossible to recognize sex based on bill length, width or depth because it reaches only 80% of maximum length in August. According to our results, the culmen still increased in second year birds, but other measurements didn't (bill depth – BD and width – BW).

The results from bill shape and bill index (BI) are interesting. This parameter did not allow ageing or sexing of white stork, since the bill turns up as the bird becomes older. At present we don't know if this is an important factor or not and it needs further investigation.

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