Sex Determination of Adult Eurasian Coots (*Fulica atra*) by Morphometric Measurements

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Sex Determination of Adult Eurasian Coots (*Fulica atra*) by Morphometric Measurements

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Abstract.—The aim of this study was to describe the size dimorphism in adult Eurasian Coots (*Fulica atra*). Appropriate discriminant functions to allow efficient sex determination on the basis of morphological measurements were developed. Breeding Eurasian Coots (n = 55) were captured from the urban population in central Poland. Eight measurements were collected from each individual, and all individuals were molecularly sexed. Head length and wing length yielded the highest discriminatory power among the collected measurements, with males larger than females. Using a jackknife procedure, a combination of these two measurements correctly sexed approximately 95% of Eurasian Coot individuals. The discriminant functions developed here could be a reliable alternative to molecular sexing techniques in the Eurasian Coot. *Received 18 October 2014, accepted 25 October 2014.*

Key words.—discriminant analysis, Eurasian Coot, *Fulica atra*, morphology, sex determination, sexual dimorphism.

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Eurasian Coots (*Fulica atra*) are a common breeder on freshwater lakes and ponds throughout large areas of Europe and Asia, and into Northern Africa and Australia. Despite being widespread, the ecology of the species remains relatively poorly studied (Guillemain *et al.* 2014). One of the features that may hamper research on this species is the lack of sexual plumage dimorphism, which makes sex determination difficult in the field. Although molecular sexing has recently become a common tool in avian studies (Ellegren and Sheldon 1997), it is an invasive technique that requires sampling of blood or other tissue that is stressful to the individual and may pose a threat (Brown and Brown 2009; McDonald and Griffith 2011). Therefore, non-invasive sexing techniques, such as morphological sex determination, are highly preferred. In fact, morphological sexing of cryptically monomorphic species is considered a reasonable choice for quick and inexpensive but efficient sex identification (Dechaume-Moncharmont *et al.* 2011). Eurasian Coot is known to be a sexually size-dimorphic species (Cramp and Simmons 1983; Baker 1993), although the extent of size dimorphism has not been explicitly evaluated. The aim of this study was to describe the level of size dimorphism in adult breeding Eurasian Coots from central Poland and to provide appropriate discriminant functions, which would allow efficient sex determination from morphological measurements.

Methods

Eurasian Coots were captured during three breeding seasons (2012-2014) in the urban area of Lodz (51° 46' N, 19° 28' E), central Poland. In total, 55 adult birds were captured from mid-March to mid-July. Individuals were captured on nests with nest traps or by hand. Using calipers, the following morphometric measurements were collected from each individual to the nearest 0.1 mm: head length (measured from tip of bill to back of skull), frontal shield length (measured from base of bill), frontal shield width, bill length, and tarsus length. Wing length (measured as maximum chord) and longest primary length were measured with a ruler to the nearest millimeter. Body mass was recorded with an electronic balance to the nearest gram.

A blood sample of 100 µl was collected from the tarsal vein of each bird and stored in 96% ethanol until analysis. Nuclear DNA from blood samples was extracted with the Thermo Scientific Genomic DNA Purification Kit (Thermo Fisher Scientific) according to the kit protocol. Amplification of the chromohelicase-DNA-binding (CHD) region was performed with the primer pair P2 and P8 (Griffiths *et al.* 1998). Polymerase chain reactions (PCR) were conducted in a final volume of 25 µl containing 12.5 µl of REDTaq ReadyMix PCR Mix (Sigma-Aldrich), 1 µl of DNA extract and 0.2 µM of each primer. All PCR amplifications followed the steps of: 1) initial denaturation at 94 °C for 1.5 min; 2) 30 cycles of denaturation at 94 °C for 30 sec followed by 45 sec at 45 °C and elongation at 72 °C for 45 sec; and 3) final elongation at 72 °C for 5 min. The PCR products were separated on 2% agarose gel until the differences in the product size were clearly visible. A male was identified by one band only (CHD-Z allele, ~500 bp), while a female was identified by two bands (CHD-Z and CHD-W alleles, ~500 bp and 400 bp). In a randomly selected 20% of birds, duplicate PCR analyses were run to

check for the repeatability of the method; the results for all repeated samples were consistent between the runs.

Between-sex differences in all collected measurements were tested with a t-test. Backward and forward stepwise discriminant analyses of all the measurements were used to identify a set of biometric traits suitable for sex determination. *A priori* classification probabilities were set equal for both sexes ($P = 0.50$). Within-group correlation coefficients for all the measurements were below 0.8, indicating a lack of strong multicollinearity between independent variables. Discriminatory power of each measurement was estimated with partial Wilk's Lambda. Validation of developed discriminant functions was conducted with two methods: 1) resubstitution, where the sex of each individual is predicted using the functions calculated from the complete data set; and 2) jackknife, where the sex of an individual is predicted from the functions calculated after that individual has been removed from the data set. The third common validation method, sample-splitting, has been reported to lead to a mean estimate of the proportion of correctly classified individuals consistent with the mean estimate using the jackknife procedure, but with a much larger variance (Dechaume-Moncharmont *et al.* 2011), and thus was not used in the analyses. All statistical analyses were performed with STATISTICA (StatSoft, Inc. 2011).

RESULTS

All measurements differed significantly between sexes, being larger in males than in females (Table 1), and were included in the discriminant analysis since they were likely to contribute to sex identification. The length and the width of the frontal shield were associated with lowest discriminatory power (partial Wilk's Lambda: 0.73 and 0.87, respectively), allowing for the correct sexing of 76.4% and 63.6% of individuals, respectively. Head length (HL) and wing length (WL) yielded the highest discriminatory power among all single measurements (partial Wilk's Lambda: 0.331 and 0.335, respectively) (Fig. 1). Each of these measurements included in the model allowed correct sexing of 92.7% of individuals and discriminant equations associated with these measurements were:

D_{HL} = 0.671*HL – 48.356 (cut-off point: D = 0.091) and

D_{WL} = 0.226*WL – 47.880 (cut-off point: D = 0.145).

The final model resulting from the backward stepwise regression included the two most discriminatory measurements: head length ($F_{1,52} = 16.6$, $P < 0.001$) and wing length ($F_{1,52} = 15.8$, $P < 0.001$). The unstandardized discriminant equation associated with this model was:

D = 0.135*HL + 0.410*WL – 58.268 (cut-off point: D = 39.641).

Both resubstitution and jackknife cross-validation showed that the equation allowed correct sexing of 94.5% of birds (92.0% of males and 96.7% of females).

The forward stepwise discriminant analysis indicated that body mass (BM, partial Wilk's Lambda: 0.45, $P = 0.021$) and tarsus length (TL, partial Wilk's Lambda: 0.49, $P = 0.16$) also could contribute to the efficiency of sex identification when included in the following discriminant equation:

D = 0.262*HL + 0.113*WL + 0.005*BM + 0.103*TL – 53.185 (cut-off point: D = -0.332).

Table 1. Morphometric measurements and body mass of male and female adult Eurasian Coots during the breeding season (from mid-March to mid-July) in central Poland, 2012-2014.

	Females ($n = 30$)		Males ($n = 25$)			
Measurement	Mean	SE	Mean	SE	t	P
Head length (mm)	70.14	0.27	74.31	0.30	10.35	< 0.001
Frontal shield length (mm)	26.47	0.56	29.96	0.52	4.48	< 0.001
Frontal shield width (mm)	19.62	0.58	22.04	0.61	2.86	0.006
Bill length (mm)	28.74	0.24	30.95	0.31	5.69	< 0.001
Tarsus length (mm)	58.14	0.41	62.76	0.48	7.38	< 0.001
Wing length (mm)	206.60	0.82	218.92	0.87	10.27	< 0.001
Longest primary length (mm)	137.73	0.92	144.24	0.85	5.11	< 0.001
Body mass (g)	690.3	13.2	839.2	12.5	8.05	< 0.001

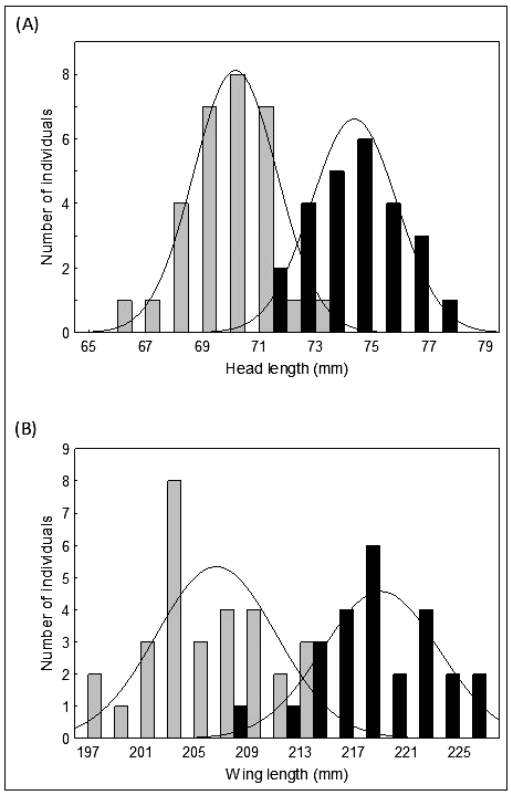

Figure 1. Frequency distributions of the two morphometric measurements, head length (A) and wing length (B), with the highest discriminatory power for sex identification of male (black bars) and female (gray bars) adult Eurasian Coots during the breeding season (from mid-March to mid-July) in central Poland, 2012-2014. Lines indicate fitted normal curves.

Resubstitution validation indicated that this function allowed correct sex identification of all measured birds ($n = 55$), whereas jackknife cross-validation indicated that 94.5% of birds (92.0% of males and 96.7% of females) were sexed correctly with this equation.

DISCUSSION

The discriminant functions developed here could be an important tool for sexing adult Eurasian Coots using morphological measurements. Head length and wing length were found to have the largest discriminatory power, and the combination of these two measurements allowed correct sexing of approximately 95% individuals. Since neither of these morphological characters is seasonally variable, I suggest that the applicability of the resulting discriminant functions should not be limited to the breeding season, but they should perform equally well for individuals captured during migration or winter. I also found that inclusion of body mass and tarsus length in the discriminant functions is likely to slightly improve efficiency of sex discrimination, as indicated by resubstitution validation of the method showing that all birds from the sample were correctly sexed. However, body mass is a highly variable trait that may show much intra- and inter-annual variation (Guillemain *et al.* 2014), and also the average values may differ substantially between populations. All Eurasian Coots measured here came from the urban population in central Poland, and the discriminant functions, including body mass, may not be appropriate for birds breeding in more natural habitats.

Contrary to expectations, the size of the frontal shield was not included in the developed discriminant functions, although this trait is known to show apparent sexual dimorphism in different species of the Rallidae family (Alvarez *et al.* 2005; Dey *et al.* 2012). Using within-pair differences in the frontal shield size to infer the sex of breeding Eurasian Coots has been reported in the field (Salathé and Boy 1987; Zhang and Ma 2012). The frontal shield in Rallidae is typically used by conspecifics as a cue of individual quality during intra- and inter-sexual interactions, primarily during fighting for mates, territory defense and courtship. There is evidence that the shield size in Rallidae is a reliable signalling ornament, as it correlates with body condition or health (Alvarez *et al.* 2005), age (Visser 1988) and dominance status (Crowley and Magrath 2004; Dey *et al.* 2014). The frontal shield size was also confirmed to be testosterone-dependent in several species of ralids (Gullion 1951; Eens *et al.* 2000), and, therefore, it has ability to change over a short period of time (Dey *et al.* 2014). The high variability of this trait seems to make it inappropriate for sex identification by discriminant analysis of morphological characters in the Eurasian Coot, and possibly in other ralids.

The results show that morphometric traits may be successfully used to identify the sex of adult Eurasian Coots from the central European population. However, it remains to be tested whether developed discriminant functions could be successfully applied to individuals from distant breeding populations.

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LITERATURE CITED

Alvarez, F., C. Sánchez and S. Angulo. 2005. The frontal shield of the moorhen: sex differences and relationship with body condition. Ethology Ecology and Evolution 17: 135-148.

Baker, K. 1993. Identification guide to European nonpasserines. British Trust for Ornithology (BTO) guide 24. BTO, Thetford, U.K.

Brown, M. B. and C. R. Brown. 2009. Blood sampling reduces annual survival in Cliff Swallows (*Petrochelidon pyrrhonota*). Auk 126: 853-861.

Cramp, S. and K. E. L. Simmons (Eds.). 1983. The birds of the Western Palearctic. Oxford University Press, Oxford, U.K.

Crowley, C. E. and R. D. Magrath. 2004. Shields of offence: signaling competitive ability in the dusky moorhen, *Gallinula tenebrosa*. Australian Journal of Zoology 52: 463-474.

Dechaume-Moncharmont, F.-X., K. Monceau and F. Cezilly. 2011. Sexing birds using discriminant function analysis: a critical appraisal. Auk 128: 78-86.

Dey, C. J., I. G. Jamieson and J. S. Quinn. 2012. Reproductive skew and female trait elaboration in a cooperatively breeding rail. Ibis 154: 452-460.

Dey, C. J., J. Dale and J. S. Quinn. 2014. Manipulating the appearance of a badge of status causes changes in true badge expression. Proceedings of the Royal Society B 281: 20132680.

Eens, M., E. Van Duyse, L. Berghman and R. Pinxten. 2000. Shield characteristics are testosterone dependent in both male and female Moorhens. Hormones and Behavior 37: 126-134.

Ellegren, H. and B. C. Sheldon. 1997. New tools for sex identification and the study of sex allocation in birds. Trends in Ecology and Evolution 12*:* 255-259.

Griffiths, R., M. C. Double, K. Orr and R. J. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7: 1071-1075.

Guillemain, M., O. Devineau, G. Simon and M. Gauthier-Clerc. 2014. Common but poorly known: information derived from 32 years of ringing Coot *Fulica atra* in Camargue, southern France. Ringing and Migration 29: 10-18.

Gullion, G. W. 1951. The frontal shield of the American Coot. Wilson Bulletin 63: 157-166.

McDonald, P. G. and S. C. Griffith. 2011. To pluck or not to pluck: the hidden ethical and scientific costs of relying on feathers as a primary source of DNA. Journal of Avian Biology 42: 197-203.

Salathé, T. and V. Boy. 1987. Territoriality and time budget of breeding Coots. Wildfowl 38: 70-76.

StatSoft, Inc. 2011. STATISTICA v. 10. Statsoft, Inc., Tulsa, Oklahoma.

Visser, J. 1988. Seasonal changes in shield size in the Coot. Ardea 76: 56-63.

Zhang, W. and J. Ma. 2012. The use of display platforms of Common Coots (*Fulica atra*) compared with the use of nests during breeding seasons. Chinese Birds 3: 82-90.