A Discriminant Function for Predicting Sex in the Balearic Shearwater

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Abstract.—We evaluate sexual adult size dimorphism and provide a sex-discriminating function for the Balearic Shearwater (*Puffinus mauretanicus*). Blood samples taken from adults were used to sex birds by the amplification of the CHD gene. Linear discriminant analysis was applied to eight morphometric characters to determine whether any single variable or combination of them could provide reliable sex determination. Females were significantly smaller than males for most tested variables. Analysis indicated that head plus bill length and minimum bill depth were the most accurate variables in a discriminant function model, predicting sex with about 90% accuracy. *Received 20 June 2002, accepted 7 September 2002.*

Key words.—Discriminant analysis, sexual dimorphism, CHD gene, morphometrics, Shearwater, *Puffinus mauretanicus.*

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The Balearic Shearwater (*Puffinus mauretanicus*) is the rarest of the three Mediterranean endemic seabird taxa: Mediterranean Shearwater (*Puffinus yelkouan*), Audouin's Gull (*Larus audouinii*) and Balearic Shearwater. Its world population is restricted to the Balearic archipelago and its size estimated to be less than 3,000 pairs (75% of the population on only one island). The species is considered critically endangered due to the dramatic decline in breeding numbers at several colonies (Arcos and Oro, in press). Studies on demography and population dynamics are crucial for the conservation of Balearic Shearwaters since they would help to evaluate the dynamics of the population and to identify the main threats of the species. Determination of sex can be useful to assess the role played by sex in some life history traits such as survival or dispersal. Body measurements have been previously used to discriminate gender through predictive functions (Witt *et al.* 1982; Coulson *et al.* 1983; Evans *et al.* 1993; Evans and Cavanagh 1995; Phillips and Furness 1997; Renner *et al.* 1998; Lo Valvo 2001). These functions are species-specific and there is strong recommendation to use them only in the studied local population if size varies over the geographical area (Evans and Cavanagh 1995).

In this paper we evaluate sexual size dimorphism in the Balearic Shearwater and we offer an easy method for sexing most adults in this species.

METHODS

Adult Balearic Shearwaters were captured during the breeding season of 2001 in two colonies on Mallorca Island (Balearic archipelago, western Mediterranean). Ten body measurements were taken by the same researcher (MM): 1) Tarsus length (from the depression in the angle of the intertarsal joint to the base of the last complete scale before the toes diverge), 2) smallest tarsus width (transversal), 3) middle toe length (distance from the first scale of the middle toe to the base of the nail on this toe), 4) head plus bill length (from the tip of the bill to the cerebellum roof), 5) bill length (from the tip of the bill to the feathering), 6) nalospi (distance from the tip to the bill to the nostril), 7) bill depth (at nostril), 8) minimum bill depth, 9) wing (maximum flattened cord) and 10) tail length (see Fig. 1 for head and bill measures). All measurements were taken with Vernier callipers (±0.02 mm) except for wing and tail length that were measured with a ruler (±0.5 mm) and body mass that was determined using a 600g Pesola (±10g) balance. From each captured bird, a small blood sample (ca. 25 µl) was taken from the leg vein. Blood was collected in a capillary tube and transferred it into a tube with approximately an equal volume of preservative buffer containing 50 mM EDTA, 2% SDS and 50 mM Tris pH8 (Griffiths *et al.* 1992). Samples were stored for several weeks at room temperature before analysis. DNA was extracted using the phenol/chloroform method, following digestion with proteinase K (Sambrook *et al.* 1989). We sexed the chicks using the polymerase chain reaction to amplify two CHD genes (Griffiths *et al.*

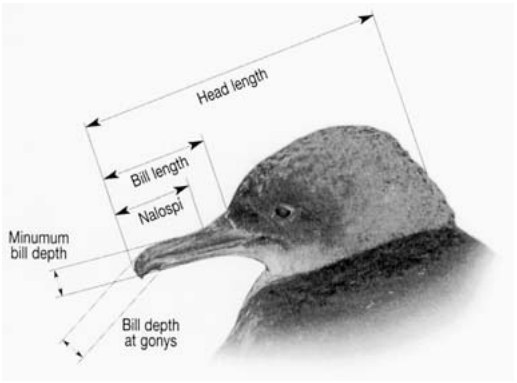

Figure 1. Head variables measurements taken in Balearic Shearwaters.

1998), detecting one band in males and two bands in females. Results were observed in a 3% agarose gel, stained with ethydium bromide.

Duplicate measurements of 20 birds were taken on two independent occasions to assess consistency (Barrett *et al.* 1989). Differences between the two measurements were tested using a paired t-test (Sokal and Rohlf 1981). Intersexual differences for each measurement were tested with a two-sample t-test. We calculated the percentage of dimorphism between sexes in each measure as:

$$\frac{\bar{x}_m - \bar{x}_f}{\bar{x}_m}$$

being $\bar{x}_m$ and $\bar{x}_f$ the mean values in males and females respectively. We also compared measurements between the members of a pair to assess how often the male was the larger of the two. Only birds for which we had all measurements were considered in the discriminant analysis. Some variables were not included in the analysis: body mass, because this can vary greatly according to the time elapsed since the last food ingestion; and wing and tail length, because they are not dimorphic in the species (see Results). The criterion used for variable selection was the statistic Wilks' Lambda. The effectiveness of the discriminant function was assessed first in terms of the proportion of adults of known sex that were classified correctly using all individuals in the analysis, second by a jacknife procedure (cross-validation) (Lachenbruch and Mickey 1968), in which each individual was classified using a function derived from the total sample minus the individual being classified (e.g., Amat *et al.* 1993; Chardine and Morris 1989; Counsilman *et al.* 1994), and third by classification of an additional sample of 12 molecular sexed birds.

RESULTS

No significant differences were found between the two sets of measurements of birds measured twice on independent occasions (see Table 1). Measurements from 72 adults sexed by PCR amplification revealed sexual size dimorphism; all bill and head measures and tarsus length were significantly larger in males than females (see Table 2). The degree of sexual dimorphism differed between the variables; minimum bill depth was the most dimorphic variable and wing, tail and weight the least dimorphic (Table 2.). Comparisons of measures within pairs revealed that males were always larger than females in the nalospi, head plus bill length and minimum bill length, but not in others (see Table 3).

The discriminant analysis was applied to eight morphometric characters of 52 adult Shearwaters (32 males and 20 females). Head plus bill length and minimum bill depth were the most accurate variables for use in a discriminant function model, predicting sex with 90% accuracy (see Table 4). Cross-validation classified adults exactly the

Table 1. Comparison of measurements of 20 birds on two independent occasions to assess consistency of data. Statistics (mean ± standard deviation), paired t-test values and level of significance are shown. All measurements are in mm.

	First measure Mean ± SD.	Second measure Mean ± SD	Mean paired difference	SE paired differences	Paired t-test	P
Bill length	38.13 ± 1.22	38.03 ± 1.17	+0.10	0.09	1.07	n.s
Nalospi	29.47 ± 1.02	29.22 ± 1.10	+0.25	0.20	1.18	n.s
Head length	89.10 ± 2.18	89.32 ± 2.05	+0.22	0.18	1.17	n.s
Bill depth at gonys	9.36 ± 0.46	9.41 ± 0.46	-0.05	0.03	-2.06	n.s
Minimum bill depth	8.80 ± 0.43	8.81 ± 1.45	-0.01	0.03	-0.07	n.s
Tarsus	50.31 ± 2.15	49.98 ± 1.60	+0.33	0.24	1.42	n.s
Tarsus width	2.78 ± 0.15	2.78 ± 0.12	0.00	0.25	0.00	n.s
Toe	55.81 ± 2.27	56.21 ± 1.76	-0.40	0.27	-1.47	n.s
Tail	74.50 ± 2.01	74.30 ± 2.08	+0.20	0.24	0.85	n.s
Wing	251.15 ± 4.95	250.95 ± 4.91	+0.20	0.37	0.53	n.s

Table 2. Descriptive statistics of the measurements of 72 adult sexed Balearic Shearwater (in mm except body mass in g). Percentage of sexual dimorphism and level of significance (P) between males and females according to the Student t-test in each variable are also shown. An asterisk (*) indicates that the variable was included in the Discriminant Analysis.

	Males		Females			
	N	Mean ± SE	N	Mean ± SE	P	% dimorphism
Bill length*	45	38.63 ± 0.17	25	37.17 ± 0.33	<0.001	3.8
Nalospi*	37	30.04 ± 0.19	20	29.04 ± 0.25	< 0.05	3.3
Head plus bill length*	45	90.45 ± 0.24	25	87.47 ± 0.43	<0.001	3.3
Bill depth at gonys*	46	9.49 ± 0.06	25	8.88 ± 0.07	<0.001	6.4
Minimum bill depth*	41	8.92 ± 0.06	23	8.18 ± 0.11	<0.001	8.3
Tarsus length*	45	50.19 ± 0.22	25	49.01 ± 0.32	< 0.05	2.4
Tarsus width*	38	2.71 ± 0.02	20	2.68 ± 0.04	n.s	0.9
Toe length*	37	55.75 ± 0.3	21	54.95 ± 0.35	n.s	1.4
Wing	38	251.75 ± 0.63	19	249.53 ± 0.99	n.s	0.9
Tail	37	74.96 ± 0.31	22	74.50 ± 0.51	n.s	0.6
Mass	46	508.55 ± 4.55	26	494.77 ± 6.11	n.s	0.3

same, so also with 90% accuracy. Applying this discriminant function to the additional sample of birds, we correctly sexed 11 from 12 adults, so accuracy was about 91%. The discriminant function (*D*) obtained was:

D = 0.41967 * head_length + 1.11984 * bill_depth - 46.88928

Wilks Lambda 0.464, $\chi^2 = 37.61$, P< 0.001. Positive discriminant scores (D) indicated male, negative values, female. When only one variable, head plus bill length or minimum bill depth, was taken into account in the discriminant function, accuracy decreased to 76% and 78% accuracy respectively.

DISCUSSION

Only in a few species of Procellariformes have significant differences in body size been detected between sexes (Warham 1990). In the Manx Shearwaters (*Puffinus puffinus*) females were found to be significantly smaller than males (Thomson 1987) although differences between sexes were smaller than those found in our study of Balearic Shearwaters. In that study, Thomson also found intersexual differences in wing length, although Brooke (1978) only found differences in bill and tarsus length working on the same species in another colony. Several head and body measurements of Short-tailed Shearwaters (*Puffinus tenuirostris)* showed that males were on average 3% larger than females (within pairs) (Meathrel *et al.* 1993). However these authors recommended against using structural size to predict the sex of adults due to assortative size mating in the species. In the closest species of the Balearic Shearwater, the Mediterranean Shearwater, Zotier (1992) concluded that the high overlapping within measurements between sexes did not allow an establishment of distinction depending on morphometrics. Instead, in Balearic Shearwaters, sexual differences between sexes were marked, males being significantly larger than females for most tested variables. In previous studies on this species, no sexual size dimorphism was found (Mayaud 1932), and only bill size gave a significant difference between sexes, but this result was probably due to small sample sizes.

Table 3. Percentage of males larger than females within pairs. N = Number of pairs examined.

	Males larger (%)	N
Bill length	90	10
Nalospi	100	10
Head length	100	11
Bill depth at gonys	91	11
Minimum bill depth	100	9
Tarsus	64	11
Tarsus width	44	9
Toe	78	9
Tail	71	7
Wing	50	8

Table 4. Accuracy of the discriminant function derived from measures of 52 Balearic Shearwaters. Frequencies and percentage (in parenthesis) of classified birds with the original function and with the cross validation (classifying leaving one out) are shown.

		Predicted sex	
	Sex	Male	Female
Original			
	Male	29 (90.6%)	3 (9.4%)
	Female	2 (10%)	18 (90%)
Cross-validation			
	Male	29 (90.6%)	3 (9.4%)
	Female	2 (10%)	18 (90%)

The best discriminant variables in the Balearic Shearwater were head length and bill depth. Both measures are consistent and easy to take in the field. These measures have been already used to discriminate sex in other seabird species such as gulls (Fox *et al.* 1981; Haners and Patton 1985; Migot 1986; Barrett *et al.* 1989; Coulson *et al.* 1993; Torlaschi *et al.* 2000), petrels (Lorentsen and Rov 1994), and Atlantic Puffins (*Fratercula artica*) (Barrett *et al.* 1989). Head length was also considered useful for sexing adults of Manx Shearwaters, although the accuracy of the discriminating function was only of 77% (Thomson 1987).

With this discriminant function, most Balearic Shearwaters can be sexed. Discriminant scores will also reveal which birds are less likely to be correctly sexed because their scores are intermediate. In such doubtful cases, the sex of the birds could be inferred by comparing head measurements with those of their partners. Otherwise, a blood sample could be taken to allow molecular sexing, but this is expensive and time consuming to apply to all individuals.

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LITERATURE CITED

Amat, J. A., J. Viñuela and M. Ferrer. 1993. Sexing Chinstrap Penguins (*Pygoscelis antarctica*) by morphological measurements. Colonial Waterbirds 16: 213-215.

Arcos, J. M. and D. Oro. In press. La Pardela Balear *Puffinus mauretanicus. In* Libro Rojo de las Aves de España (A. Madroño and R. Martí, Eds.). Sociedad Española de Ornitología SEO/BirdLife, Madrid.

Barrett, R. T., M. Peterz, R. W. Furness and J. Durinck. 1989. The variability of biometric measurements. Ringing and Migration 10: 13-16.

Brooke, M. de L. 1978. Weights and measurements of the Manx Shearwater (*Puffinus puffinus*). Journal of Zoology, London 186: 359-374.

Brooke, M. L. 1990. The Manx Shearwater. Academic Press, London.

Chardine, J. W. and R. D. Morris. 1989. Sexual size dimorphism and assortative mating in the Brown Noddy. Condor 91: 868-874.

Coulson, J. C., C. S. Thomas, J. E. L. Butterfield, N. Duncan, P. Monaghan and C. Shedden. 1983. The use of head and bill length to sex live gulls Laridae. Ibis 125: 549-557.

Counsilman, J. J., K. Nee, A. K. Jalil and W. L. Keng. 1994. Discriminant analysis of morphometric characters as a means of sexing Mynas. Journal of Field Ornithology 65: 1-7.

Del Hoyo, J., A. Elliot and J. Sargatal. 1992. Handbook of the Birds of the World, Vol. 1. Lynx Edicions, Barcelona.

Evans, D. R., E. M. Hoopes and C. R. Griffin. 1993. Discriminating the sex of Laughing Gulls by linear measurements. Journal of Field Ornithology 64: 472-476.

Evans, D. R. and P. M. Cavanagh. 1995. Identifying the sex of Massachusetts Herring Gull by linear measurements. Journal of Field Ornithology 66: 128-132.

Fox, G. A., C. R. Cooper and J. P. Ryder. 1981. Predicting the sex of Herring Gulls by using external measurements. Journal of Field Ornithology 52: 1-9.

Griffiths, R., B. Tiwari and S. A. Becker. 1992. The identification of sex in the starling *Sturnus vulgaris* using a molecular technique. Molecular Ecology 1: 191-194.

Griffiths, R., M. Double, K. Orr and R. Dawson. 1998. A simple DNA test to sex most birds. Molecular Ecology 7: 1071-1076.

Haners, L. A. and S. R. Patton. 1985. Sexing Laughing Gulls using external measurements and discriminant analysis. Journal of Field Ornithology 56: 158-164.

Lachenbruch, P. A. and M. R. Mickey. 1968. Estimation of error rates in discriminant analysis. Technometrics 10: 1-11.

Lo Valvo, M. 2001. Sexing Adult Cory's Shearwater by Discriminant Analysis of Body Measurements on Linosa Island (Sicilian Channel), Italy. Waterbirds 24: 169-174.

Lorentsen, S.-H. and N. Rov. 1994. Sex determination of Antarctic petrels *Thalassoica antarctica* by discriminant analysis of morphometric characters. Polar Biology 14: 143-145.

Mayaud, N. 1932. Considérations sur la morphologie et la systematique de quelques puffins. Alauda 4: 41-78.

Meathrel, C. E., J. S. Bradley, R. D. Wooler and I. J. Skira. 1993. The effect of parental condition on egg-size and reproductive success in Short-tailed Shearwaters *Puffinus tenuirostris*. Oecologia 93: 162-164.

Migot, P. 1986. Le Goéland Argenté *Larus argentatus argentatus* Brehm en Bretagne: Caracteristiques biométriques des reproducteurs. Alauda 54: 268-278.

Phillips, R. A. and R. W. Furness. 1997. Predicting the sex of Parasitic Jaegers by discriminant Analysis. Colonial Waterbirds 20: 14-23.

Renner, M., J. Valencia, L. S. Davis, D. Saez and O. Cifuentes. 1998. Sexing of Adult Gentoo Penguins in Antarctica Using Morphometrics. Colonial Waterbirds 21: 444-449

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning. A laboratory manual. Second edition. Cold Spring Harbor Laboratory Press, New York.

Sokal, R. R. and F. J. Rolf. 1989. Biometry. W. H. Freeman, New York.

Thomson, K. R. 1987. The Ecology of the Manx Shearwater *Puffinus puffinus* on Rhum, West Scotland. Unpublished Ph.D. Thesis, University of Glasgow.

Torlaschi, Ch., P. Gandini, E. Frere and R. M. Peck. 2000. Predicting the Sex of Kelp Gulls by External Measurements. Waterbirds 23: 518-520.

Warham, J. 1990. The Petrels. Their Ecology and Breeding Systems. Academic Press, London.

Witt, H-H., J. Crespo, E. de Juana and J. M. Varela. 1982. Geschlechtsunterschiede bei der Korallenmöwe (*Larus audouinii*) nach meßbaren Merkmalen. Vogelwarte 31: 457-460.

Zotier, R. 1992. Biogéographie des oiseaux marins en Méditerranée et écologie d'un Procellariiforme endémique: Le puffin de Méditerranée *Puffinus yelkouan*. Unpublished Ph.D. Thesis, Université Montpellier II.