



Sex differentiation of Corn Buntings *Miliaria calandra* wintering in northern Spain

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A discriminant function was developed for sex differentiation of Corn Buntings *Miliaria calandra* wintering in northern Spain. The function, $y = 0.136A + 0.288W - 26.837$, where A is wing length in mm and W is body weight in g, gives positive values for males and negative values for females. The error of this function was 3.9% lower than other published biometric criteria for sex differentiation in Corn Buntings.

The Corn Bunting *Miliaria calandra* has no obvious sexual dimorphism in plumage, but males are about 10% heavier than females (Boddy & Blackburn 1978) and wing length is about 10% longer (Prys-Jones 1976). Several authors have tried to differentiate the sexes of this species according to wing length and body weight (Follows 1969, Prys-Jones 1976, Boddy & Blackburn 1978, Svensson 1996), but some specimens showed intermediate values. According to Follows (1969), males in the United Kingdom had a wing length greater than 95 mm and body weight less than 46 g, while females were less than 95 mm in wing length and weighed less than 46 g. Boddy & Blackburn (1978) found that males in the United Kingdom in winter had a wing length greater than 97 mm or could range from 95 - 97 mm if they weighed over 53 g, whereas females had a wing length less than 95 mm or between 95 - 97 mm if they weighed less than 45 g. Svensson (1996) only reports wing length (males: 96.5 - 105 mm, females: 88 - 96 mm).

Some populations of Corn Buntings are partially migratory. Recovery data confirm that some individuals from central Europe overwinter in southern France and northern Spain (Cramp & Perrins 1994). Individuals of unknown origin migrate further southwards, and hundreds of Corn Buntings have been recorded passing through Gibraltar on their way to Africa (Tellería 1981, Finlayson 1992). Thus, it is expected that wintering flocks in areas such as the north of Spain probably comprise both locally bred and immigrant birds.

The aim of this paper is to present a method to distinguish between male and female Corn Buntings based on their biometry and weight. Studies of winter survival, migratory behaviour or breeding strategies for

this species will clearly be improved with effective methods of sex differentiation. Similarly, changes in agricultural techniques and crop types are among the main reasons for changes in Corn Bunting populations, and information on differences between the sexes may inform appropriate management and conservation techniques (McGregor & Peake 1998).

METHODS

Between March 2000 and March 2003, 103 Corn Buntings were trapped with mist nets at roosts near Olite (42°29'N 1°39'W), northern Spain. All the birds were ringed with conventional metal rings. Following Svensson (1996), wing length (maximum chord) was measured to the nearest 0.5 mm with a stopped rule, and birds were weighed to the nearest 0.1 g.

Sex determination

Approximately 10 µl of blood was taken by brachial venipuncture from 103 Corn Buntings. Blood was placed on FTA® Classic Cards for DNA extraction according to the protocol used by Gutiérrez-Corchero *et al* (2002). DNA sequences corresponding to the Chromo-Helicase-DNA-Binding (CHD) protein, present in Z and W sex chromosomes, were amplified using the polymerase chain reaction (PCR). PCR fragments were separated by gel electrophoresis on a 2.5% agarose gel. Gels were viewed under UV light and photographed. According to Griffiths *et al* (1998), samples from males show one DNA band on the gel, corresponding to the CHD-Z gene, and samples from females show two bands, corresponding to CHD-Z and CHD-W genes.

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Wing length and body weight were used in a discriminant function analysis on individuals that had been sexed by the molecular method. From these values we obtained a linear function and a limit value above or below which birds were classified as males or females, respectively (Hair *et al* 1999). The discriminant function analysis is a multivariate technique that allows analysis of the degree of aggregation of some groups considering a number of variables jointly. From the analysis, a discriminant function is obtained that allows classification of the cases analysed, and other new cases, within the studied groups (Klecka 1980). This multivariate technique has been used to differentiate the sex of other birds (eg Lezana *et al* 2000).

The difference in average wing length of birds from several European countries was verified by means of Wilcoxon Matched-Pairs Signed-Ranks test for males (symmetry coefficient $g_1 = -0.389$) and Sign Test for females (symmetry coefficient $g_1 = 1.736$).

RESULTS

We sexed 103 Corn Buntings (52 males, 51 females) by molecular methods. Data on their body weight and wing length were distributed into two distinct groups (Fig 1). In five females wing length was greater than 100 mm, but measurement error is not the cause of these unusual wing lengths. The following discriminant function was obtained (Eq 1)

$$y = 0.136A + 0.288W - 26.837 \quad (\text{Eq 1})$$

where A is the wing length (mm) and W the body weight (g). According to this function, birds were males when $y > 0$ and females when $y < 0$ (Fig 1).

We compared the accuracy of our model (Table 1) and other methods to differentiate sexes based on biometric characters (Follows 1969, Boddy & Blackburn 1978, Svensson 1996). Almost all the birds in our study were sexed correctly by combining wing length and body weight. Only two females and two males were incorrectly classified. In these cases the values of the discriminant function were slightly lower for males ($y = -0.237$, $y = -0.317$) and slightly higher for females ($y = 0.107$, $y = 0.089$) (Fig 1). Thus, the discriminant function was more reliable than other criteria proposed in previous studies. Follows (1969) and Boddy & Blackburn (1978) used wing length and body weight, and Svensson (1996) only wing length. The method described by Follows (1969) and Svensson (1996) lead to 9.7% error and that one proposed by Boddy & Blackburn (1978) had a 7.8% error. Our model reduced

this error to 3.9% (Table 1). From the overall sample, we reclassified 10 random subsamples of 50 individuals and the classification success was always greater than 95% (mean 97.8%).

DISCUSSION

Central European populations of Corn Buntings are partially migratory (Donald & Hustings 1997) and some winter in the Iberian Peninsula (Muñoz-Cobo 1997, Tellería *et al* 1999). The number of migrants is unclear but it appears that the population size of wintering birds can vary greatly (Cramp & Perrins 1994). In our study area, the few errors in sexing (Table 1) could correspond to males in the most meridian populations, including the native ones, or to females in the northern parts.

Sex determination could be further complicated by differences in age classes. In summer, Corn Buntings undergo a complete moult, so that specific age data are difficult to obtain from August-September (Svensson 1996), making it impossible to separate biometric data according to age classes. In many species, including buntings, young birds have shorter wings than adults and are generally smaller (Alatalo *et al* 1984, Aymí 1993). Thus, during the winter season young native male buntings may be difficult to differentiate from adult females from northern Europe.

No differences in wing length were observed between the samples in this study and reference mean values from populations from Holland ($Z = -0.488$, $P = 0.683$ for males; $Z = -1.803$, $P = 0.071$ for females) and Germany ($Z = -2.642$, $P = 0.080$ for males; $Z = -1.429$, $P = 0.153$ for females). Significant differences were found between the populations from Spain and Great

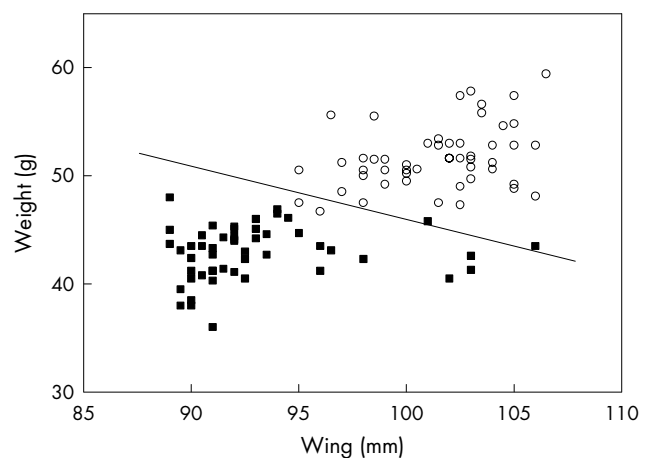


Figure 1. Wing length and body weight distributions of Corn Buntings of known sex. Circles = male; squares = female. The line represents the discriminant function (see text for details).

Table 1. Characteristics of the 15 Corn Buntings where the sex determination failed in at least one of the four methods used. The sex shown is that determined by the molecular method.

Ring number	Date	Wing (mm)	Weight (g)	Sex	Follows (1969)	Boddy & Blackburn (1978)	Svensson (1992)	This paper
V051999	20.02.03	89.0	48.0	Female	Error			
V051954	16.11.02	96.0	41.2	Female	Error			
V044279	29.11.02	96.0	43.5	Female	Error			
V044286	28.02.03	96.5	43.1	Female	Error		Error	
V051991	06.02.03	98.0	42.3	Female	Error	Error	Error	
V051993	06.02.03	101.0	45.8	Female	Error	Error	Error	Error
V051989	06.02.03	102.0	40.5	Female	Error	Error	Error	
V044280	29.11.02	103.0	41.3	Female	Error	Error	Error	
V051990	06.02.03	103.0	42.6	Female	Error	Error	Error	
V051964	21.11.02	106.0	43.5	Female	Error	Error	Error	Error
V027429	01.03.00	95.0	47.5	Male			Error	Error
V027461	27.10.00	95.0	50.5	Male			Error	
V027439	06.03.00	96.0	46.8	Male			Error	Error
V027467	27.10.00	97.0	48.5	Male		Error		
V027445	06.03.00	97.0	51.3	Male		Error		
Error (%)					9.7	7.8	9.7	3.9

Britain ($Z = -2.584$, $P = 0.010$ for males, $Z = -3.467$, $P = 0.001$ for females; Table 2). Even though birds from southern Europe may be expected to be smaller than birds from northern regions (Cramp & Perrins 1994), possible wintering Corn Buntings from central Europe may have increased the mean wing length in our study population.

The range of biometric data suggests that Corn Buntings from different locations are present in Northern Spain in winter. The recovery in our study area of an individual ringed in France confirms this.

Table 2. Wing lengths (mean \pm SD) in male and female Corn Buntings in several European countries. Range of values in brackets. N is sample size.

Country	Males	Females	Source
Netherlands	101.5 \pm 2.23 (96 - 107) N = 48	92.7 \pm 2.54 (87 - 98) N = 25	Cramp & Perrins (1994)
Germany	102.7 \pm 2.65 (96 - 109) N = 53	92.5 \pm 1.96 (90 - 96) N = 10	Eck (1985)
Great Britain	100.1 \pm 1.8 (96 - 106) N = 68	90.3 \pm 2.0 (85 - 96) N = 202	Boddy & Blackburn (1978)
Spain	101.3 \pm 2.97 (95 - 106.5) N = 52	93.0 \pm 3.91 (89 - 106) N = 51	This paper

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REFERENCES

- Alatalo, R.V., Gustafsson, L. & Lundberg, A.** (1984) Why do young passerine birds have shorter wings than older birds? *Ibis* **126**, 410-415.
- Aymí, R.** (1993) Sex and age related differences in the biometrics of a wintering population of Yellowhammers *Emberiza citrinella*. *Bulletin GCA* **10**, 1-8.
- Boddy, M. & Blackburn, A.C.** (1978) Ringing studies at a Nottinghamshire Corn Bunting Roost. *Ringing & Migration* **2**, 27-33.
- Cramp, S. & Perrins, C.M.** (eds) (1994) *The Birds of the Western Palearctic*. Vol IX. Oxford University Press, Oxford.
- Donald, P. & Hustings, F.** (1997) Corn Bunting. In *The EBCC Atlas of European Breeding Birds* (eds Hagemeyer, W. J. M. & Blair, M. J.) pp 762-763. T. & A.D. Poyser, London.
- Eck, S.** (1985) Katalog der ornithologischen Sammlung Dr Udo Bahrmanns. (6. Fortsetzung). *Zoologische-Abhandlungen Staatl. Mus. Tierkde Dresden* **41**, 1-32.
- Finlayson, J.C.** (1992) *Birds of the Strait of Gibraltar*. T. & A.D. Poyser, London.
- Follows, G.** (1969) Weights and measurements of Corn Buntings. *Ringers' Bulletin* **3**, 11-12.
- Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J.G.** (1998) A DNA test to sex most birds. *Molecular Ecology* **7**, 1071-1075.

- Gutiérrez-Corchero, F., Arruga, M.V., Sanz, L., García, C., Hernández, M.A. & Campos, F.** (2002) Using FTA cards to store avian blood samples for genetic studies. Their application in sex determination. *Molecular Ecology Notes* **2**, 75-77.
- Hair, J.F., Anderson, R.E., Tatham, R.L. & Black, W.C.** (1999) *Análisis Multivariante*. Prentice Hall Iberia, Madrid.
- Klecka, W.R.** (1980) *Discriminant Analysis*. Sage Publications Beverly Hills.
- Lezana, L., Miranda, R., Campos, F. & Peris, S.J.** (2000) Sex differentiation in the spotless starling (*Sturnus unicolor*, Temminck 1820). *Belgian Journal of Zoology* **130**, 139-142.
- McGregor, P.K. & Peake, T.M.** (1998) The role of individual identification in conservation biology. In *Behavioural Ecology and Conservation Biology*. (ed Caro, T.) pp 31-55. Oxford University Press, New York & Oxford.
- Muñoz-Cobo, J.** (1997) Triguero. In *Atlas de las aves de España (1975-1995)*. (ed Purroy, F.) pp 542-543. Lynx Edicions, Barcelona.
- Prys-Jones, R.** (1976) Wing-length in the corn bunting. *Bird Study* **23**, 294.
- Svensson, L.** (1996) *Guía de identificación de los Paseriformes de Europa*. SEO-Birdlife, Madrid.
- Tellería, J.L.** (1981) *La migración de las aves en el Estrecho de Gibraltar. Volumen II. Aves no planeadoras*. Universidad Complutense, Madrid.
- Tellería, J.L., Asensio, B. & Díaz, M.** (1999) *Aves Ibéricas II. Paseriformes*. Revero, Madrid.

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