



Sex determination of Iberian Azure-winged Magpies *Cyanopica cyanus cooki* by discriminant analysis of external measurements

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Like all corvids, the Azure-winged Magpie *Cyanopica cyanus* is sexually monomorphic in plumage, making it difficult to distinguish between males and females in the field. We examined sexual size dimorphism in a population of Iberian Azure-winged Magpies *C.c. cooki* and used a discriminant function analysis based on five morphological characters to determine the sex of first-year and adult individuals. Females were significantly smaller than males for all tested variables within each age class except for keel length of first-year individuals. In both sexes, wing length of adults was greater than that of yearlings, but only males showed significant differences in tail length between age classes (longer in first-year birds). Stepwise discriminant analysis indicated that tail length and head plus bill length were the most accurate variables in a discriminant function model, predicting the sex of yearlings with about 90% accuracy. Adding wing length to the model yielded the same level of accuracy for adults. We propose a simple system to sex Iberian Azure-winged Magpies, using only two or three variables easily measured in the field, which provides classification with a high level of accuracy.

Behavioural and ecological studies often require knowledge of the sexes of the individuals being studied. Many bird species, however, are not obviously dimorphic in size or plumage, so field identification of males and females is difficult. In such cases, individuals may be sexed by breeding behaviour (Baeyens 1981) or by the presence of a brood patch on incubating females (Green 1982, Reese & Kadlec 1982), but this is possible only for sexually active individuals during the breeding season (Coulson *et al* 1983). When sex determination in the field is impossible, alternative methods include genetic analysis (Griffiths *et al* 1998), hormone analysis (Eason *et al* 2001) and laparoscopy (Risser 1971); however, these techniques are expensive, time-consuming, highly invasive, and require trained researchers and specialised equipment (Edgington 1989). Quick, non-invasive techniques for sexing birds are, therefore, of great value to field researchers. Discriminant analysis, for example, is a statistical method that can provide a simple method for sexing birds (Anderson 1975, Fox *et al* 1981, Green 1982, Green & Theobald 1989). This type of multivariate analysis has previously been used in other monomorphic bird species, including corvids, with varying success (Reese & Kadlec 1982, Green 1982, Scharf 1987, Kavanagh 1988, Clark *et al* 1991, Kenward *et al* 2004). The analysis constructs a formula that calculates a discriminant

score for each individual based on its morphological measurements, determines the probability that the individual belongs to a predefined category, and assigns the individual to the category of highest probability. Forward stepwise discriminant analysis sequentially identifies the variables that contribute most to the differences between the groups, placing an unknown case into one of the two groups (Sokal & Rohlf 1981).

The Azure-winged Magpie *Cyanopica cyanus*, Pallas 1776, is a small corvid that is sexually monomorphic in plumage and nearly monomorphic in size (Cramp & Perrins 1994). This gregarious bird has a fragmented distribution, being present only in eastern Palaearctic Asia and in the southwestern part of the Iberian Peninsula (Goodwin 1986, Cramp & Perrins 1994); cooperative breeding has been reported in both the Japanese (Hosono 1983, Komeda *et al* 1987) and Iberian subspecies (Valencia *et al* 2003). However, large morphological differences exist between the Asiatic and Iberian populations, and Fock *et al* (2002) and Kryukov *et al* (2004) have recently reported sufficient genetic differences to warrant recognition of two different species, designating the western populations *Cyanopica cooki*.

Few data exist on biometric measurements and sexual size dimorphism in these magpies in Iberian populations (Dos Santos 1965, Álvarez & Aguilera 1988, Cruz 1988), and none of the literature on morphometric

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differences between the sexes attempts to account for the age of individuals. In this study, our aim was to test for biometric differences between the sexes and to quantify size dimorphism by sex and age class. Although discriminant function analyses can be applied only to the populations from which they are derived (Mawhinney & Diamond 1999), these data may provide an easy method of sexing Iberian Azure-winged Magpies in the field.

METHODS

Capture, sex and age

As part of a long-term study on the reproductive biology of a colony of Azure-winged Magpies in Extremadura, southwestern Spain (39° 03'N 6° 48'W; Cruz 1988) a large proportion of individuals were captured and tagged with a metal ring and an unique combination of colour plastic rings that allowed recognition of individuals. The birds were captured using a specially designed trap (1.8 x 1.8 x 1.8 m) with two funnel-like entries and baited with fodder, fruit and bread. We took external measurements from 123 individuals caught during 2002–04. Each individual was sexed during the breeding season by focal observations of its behaviour: individuals that incubated eggs (Hosono 1983, Komeda *et al* 1987, Cruz & Valencia 2004) or had a brood patch (Reese & Kadlec 1982) were assumed to be females. The age of each individual was determined from moult limits in feather tracts, after Cruz *et al* (1992), or by knowledge of the year that the bird had been tagged as a nestling. We used age–moult class instead of Euring age codes because the latter method considers calendar-year classes rather than age in relation to moult, thereby classifying individuals in different stages of moult in the same age–year classes (Fig 1).

We defined two classes of age–moult: first-year birds and adults. First-year birds were defined as individuals that had completed the first partial post-juvenile moult but had not yet undergone the complete post-nuptial moult. This category encompassed the period from November to December of the first calendar year (equivalent to Euring code 3 under the alternative system) as well as January to July of the second calendar year (Euring code 5). Adults were defined as individuals that had completed a post-nuptial moult. This category encompassed the period from September to December of the second calendar year (Euring code 5) and all subsequent years. Individuals captured during the moult season (from July to September) and during the breeding season (from April to June), as well as individuals of unknown sex or age, were excluded from the analysis to avoid any possible bias in the data.

Sample size and biometrics

A total of 62 males (34 adults and 28 first-year birds) and 61 females (30 adults and 31 first-year birds) were captured in the field. Sample size differences between analyses reflect missing values; only one measure for each individual was included in the analysis to avoid pseudoreplication (Hurlbert 1984). For consistency, all the measurements were always taken by the same person.

The following variables were recorded for each individual (Fig 2): wing length (WL), calculated as the distance from the carpal joint to the tip of the longest primary; tail length (TLL), from the insertion of the middle rectrices to the tip of the longest central rectrix; head+bill length (HBL), from the posterior ridge formed by the parietal–supraoccipital junction to the front edge of the bill (measured with the callipers held perpendicular to the top of the head); tarsus length (TSL), from the middle of the mid-tarsal joint to the distal end of the tarsometatarsus. Keel length (KL), the length of the raised central portion of the breastbone, was measured as the distance from the base of the sternum to the base of the furcula (see Fig 1 in Cartar 1984). Body mass (± 1 g) was measured with a 100 g Pesola balance. We used a ruler (± 1 mm) to measure WL, BL and TLL and digital callipers (± 0.01 mm) to measure HBL, TSL and KL.

Statistical analysis

We calculated the percentage of dimorphism (%D) between sexes for each morphometric character within each age class as: $(\text{mean}_m - \text{mean}_f) / \text{mean}_m$, where mean_m and mean_f are the mean values for males and females, respectively (Genovart *et al* 2003).

We employed two-way ANOVAs to compare sex and age differences in body size as well as interactions between both factors. Differences between sexes within age–moult classes, and between first-year birds and adults within sex classes, were analysed by t-tests. Stepwise discriminant function analysis was applied to morphometric characters to determine the best linear combination that could reliably predict sex. The discriminant analysis follows the principle of maximising the ratio of between-group variance to the pooled within-group variance and utilises the biometric data from birds of known sex to obtain an equation which can be used to predict the sex of unsexed individuals whose body measurements are known. Equations derived from measurements of birds of known sex were used to determine a discriminant score for the measurements of unsexed birds. If the discriminant score was greater than zero, the bird was classified as a male; otherwise, it was classified as a female.

'Posterior probabilities' refer to the probabilities of group affiliation assigned by the analysis after the best discrimination had been achieved using the *a priori* groupings. We eliminated body mass for the discriminant

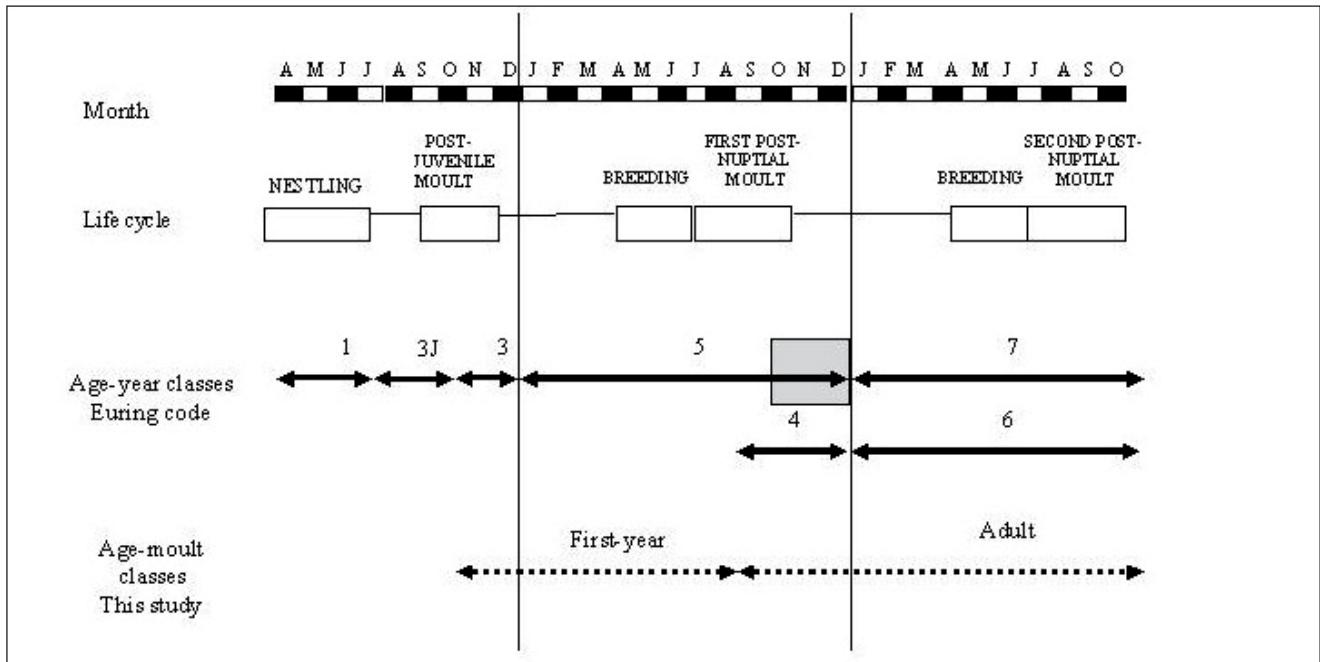


Figure 1. Age-year and age-moult classes by year and life cycle in Iberian Azure-winged Magpies. The grey box indicates individuals that are in adult plumage, but which are defined as juveniles (5) by the Euring code.

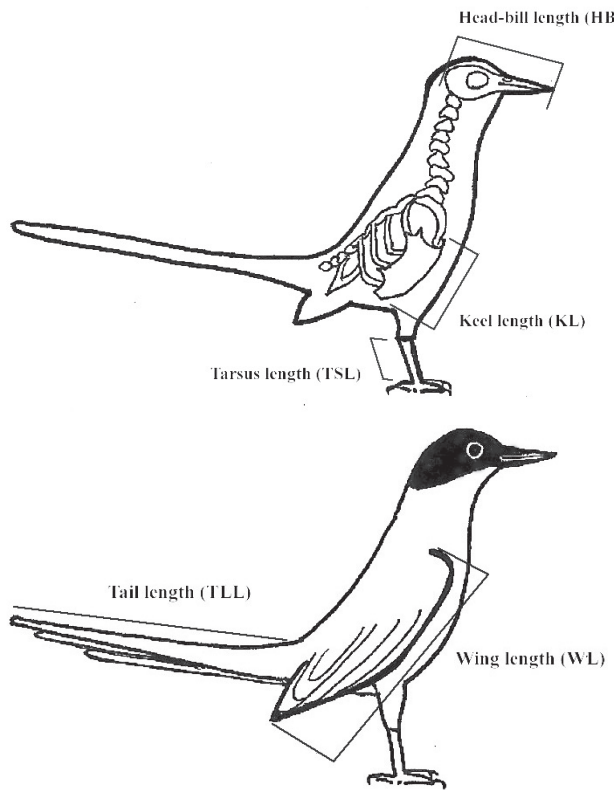


Figure 2. Scheme of variables recorded for each individual bird: wing length (WL), tail length (TLL), head-bill length (HBL), tarsus length (TSL), and keel length (KL) (see the text for descriptions).

analysis due to its great variability (Green 1982), thus controlling for the effects of body condition on sex identification. The variables retained for the discriminant analysis had no correlations greater than 0.75 and characters were not linear combinations of each other, thereby excluding any possibly redundant information.

Since we could visually distinguish first-year birds and adults, and since there were size differences between age classes in some of the variables, we developed two different discriminant functions for each age group. We validated the discriminant linear function by testing it on a different sample of 52 females (26 first-year birds, 26 adult) and 76 males (44 first-year birds, 32 adult) caught in the same place before 2001 and not used in the discriminant analysis (Frank *et al.* 1965).

Normality and homoscedascity were checked using Kolmogorov–Smirnov and Levene’s tests to ensure that the data set satisfied the assumptions necessary for the analysis. We used the statistical package STATISTICA for Windows (Statsoft 2001) to perform data analysis.

RESULTS

Sex and age differences in morphometric variables

Although there was a slight overlap in linear measurements, males were on average larger than females for discriminant characters within age-moult classes except for keel length in first-year individuals (Table 1). Body mass of yearlings

Table 1. Body measurements of first-year and adult Iberian Azure-winged Magpies, for both sexes. All linear measures are in millimetres; body weight is in grams. WL = wing length; TLL = tail length; HBL = head+bill length; KL = keel length; TSL = tarsus length; BM = body mass. * denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$. %D = percentage of dimorphism.

| | Males | | | Females | | | t-test | P | %D |
|------------------|-------|-----------------|-----------|---------|-----------------|-----------|--------|-----|-----|
| | N | Mean \pm SD | range | N | Mean \pm SD | range | | | |
| First-year birds | | | | | | | | | |
| WL | 27 | 129.5 \pm 3.4 | 122–136 | 31 | 126.4 \pm 3.8 | 119–135 | -3.16 | ** | 2.4 |
| TLL | 26 | 189.4 \pm 5.2 | 175–198 | 31 | 175.1 \pm 6.5 | 162–190 | -9.30 | *** | 7.5 |
| HBL | 26 | 53.7 \pm 1.1 | 51.9–56.0 | 28 | 51.7 \pm 0.9 | 50.0–53.6 | -7.40 | *** | 3.7 |
| TSL | 28 | 34.8 \pm 1.2 | 31.7–37.8 | 27 | 34.1 \pm 1.3 | 31.5–36.0 | -2.14 | * | 2.0 |
| KL | 28 | 28.6 \pm 1.1 | 27.2–31.4 | 28 | 28.3 \pm 1.2 | 26.0–31.4 | -0.93 | ns | 1.0 |
| BM | 26 | 71.5 \pm 3.8 | 63.5–77.0 | 29 | 68.2 \pm 5.1 | 60.0–84.5 | -2.65 | ** | 4.6 |
| Adult birds | | | | | | | | | |
| WL | 34 | 134.4 \pm 3.3 | 127–142 | 30 | 129.4 \pm 4.4 | 120–140 | -5.12 | *** | 3.7 |
| TLL | 32 | 185.9 \pm 6.5 | 171–200 | 30 | 174.2 \pm 5.5 | 163–185 | -7.55 | *** | 6.3 |
| HBL | 33 | 54.1 \pm 0.9 | 52.0–56.1 | 30 | 51.7 \pm 1.2 | 48.5–54.4 | -8.65 | *** | 6.3 |
| TSL | 33 | 35.3 \pm 0.8 | 33.6–37.0 | 29 | 34.3 \pm 1.1 | 30.4–35.6 | -4.03 | *** | 2.8 |
| KL | 33 | 28.8 \pm 0.7 | 27.2–30.5 | 29 | 27.7 \pm 1.6 | 23.0–32.5 | -3.47 | ** | 3.1 |
| BM | 33 | 70.4 \pm 4.5 | 61.5–78.0 | 29 | 70.4 \pm 5.8 | 61.0–84.0 | 0.02 | ns | 0 |

was significantly different between sexes, but we did not find such differences in adults (Table 1).

For both sexes, adults exhibited longer wings than first-year birds (males: $t_{59} = 5.62$, $P < 0.001$; females: $t_{59} = 5.03$, $P < 0.01$). Yearling males had significantly longer tails than did adult males, but the tail length of females did not differ between age classes (males: $t_{56} = -2.21$, $P < 0.05$; females: $t_{59} = -0.57$, ns). There were no differences between age classes for either sex for the remainder of the variables.

There were no significant interactions between sex and age factors (two-way ANOVAs: WL, $F_{1,103} = 1.27$, ns; TLL, $F_{1,103} = 0.32$, ns; HBL, $F_{1,103} = 0.52$, ns; KL, $F_{1,103} = 0.46$, ns; TSL, $F_{1,103} = 3.11$, ns; BM, $F_{1,103} = 2.31$, ns).

The degree of sexual size dimorphism varied among variables within each age class (Table 1). Tail length was the most strongly dimorphic variable for both adults and first-year birds, followed by head+bill length and wing length (excluding body mass in first-year birds).

Sex determination by discriminant analysis

Due to overlap in the ranges of morphometric values, no single variable could correctly determine the sex of individuals in either age class. Forward stepwise

discriminant analysis indicated that tail length (TLL) and head+bill length (HBL) were the two most accurate variables, predicting the sex of first-year birds with 90% accuracy (Table 2). This function correctly classified 85% of first-year females and 96% of first-year males. Fig 3a shows, after Green & Theobald (1989), a scatterplot for these variables with the probability contours of being male for first-year magpies.

For adults, the analysis selected wing length (WL) as well as head+bill length (HBL) and tail length (TLL) as the most parsimonious subset of variables that best predicted the sex of adults (Table 2). This linear discriminant function was 90% effective in separating the two sexes and correctly classified 86% of adult females and 93% of adult males. Fig 3b shows a scatterplot with only head+bill length (HBL) and tail length (TLL) for adult birds.

Applying discriminant functions to the additional sample of individuals to validate the model resulted in 92% (24/26) correct classification of first-year females but only 79% (35/44) of first-year males. However, adult functions achieved approximately the same percentage for both sexes: 88% accuracy for adult females (23/26) and 81% for adult males (26/32).

Table 2. Discriminant functions for sexing first-year and adult Iberian Magpies based on morphological measurements. Variables used in the model include WL (wing length), TLL (tail length), and HBL (head+bill length). Males: $D > 0$; females: $D < 0$.

| | Discriminant function | Wilks' lambda | F | P | df | Accuracy |
|-----------|--|---------------|-------|-----------|------|----------|
| Yearlings | $D = (0.28 \times \text{TLL}) + (1.12 \times \text{HBL}) - 111.66$ | 0.378 | 41.17 | < 0.001 | 2.50 | 90% |
| Adults | $D = (1.71 \times \text{HBL}) + (0.12 \times \text{TLL}) + (0.15 \times \text{WL}) - 132.86$ | 0.381 | 30.75 | < 0.001 | 3.57 | 90% |

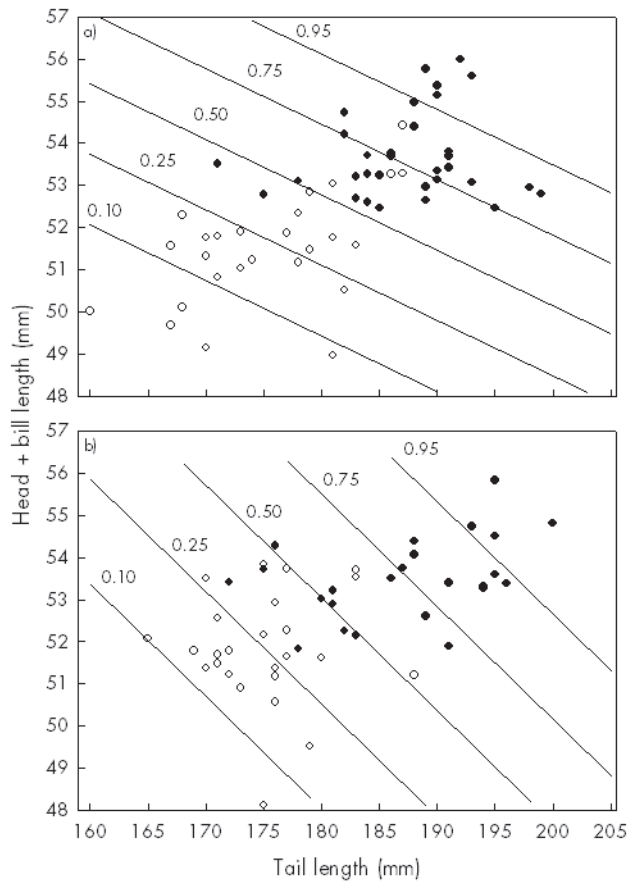


Figure 3. Size segregation of a) first-year, and b) adult, male (filled symbols) and female (open symbols) Azure-winged Magpies, with contours corresponding to given probabilities of being male.

DISCUSSION

Differences between age classes

For both sexes, first-year Iberian Azure-winged Magpies have shorter wing chords than adults; this is not unexpected (eg Pienkowski & Minton 1973). First-year males had longer tails than adults, but first-year females did not. First-year birds frequently retain remiges in the first moult, usually primaries (Cruz *et al* 1992). For this reason, primaries retained from the nestling plumage are shorter than the fresh primaries of adult birds. However, central rectrices are always moulted during the post-juvenile moult, so new feathers are as long or longer than adult feathers. The differences in tail length between age classes could be due to more pronounced wear of the central rectrices of adults, which were moulted two or three months before those of the first-year birds (Cruz *et al* 1991, 1992).

Sexual dimorphism

Male Iberian Azure-winged Magpies were larger than females in both first-year and adult age classes, a trend shared by

other corvids (Coombs 1978). Álvarez & Aguilera (1988) described sexual biometric differences in another Iberian population of Azure-winged Magpies. They attempted to explain these differences by trophic segregation between the sexes; however, they failed to find supporting differences in diet between the sexes. In addition, if this hypothesis were correct, patterns of sexual dimorphism would be stronger in bill morphology and other areas related to feeding than in other structures (McCracken *et al* 2000). The authors therefore rejected the hypothesis that food segregation may have given rise to or maintained sexual size dimorphism. They suggested that sexual size dimorphism may instead be related to the reduced nutritional requirements of the female during the breeding season or to male-male competition. Our results may support this hypothesis, since size dimorphism is present from an early age and first-year birds are able to breed in their first year (Radford & du Plessis 2004).

Though head+bill length has not been used regularly in ornithology, this measure may be very useful in distinguishing between the sexes of other bird species, since it can be measured with a high degree of consistency (Coulson *et al* 1983, Hanners & Patton 1985, Clark *et al* 1991, Franeker & ter Braak 1993, Shephard *et al* 2004). Tail length may also be useful in sexing corvids, since most have a long tail that is prominently displayed in both inter- and intra-sexual contexts by flicking the tail into an exaggeratedly upright position (Coombs 1978; pers obs).

Our discriminant function analysis was limited to five morphological measures, but previous studies show that the consistent measurement of a few basic characteristics is more powerful than the addition of numerous additional measures which might not be statistically independent (Byers & Cary 1991, Franeker & ter Braak 1993, Orthmeyer & Takekawa 1995). In conclusion, we propose a simple system to sex Iberian Azure-winged Magpies. The discriminant analysis described here uses only two or three variables, which are easily measured in the field, and provides classification with a high level of accuracy.

ACKNOWLEDGEMENTS

We appreciate the improvements in English usage made by Christina Riehl through the Association of Field Ornithologists' programme of editorial assistance. We thank Miguel Angel Pitarch, Valdesequera owner, for allowing us to work in his estate. Many students of Extremadura University helped us in data collection. Special thanks to Olga, Pipe, and Chus for helping with fieldwork. Financial support came from Junta de Extremadura projects (IPR99A020 and 2PR02A080 to C.C.) S.A. and E.S. were supported by a predoctoral grant of the Autonomic Government of Extremadura.

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(MS received 7 June 2007; accepted 27 August 2007)