

SEX DIFFERENTIATION
OF BONELLI'S EAGLE *AQUILA FASCIATA*
IN WESTERN EUROPE USING MORPHOMETRICS
AND PLUMAGE COLOUR PATTERNS

DIFERENCIACIÓN DE SEXOS
EN EL ÁGUILA PERDICERA *AQUILA FASCIATA*
EN EUROPA OCCIDENTAL UTILIZANDO MORFOMETRÍA
Y PATRONES DEL COLOR DEL PLUMAJE

Víctor GARCÍA¹ *, Rubén MORENO-OPÓ^{1,2} and Albert TINTÓ³

SUMMARY.—The study of biometric variables and the colour patterns of different plumage parts allow sex discrimination of Bonelli's eagles, both in the hand and at a distance. Between 1999 and 2012, 175 Bonelli's eagles were captured in Spain, Portugal and France and sexed on the basis of genetic analysis or by verification of reproductive behaviour. Biometric variables were measured and coloration patterns were assigned to four plumage parts (tail feathers, undertail coverts, leg feathers and throat). Females were larger than males, all variables analysed, with the exception of wing length, showing significant differences. The greatest differences were in body length, tarsus width, body mass and hindclaw length. The study of coloration revealed differences in plumage patterns between males and females. Males had paler underparts than females. Bonelli's eagles may be sexed reliably through biometric measurements and by assessing and identifying colour patterns. This is the first study quantifying sexual dimorphism of the endangered Bonelli's eagle, for which several research and conservation programmes are now being implemented.

Key words: biometrics, plumage colour, raptor, sexual dimorphism, western Mediterranean.

RESUMEN.—El estudio de variables biométricas y de patrones de coloración de distintas partes del plumaje permite la discriminación entre sexos en el águila perdicera, tanto en mano como a distancia. Entre 1999 y 2012 se capturaron 175 águilas perdiceras en España, Portugal y Francia, que fueron sexadas por medio de análisis genéticos o mediante la verificación del comportamiento reproductivo. Se tomaron distintas medidas biométricas y se asignaron los patrones de coloración de cuatro partes

¹ Deputy General Directorate on Nature. Ministry of Agriculture, Food and Environment. Pza. San Juan de la Cruz s/n., E-28071 Madrid, Spain.

² Vertebrate Biology and Conservation Group. University Complutense of Madrid, C/ José Antonio Novais 2, E-28040 Madrid, Spain.

³ Department of Animal Biology. University of Barcelona, Av. Diagonal 643, E-08028. Barcelona, Spain.

* Corresponding author: victorgarciam@yaho.es

del plumaje (rectrices, infracobertoras caudales, calzas y garganta). Las hembras resultaron más grandes que los machos, mostrando todas las variables diferencias significativas excepto la longitud del ala. Las variables que evidenciaron mayor divergencia entre sexos fueron el tamaño de cuerpo, la anchura de tarso, el peso y la uña posterior de la garras. El estudio de la coloración del plumaje reveló diferencias entre machos y hembras. Los machos presentaron, en general, un plumaje más claro en comparación con las hembras. La determinación del sexo del águila perdicera es posible a través de medidas morfométricas y de la identificación de patrones de coloración del plumaje. Este estudio es el primero que cuantifica el dimorfismo sexual en una especie amenazada como es el águila perdicera, para la que se aplican distintos programas de seguimiento y conservación en la actualidad.

Palabras clave: biometría, color del plumaje, dimorfismo sexual, Mediterráneo occidental, rapaces.

INTRODUCTION

In bird species there is often sex-based variation in morphological characteristics as an outcome of evolutionary processes (Slatkin, 1984). These intersexual differences also include ecological parameters such as diet, parental investment, survival rates or foraging and migration patterns (Promislow *et al.*, 1992; Temeles and Roberts, 1993; Margalida *et al.*, 2007). In the case of raptors, morphological differences exist between sexes, with females generally being larger than males (Newton, 2010). This sexual size dimorphism is caused by differences in ecological and evolutionary pressures on both sexes (Wheeler and Greenwood, 1983; Slatkin, 1984). As a result of these pressures, females cannot reduce their size due to the demands of egg-laying, while male size may be influenced by other adaptive mechanisms as in the case of the size decrease that occurs principally in bird-eating raptors, in which the large intersexual size discrepancy allows the pair access to a wider range of prey (Newton, 2010). The sexing of individuals is key to developing comparative studies on physiological, ecological and behavioural processes, to evaluating the impact of threatening factors and to applying management measures.

In general, there is adequate knowledge of patterns that allow sex identification of

western Palearctic raptors, mostly through biometric and plumage colour differentiation (Cramp, 1998; Ferguson-Lees and Christie, 2006; Forsman, 2007). Nonetheless, few studies have identified the most representative morphological variables distinguishing the sex of large Palearctic eagles or the variation in these variables (e.g., Ferrer and de le Court, 1992; Balbontín *et al.*, 2001). Likewise, studies analysing plumage colour differences between the sexes are lacking, except for species with obvious colour differences, such as harriers *Circus aeruginosus*, *C. pygargus*, *C. cyaneus*, *C. macrourus*; kestrels *Falco tinnunculus*, *F. naumanni*, *F. vespertinus*; the merlin *Falco columbarius*; the goshawk *Accipiter gentilis* and the sparrowhawk *Accipiter nisus*.

Bonelli's eagle *Aquila fasciata* is widely distributed across the Palearctic although the western populations (those situated around the Mediterranean Basin) are scattered and have an unfavourable conservation status (BirdLife International, 2004). In Europe it is considered 'Endangered', as its western European population does not exceed 1,000 pairs (105-115 in Portugal, 733-768 in Spain and 29 in France) and numbers are decreasing (Del Moral, 2006; Scher and Lecacheur, 2011). Different causes have been proposed for the decline of the species: i) an imbalance between survival and breeding success caused by an increase in non-natural mortality (ca-

sualties from power lines and direct persecution) and a decrease in productivity due to the decline of its main prey, the wild rabbit *Oryctolagus cuniculus* (Real *et al.*, 2001; Moleón *et al.*, 2012a); and ii) habitat loss in the breeding and foraging areas caused by human disturbance during the breeding season and, conversely, land abandonment affecting prey availability (Ontiveros *et al.*, 2004, 2005).

Due to its unfavourable conservation status, the species has been the subject of numerous studies on breeding biology, dispersal patterns, habitat and prey selection and threats among others (e.g. Real *et al.*, 2001; Moleón *et al.*, 2007; Caro, 2010; Hernández-Matías *et al.*, 2011, 2013). Moreover, Bonelli's eagle has been the subject of different conservation programmes (e.g. Arroyo and Ferreiro, 2007; Gobierno de Aragón, 2011; Palma and Cangarato, 2011). The correct sexing and ageing of individuals is necessary both when managing the birds in the hand for radio or satellite-tracking purposes and when monitoring birds in the field during studies that aim to evaluate population dynamics and survival rates. Thus, while the age patterns of the nominal subspecies *A. f. fasciata* are well-known (Cramp, 1998; Ferguson-Lees and Christie, 2006; Forsman, 2007), there is only basic information about sexing aspects (Parellada, 1984). In particular, no work has been carried out on morphometric variables or on possible differences in plumage coloration patterns aimed at differentiating males from females. Determination of the sexes in Bonelli's eagle is a challenge in long-term monitoring programmes and has been achieved only in chicks through molecular techniques (Palma *et al.*, 2001) or visually through noting the general size differences when both members of a breeding pair are observed together (Forsman, 2007). Precise identification guidelines, of known reliability, could be very useful in establishing intersexual differences

without the need to carry out genetic studies or to observe both members of a territorial pair at the same time.

The main aim of this work is to establish criteria for sexing Bonelli's eagle based on biometric characteristics (of birds in the hand) or through the colour patterns of distinctive parts of the plumage (both for field and in the hand observations). The study of age-related and geographical variation in biometrics and plumage characteristics was a complementary objective. All this information could improve the management and monitoring techniques of this endangered species by identifying sexes more accurately, thus providing a useful tool for researchers and conservation workers.

METHODS

Fieldwork and variables studied

Between June 1999 and January 2012, 175 Bonelli's eagles fulfilling the following conditions to achieve the selected objectives of this study were live-trapped: 1) birds were not chicks in the nest, and 2) they could be sexed on the basis of genetic analyses (Palma *et al.*, 2001) or detailed observation of the reproductive behaviour of marked/ringed individuals with respect to mating position (male on top) and parental investment: the female incubates for longer periods and leaves the nest to feed only briefly (Arroyo *et al.*, 1976; Newton, 2010). This work was performed within the framework of different research and conservation projects for which technical assistance from the Spanish Ministry of the Environment was requested, in several regions of Spain, Portugal and France (fig. 1).

Trapping of Bonelli's eagles was carried out in areas occupied by territorial pairs but avoided the period, generally from February to April, when the birds were incubating and chicks were not yet well feathered. Live baits

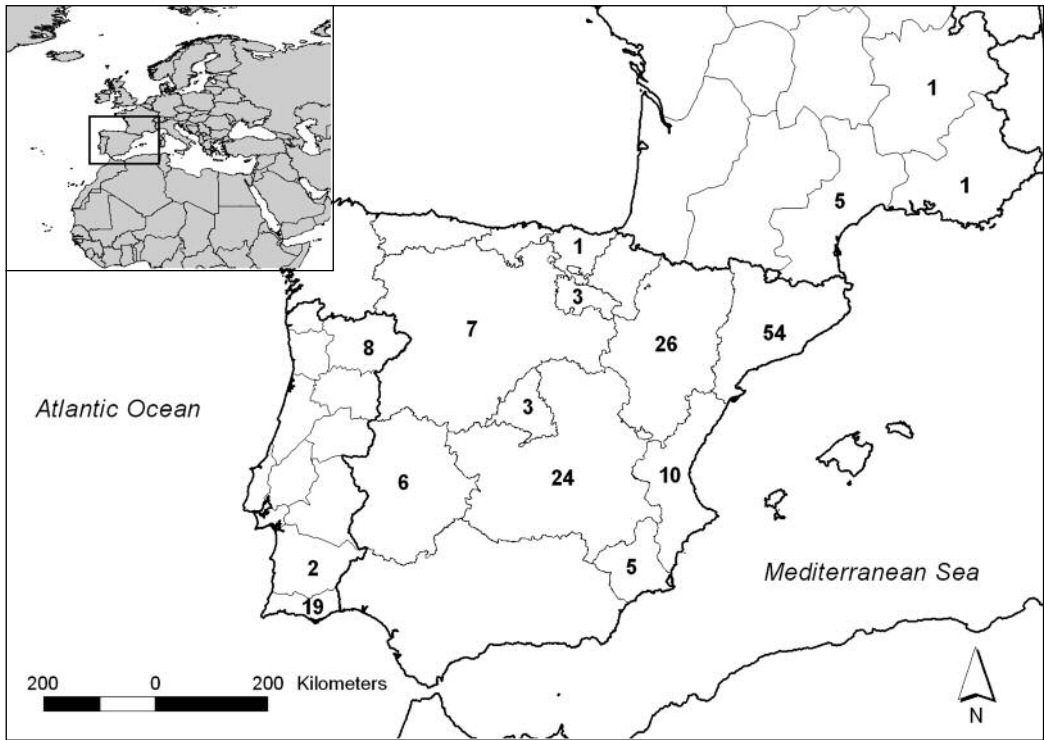


FIG. 1.—Geographical distribution in administrative regions of Spain, Portugal and France, of Bonelli's eagle *Aquila fasciata* captures, during 1999-2012. There were no captures in regions without numbers. [Distribución geográfica, en regiones administrativas de España, Portugal y Francia, de las capturas de águila perdicera *Aquila fasciata*, en el período 1999-2012. No hubo capturas en las regiones sin números.]

were used along with remote-controlled floor net traps, activated by a field technician. Biometric data and biological and photographic samples were gathered as quickly as possible, before the birds were released into the wild.

The same field technician took different morphometric measurements of the captured individuals (table 1), with the help of precision tools: digital weighing scales (± 1 g), metal rulers (± 0.5 mm) and digital callipers (± 0.01 mm). Photographs of different plumage parts of the trapped individuals were taken; of the lower and upper surfaces of tail feathers, leg feathers, the throat, wings, undertail coverts, uppertail coverts,

mantle, breast-abdomen and head. Four of the plumage parts were selected (table 2) on the basis of the preliminary detection of colour patterns that could be hypothesised to be sex-related. Based on these pictures, identifying plumage features were grouped into categories including the greatest possible variability, based upon criteria of extension/proportion of feathers with light versus dark tonalities, as shown in the technical drawings (table 2, fig. 2). In this process, individuals were assigned to predetermined categories of plumage coloration, to study their frequencies of occurrence. With respect to the *tail feathers* variable, the mean value

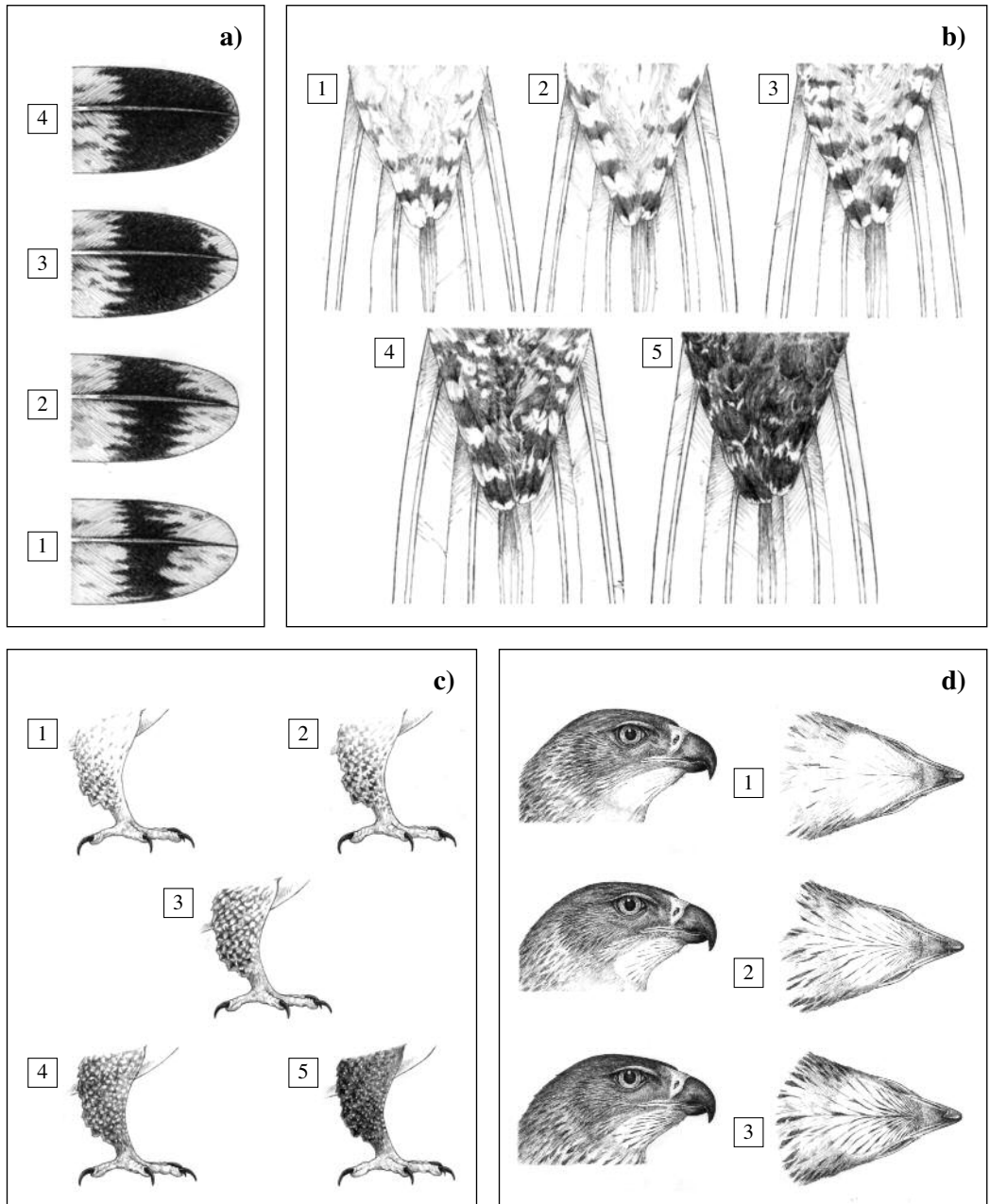


FIG. 2.—Colour patterns of the different categories (in numbers) of the plumage parts of the Bonelli's eagle *Aquila fasciata* selected to evaluate their relation to sex and age of the birds (see table 2): a) tail code, b) under-tail coverts, c) leg feathers, and d) throat.

[Patrones de coloración de distintas categorías (en números) de partes del plumaje del águila perdicera *Aquila fasciata* seleccionadas para evaluar su relación con el sexo y la edad de las aves (véase tabla 2): a) código de plumas rectrices, b) infracobertoras caudales, c) calzas y d) garganta.]

TABLE 1

Biometric variables selected to study their relationship to sexual dimorphism in Bonelli's eagle *Aquila fasciata*, descriptions of the procedures used for data collection and the units of measurement.

[*Variables biométricas seleccionadas para el estudio de su relación con el dimorfismo sexual del águila perdicera Aquila fasciata, la descripción del procedimiento empleado para su toma en campo y las unidades de referencia empleadas en el presente artículo.*]

Name	Description	Units
Body mass	Weight	g
Tarsus	Lateral width of tarsus-metatarsus in the narrowing part situated above the beginning of the metatarsal angle	mm
Wing	Maximum wing length from its junction with the body to the tip of the longest primary	cm
Tail	Length of the longest rectrix	cm
Wingspan	Maximum length from the tip of one wing to the other tip, both wings being completely drawn	cm
Body length	Length from the tip of the bill to the tip of the tail	cm
Bill length	From the distal tip up to the point where it joins the skull	mm
Bill height	In the part of the cornea with maximum distance	mm
Head length	Length from occipital part of the head to the tip of bill	mm
Head width	Head width at the widest part	mm
Hindclaw	Length of the nail of the back finger of the claw	mm
Forearm	Length of the radius and ulna bones, from its junction with the humerus to the elbow formed with the carpus-metacarpus	cm
7 th primary	Length of the seventh primary feather	cm

of the categories of the central eight tail feathers was calculated, discarding the two outermost pairs due to their uniformity in the extension of the dark colour (table 2). In this way, a numeric value was obtained for quantitative analysis. Birds with heavily worn rectrices, impeding the assessment of the feather edges (fig. 2), were not included in the analyses.

With the aim of determining age-related plumage differences, 85 Bonelli's eagles

were placed into the following groups according to their moult patterns and general coloration (Parellada, 1984; Forsman, 2007; Caro, 2010): second-third calendar year (immature, n = 8 birds), third-fourth calendar year (subadult, n = 24), and fifth or more than fifth calendar year (adult, n = 53). The effect of age classes on biometrics was also evaluated despite the fact that, in general, once birds have fledged they have acquired their adult size (Newton, 2010).

TABLE 2

Parts of the plumage considered as study variables to evaluate its relation to the sex and age of the Bonelli's eagle *Aquila fasciata*, and the description of the distinctive categories in which each has been distinguished.

[Partes del plumaje consideradas como variables de estudio para evaluar su relación con la edad y el sexo del águila perdicera *Aquila fasciata*, y la descripción de las categorías en las cuáles se han distinguido dichas variables.]

Variable	Category descriptions	Figure
Tail feathers code	<p>Average value of the terminal part of the rectrix at its lower part from the start of the last dark bar to the tip of the feather- in the 8 central tail feathers</p> <p>1 = the dark part extends less than 50%</p> <p>2 = the dark part extends over 50% until 75%</p> <p>3 = the dark part extends more than 75% until 95%</p> <p>4 = the dark part extends over 95%</p>	2a
Under-tail coverts	<p>1 = narrow and non-continuous dark stripes along the outer feathers, with no dark colour in the inner part of the under-tail coverts. Predominantly white colour</p> <p>2 = dark stripes creating continuous bars along the feather, and spread out over the whole under-tail coverts, although less extended than the white parts</p> <p>3 = dark bars with similar width and extension to white strips in all under-tail coverts</p> <p>4 = dark parts form connected strips within the same feather and are more extended than the white strips</p> <p>5 = dark parts prevailing and occupying more than 75% of the under-tail coverts. White parts not forming stripes and only dispersed spots</p>	2b
Leg feathers	<p>1 = dark parts extended less than a third of the leg feathers. More than half of the feathers do not have dark marks</p> <p>2 = dark parts occupy between one-third and a half of the leg feathers. Less than half of feathers do not have dark marks</p> <p>3 = dark parts occupy between half and two-thirds of the leg feathers. All feathers have light and dark patches</p> <p>4 = dark parts occupy more than two-thirds of the leg feathers, and white spots and stripes do not form bands and are presented as isolated spots in almost all feathers</p> <p>5 = dark parts occupy all the leg feathers and white spots do not appear or appear as tiny specks</p>	2c
Throat	<p>1 = feathers of the lower jaw, chin and throat do not show dark stripes or are difficult to distinguish</p> <p>2 = dark stripes slightly marked around less than half of the feathers of the area defined by the chin and the boundaries of the lower jaw</p> <p>3 = easily distinguishable dark strips occupying more than half of the feathers of the area defined by the chin and the boundaries of the lower jaw</p>	2d

Statistical analyses

With respect to morphometric analysis, we first checked the fit of data of the 13 studied variables into a normal distribution (Shapiro-Wilks test, $p > 0.05$). Differences between sexes for each variable were evaluated by means of a Student's t-test (Sokal and Rohlf, 1995). To determine biometric differences between the sampled areas and age-classes, each bird was assigned to its country of origin (*Portugal, Spain or France*) and age-class (*immature, subadult or adult*) and an analysis of variance (one-way ANOVA) was performed for the different variables (table 1), for males and females separately. Also, to obtain a discriminating formula for the classification of individuals by sex, a step-wise Discriminant Function Analysis was applied (DFA; Dillon and Goldstein, 1984). In this DFA, those variables that showed significant sex-related differences in the t-test analysis were included ($n = 12$). This analysis allowed the generation of a function for each sex, which showed the discriminating power of each variable through a standardised coefficient (Dillon and Goldstein, 1984; Hermosell *et al.*, 2007; Arizaga *et al.*, 2008). A subsequent DFA was performed for each geographical or age category in cases of resulting significant differences in biometrics between countries of origin or age-classes.

Two types of analysis were conducted to review coloration patterns, one for the *tail feathers* variable and another for the other three variables. The first variable offered quantitative values for the categories for each individual (response variable), and thus an analysis of variance ANOVA was carried out using, sex, age and their interaction (sex \times age) as independent variables. A Generalised Linear Model (GLM) analysis with a Poisson distribution and a log-link function was performed for the remaining three variables (*undertail coverts, leg feathers and throat*) in order to assess the

influence of sex and age in colour patterns jointly, taking as responses the categories of the three variables (*undertail coverts, leg feathers and throat*) and sex, age and their interaction as independent variables. Statistica 6.1 software (Statsoft, 2002) was used for all analyses.

RESULTS

Morphometrics

All studied biometric variables, except *wing*, differed significantly between sexes, with female values being greater (table 3). Moreover, significant differences were registered in some biometric variables according to country of origin. Thus, the *head width* differed both for males and females ($F_{2,28} = 4.32$, $p = 0.023$ for males; $F_{2,27} = 12.70$, $p < 0.001$ for females), with Portuguese individuals showing greater values. The *body length* was greater for males and females in Portugal than, in turn, Spain and France ($F_{2,32} = 8.22$, $p = 0.001$ for males; $F_{2,30} = 6.83$, $p = 0.003$ for females). On the other hand, females were heavier in France and Spain than in Portugal ($F_{2,80} = 3.50$, $p = 0.034$) and the *bill depth* was greater in France than in Portugal and Spain ($F_{2,28} = 4.27$, $p = 0.024$). In relation to age-class, no significant differences were found for males or females in any of the studied biometric variables.

For all studied birds, the DFA provided a classification matrix in which the variables *body mass, tarsus, wingspan, body length* and *7th primary* correctly grouped more than 90% of cases (table 4). Thus, a forward step-wise DFA offered the following standardised canonical discriminant classification function, with values $D < 0$ corresponding to males and values $D > 0$ to females. This function classified 100% of individuals correctly ($n = 49$ with data for all variables) using a jackknife validation procedure:

TABLE 3

Sex-associated morphometric variables of Bonelli's eagle *Aquila fasciata* in the western Mediterranean.
 [Valores de las variables morfológicas empleadas para el sexado del águila perdicera *Aquila fasciata* en la región oeste del Mediterráneo.]

	Males			Females			Statistics		
	N	mean ± SE	range	N	mean ± SE	range	t value	p	
Body mass	91	1941.37 g ± 157.12	1400-2240	83	2618.61 g ± 198.95	2100-3025	-25.026	<0.001	
Tarsus	88	9.95 mm ± 0.41	9.1-12.0	81	11.91 mm ± 0.40	11.0-12.7	-30.832	<0.001	
Wing	71	48.04 cm ± 11.07	30.8-53.0	70	48.12 cm ± 6.46	28.0-58.0	-0.054	0.956	
Tail	84	26.81 cm ± 0.88	24.5-28.7	79	28.85 cm ± 1.52	26.4-31.9	-10.523	<0.001	
Wingspan	81	155.40 cm ± 3.83	143.0-163.0	76	167.76 cm ± 4.15	156.0-176.0	-19.383	<0.001	
Body length	35	65.01 cm ± 1.17	63.0-67.0	33	70.72 cm ± 1.56	68.0-74.0	-17.049	<0.001	
Bill length	33	43.32 mm ± 1.33	40.4-45.3	31	46.61 mm ± 3.29	41.3-51.8	-5.300	0.002	
Bill height	33	22.45 mm ± 1.45	21.0-23.8	31	24.10 mm ± 0.60	22.8-25.3	-5.864	<0.001	
Head length	33	99.05 mm ± 2.95	92.2-108.8	31	108.55 mm ± 6.50	100.6-122.4	-25.026	<0.001	
Head width	31	53.37 mm ± 2.58	48.4-57.2	30	55.92 mm ± 2.75	51.6-59.8	-3.728	0.004	
Hind claw	32	37.21 mm ± 6.28	37.1-39.4	30	43.10 mm ± 2.75	40.3-46.7	-4.785	<0.001	
Forearm	75	16.68 cm ± 0.41	15.6-17.8	69	17.70 cm ± 0.46	16.9-19.8	-13.985	<0.001	
7 ^a primary	81	34.74 cm ± 1.42	31.5-38.8	70	37.08 cm ± 1.25	34.0-40.8	-10.640	<0.001	

$$D = (0.002 \times \text{biomass}) + (1.813 \times \text{tarsus}) + (0.117 \times \text{wingspan}) + (0.136 \times \text{bill length}) + (0.315 \times \text{body length}) - 71.213$$

As significant biometric variation between birds of different countries of origin was observed, we performed subsequent standardised canonical discriminant classification functions for each geographical area considered. Males generated D values < 0 and females D > 0. Functions correctly classified 100% of the individuals for which

values for all the variables were available (n = 49 for Spain; n = 12 for Portugal; n = 5 for France):

$$D = (0.002 \times \text{biomass}) + (1.984 \times \text{tarsus}) + (0.165 \times \text{wingspan}) + (0.230 \times \text{bill length}) + (0.214 \times \text{head width}) - 75.920 \text{ (Spain)}$$

$$D = (2.546 \times \text{tarsus}) + (0.887 \times \text{body length}) - 89.890 \text{ (Portugal)}$$

$$D = (6.729 \times \text{tarsus}) - 72.003 \text{ (France)}$$

TABLE 4

Results of the Discriminant Function Analysis (DFA) of the morphometric variables of the Bonelli's eagle *Aquila fasciata*. Wilk's Lambda (λ_{Wilk}) values and the percentages of correct classification by sex and for both sexes for each variable are shown.

[Resultados del Análisis de Función Discriminante (DFA) de las variables morfológicas del águila perdicera *Aquila fasciata*. Se muestran los valores de Wilk's Lambda (λ_{Wilk}) y los porcentajes de clasificación correcta por sexo, y para ambos sexos, de cada variable.]

	λ_{Wilk}	Males (%)	Females (%)	Overall (%)
Body mass	0.2154	100.0	95.1	97.7
Tarsus	0.1494	97.7	100.0	98.8
Tail	0.5924	90.4	79.7	85.2
Wingspan	0.2920	95.0	89.4	92.3
Body length	0.1850	100.0	100.0	100.0
Bill length	0.6888	96.0	74.2	85.9
Bill height	0.6432	87.8	90.3	89.0
Head length	0.5173	96.9	61.3	79.6
Head width	0.8092	58.0	56.5	57.3
Hindclaw	0.7237	96.0	93.3	95.1
Forearm	0.4206	93.3	82.6	88.2
7 ^a primary	0.5682	82.7	78.5	80.8

Plumage colour patterns

The study of the different plumage parts to distinguish coloration differences between male and female Bonelli's eagles revealed sex-related differences in the frequencies of occurrence of the colour patterns (table 5). Thus, the sex determined the existence of coloration distinctiveness, while neither age nor interaction between the sex and age of birds had an influence on plumage patterns (table 6).

In general, males were paler below than females, so that their undertail coverts, leg feathers and throats were whiter and less marked with brown or dark streaks or spots than in females, which appear to have predominantly darker coloration (fig. 3). Similarly, the extent of pale coloration on the margins of the tail feathers was greater in males than in females, presenting lower mean scores in males (fig. 3).

DISCUSSION

Morphometrics

The study of morphometrics showed clear sexual dimorphism, as was already evident for Bonelli's eagle from previous studies (Cramp, 1998; Ferguson-Lees and Christie, 2006; Forsman, 2007). As many as 12 biometric variables differed significantly between males and females, even demonstrating a

high discriminatory power. Of these, those recommended for sexing are the body length and the hindclaw length because no measurements of these for any of the specimens analysed (68 and 62, respectively) overlapped between sexes. The tarsus width and body mass also offered a high discriminatory power, with a classification percentage of 98.8% and 97.7%, respectively, according to the DFA.

In relation to body mass, only one male exceeded 2,200 g, and one female was below this weight. Despite this low overlap, it is interesting to highlight the inherent variability in the body mass values, which could reduce the proportion of correct classification by mass. This variability is due to such factors as the physical condition of the bird, the ecological characteristics of the home range or intra-annual variation according to the life-

TABLE 5

Distribution of the studied individuals of Bonelli's eagle *Aquila fasciata* within the different plumage coloration categories (see fig. 2). See table 2 for *Tail feathers score* categories.

[Distribución de los individuos estudiados de águila perdicera *Aquila fasciata* en distintas categorías de coloración del plumaje (véase fig. 2). Para la variable Tail feathers code, las categorías están referidas a intervalos de valores promedio de la parte terminal de las rectrices en su parte más exterior para las ocho plumas centrales de la cola.]

Variable	Category	Males			Females		
		n	% respect to females	% respect to all males	n	% respect to males	% respect to all females
Under-tail coverts	1	10	90.1	16.4	1	9.9	1.7
	2	34	97.1	55.7	1	2.9	1.7
	3	13	65.0	21.3	7	35.0	12.5
	4	4	11.5	6.6	31	88.5	55.4
	5	0	0.0	0.0	16	100.0	28.7
Leg feathers	1	3	100.0	5.1	0	0.0	0.0
	2	10	83.3	17.2	2	16.7	3.7
	3	25	86.2	43.1	4	13.8	7.4
	4	19	46.3	32.8	22	53.7	40.7
	5	1	3.7	1.7	26	96.3	48.2
Throat	1	37	86.0	63.8	6	14.0	10.5
	2	14	56.0	24.1	11	44.0	19.3
	3	7	14.9	12.1	40	85.1	70.2
Tail feathers code	1 to 2	7	87.5	12.3	1	12.5	1.7
	> 2 to 3	41	82.0	71.9	9	18.0	15.5
	> 3 to 4	9	15.7	15.8	48	84.3	82.8

TABLE 6

Analysis of variance (ANOVA) for the *tail score* variable and GLZ analysis of plumage coloration on the basis of sex, age and the interaction between sex and age for the remaining exposed variables in individual Bonelli's eagles *Aquila fasciata*.

[Análisis de la varianza (ANOVA) para la variable código de rectrices, y análisis GLZ de la coloración del plumaje en función del sexo, la edad y la interacción entre ambos (*sexo*edad*) para el resto de variables, en ejemplares de águila perdicera *Aquila fasciata*.]

	Sex		Age		Sex*age	
	F	p	F	p	F	p
Tail feathers code	27.99	< 0.0001	0.98	0.4066	1.37	0.2601
	Wald	p	Wald	p	Wald	p
Under-tail coverts	46.40	< 0.0001	0.43	0.9335	3.21	0.3593
Leg feathers	24.56	< 0.0001	2.56	0.4638	3.97	0.2643
Throat	24.91	< 0.0001	5.68	0.1282	3.34	0.3417

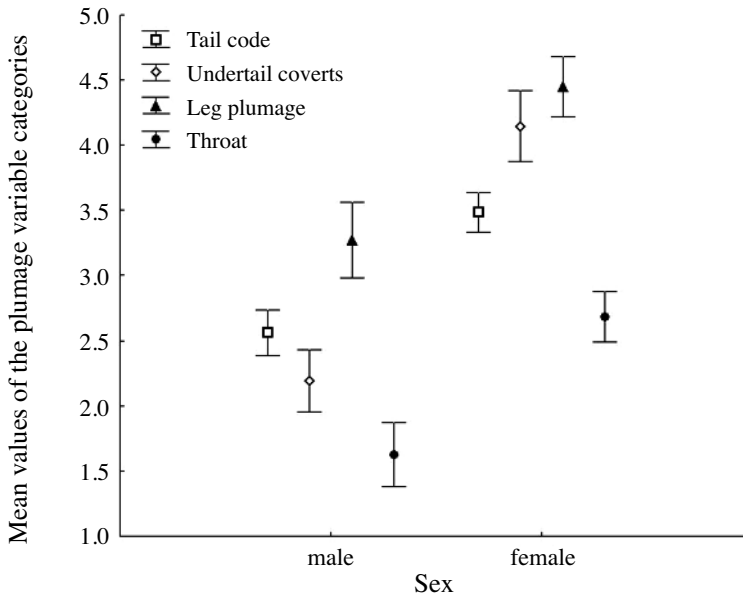


FIG. 3.—Mean values \pm 95% CI of the plumage variable categories studied for sexing Bonelli's eagle *Aquila fasciata* (1 to 5 for *undertail coverts* and *leg plumage*; 1 to 4 for *tail score*; and 1 to 3 for *throat*, see table 2).

[Valores de la mediana \pm 95% CI de las categorías (1 a 5 para *infracobertoras caudales* y *calzas*; 1 a 4 para *rectrices*; y 1 a 3 para *garganta*, véase tabla 2) de distintas variables del plumaje estudiadas para el sexado del águila perdicera *Aquila fasciata*.]

cycle phase (Zwarts *et al.*, 1996; Koenig *et al.*, 2005). Thus, there are fluctuations of about 10% in the body mass of individuals depending on the period of the year, as was detected by trapping the same individuals several times during different seasons (authors' unpublished data). The highest body mass values were registered in months prior to reproduction (October to February) and the lowest during spring-summer (May to August; Lima, 1986; Cox *et al.*, 2011).

Morphometric variables different from those usually used for in-the-hand studies (i.e. wing length, tarsus length, body mass; Barlein, 1995) can also be very useful for sexing individuals with certainty. In this case, Bonelli's eagle is one of the Palearctic species with greatest sexual size dimorphism and, for this reason, could offer discriminatory results based on analysis from a higher number of variables in comparison to other species (Arizaga *et al.*, 2008; Hermosell *et al.*, 2007).

Moreover, the existence of size differences between individuals from the different countries studied could reflect adaptive variation in response to conditions in their habitats (Laiolo and Rolando, 2001; Tieleman *et al.*, 2003). Although the delimitation of populations between countries does not fit biogeographical patterns (populations studied in the north of Portugal are contiguous with those in northwestern Spain), the general consideration of the three groups of birds by countries could indicate sub-regional differences. Among all variables, the head width and the body length differed in males and females between different countries, with the Portuguese birds being the largest. Why these variables, and not others, were indicative of differences could be due to adaptive traits related to habitat and prey selection by Portuguese populations (Palma *et al.*, 2006). The diet of Bonelli's eagle is centred on rabbits in Spain (Moleón *et al.*, 2009, 2012b; Caro *et al.*, 2011), whereas in southern Portugal the abundance of rabbits

in the study areas is very low and pigeons *Columba livia* constitute the main prey (Palma *et al.*, 2006). In the same way, the nests of the species in Spain and France are mostly located on cliffs, while in southern Portugal, due to limited availability of rocky areas, nests are principally found in trees (*Eucalyptus* spp., Palma *et al.*, 2006). Thus, our results showed that when analysing measurements of tree-nesting birds from the south of Portugal with respect to individuals in the other regions, together with significant differences in head width and body length, the tails of females were significantly longer ($F_{1,77} = 4.71, p = 0.033$). This could indicate that adaptation to forest environments and to capturing birds requires greater flight manoeuvrability and, for this reason, morphological differences could exist within western Mediterranean sub-populations. However, the philopatric patterns of the species show that there is fluent exchange of individuals between different Iberian and French regions (Real and Mañosa, 2001; Cadahía *et al.*, 2009, 2010; Hernández-Matías *et al.*, 2010), which would counter any morphological differentiation on the scale of the western Mediterranean region (Mayr, 1963; James, 1983; Senar *et al.*, 2006).

Colour patterns

Male Bonelli's eagles show a lighter tonality in the plumage of the studied parts, as generally found by previous studies (Parellada, 1984; Forsman, 2007). Nevertheless, differences between males and females regarding the plumage colour of the undertail coverts, legs, throat and tail have not previously been assessed precisely. Similar patterns of coloration have been found in closely related species such as the African hawk-eagle *Aquila spilogaster*, in which underparts of females are more heavily streaked than in males (Hockey *et al.*, 2006), or in

other whitish raptors such as the short-toed eagle *Circaetus gallicus* in which females are generally darker than males (Choussy, 1970, 1973; Joubert, 2001). It is also worth noting that quantifying the existing variability and assigning it to concrete categories is, in many cases, a subjective exercise. This being so, it is advisable to be cautious when assigning sexes to birds in the field, particularly when observation conditions are not optimal, since some colour categories may overlap between sexes.

Although the general plumage pattern of Bonelli's eagle differs between immature, subadult and adult cohorts (Parellada, 1984; Ferguson-Lees and Christie, 2006; Forsman, 2007), the acquisition of contrasting patterns between dark and light feathers does not vary with age. In the case of immature birds, the general coloration of the underparts, including the leg feathers, throat and undertail coverts, was more ochre or pale brown, and was considered as equivalent to the white tones of the adults for this study. The ochre or pale brown parts already contrast with the dark tones after the first body moults (Blasco-Zumeta, 2012).

In conclusion, the morphometrics and plumage colour patterns of non-juvenile Bonelli's eagles enable sexual identification both in the hand and in the field. These discrimination characters constitute a useful tool for the management and conservation of this endangered species and for an accurate implementation of research projects and monitoring programmes.

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