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Sex identification of Eurasian Scops Owl *Otus scops* using morphometric analysis

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Abstract

The Eurasian Scops Owl *Otus scops* is a migratory owl whose population is declining throughout a large part of its breeding range, due to intensification of farming practices and land-use changes. For this reason, it is considered the most threatened owl in Europe. Several raptor species like Scops Owl present limited possibility to differentiate between sexes, due to the reduced biometric and morphometric differences. The availability of a reliable method to sex Scops Owl through biometric measurements would facilitate the study of sex-related survival, movements and behavior characteristics of this species, improving conservation management projects. In the current study, we developed a cost-effective and accurate method of sexing Scops Owl, based on discriminant analysis of morphometry. One hundred and five birds were captured, sexed using genetic methods and biometric measures were taken. A GLM model was built to evaluate the biometric measures statistically linked to the sex. The best model (AIC = 49.211) selected three biometric measures: wing length, tail length, weight. The results of the model were used to derive models scores, and the predictive probability of male and female to be correctly identified was estimated. The discriminant function provides an accurate method of sexing Scops Owl in the hand.

INTRODUCTION

The Eurasian Scops Owl *Otus scops* (hereafter Scops Owl) is a migratory owl distributed as a breeding bird throughout the Southern Palearctic, from Portugal to Central Asia, with a Southern limit in North Africa; the species spends the winter period mainly in sub-Saharan Africa and, with limited numbers in southern Europe (Glutz von Boltzheim & Bauer 1980, Cramp 1985, del Hoyo *et al*. 1999). The species has shown widespread declines throughout a large part of its Western European breeding range, especially in Switzerland (Arlettaz 1990, Arlettaz *et al.* 1991), Spain (Martinez *et al.* 2007) and Northern Italy (Galeotti & Sacchi 2001, Treggiari *et al.* 2013), mainly due to intensification of farming practices and land-use changes (Sergio *et al.* 2009), so it was considered species of European Conservation Concern (BirdLife International 2004); but in the last years signs of local recovery (e.g. Caula & Beraudo 2014, Boano & Silvano 2015, Knaus *et al.* 2018) and even of spreading towards North (Mebs & Nicklaus 2014) have been detected.

In ecology, the identification of sex in natural populations is useful in both theoretical studies and practical applications (Arcese & Keller 2018). Survival and dispersal parameters can noticeably affect sex ratio (Székely *et al*. 2014), migration and wintering patterns (Brides *et al*. 2017), habitat selection (Ruckstuhl & Neuhaus 2006), mating systems and life history traits (Liker & Székely 2005, Liker *et al* 2014). It is well known that in several bird species the survival rate is higher in males (Lack 1966, Greenwood 1980), while natal and breeding dispersal are greater in females (Clarke *et al*. 1997). Sex-related differences have been highlighted also in some Strigiformes species in dispersion patterns (Hakkarainen 2002), winter distribution (Kerlinger & Lein 1986), and survival (León-Ortega 2016).

Several methods are available to sex monochromatic birds, i.e. laparotomy, behaviour observations and voice analysis (Galeotti *et al*. 1997, Bourgeois *et al*. 2007). Moreover, DNA-based tests have been successfully applied to sex identification in many species (Griffiths *et al*. 1998, Sacchi *et al.* 2004, Morinha *et al*. 2012, Çakmak *et al.* 2017).

Biometric measurements are commonly taken during ringing operations (Cucco *et al*. 1999, Scebba 2001, Gordo *et al*. 2017), however, in absence of a standardized protocol, it remains problematic to use morphometric criteria for sex determination.

This is true for several raptor species that present limited biometric differences, and no differences at all by plumage between sexes (del Hoyo *et al.* 1994, 1999). In European owls only brood patch is considered a useful sexing criterium in breeding period, whereas minor biometric measurements could be useful for some species, and color differences are limited to the genus *Asio* (Baker 2016). In particular, according to Cramp & Simmons (1985), only minor differences in bill length are observable in adult Scops Owl, whereas Martinez *et al*. (2002) state that, differences in

coloration between sexes have not yet been described, and that only the females can be identified during the breeding season by the presence of the brood patch.

One successful approach to sex monomorphic bird in field studies involves discriminant analysis using morphological measurements (Balbontin *et al*. 2001, Bertellotti *et al*. 2002, Rodriguez & Martinez 2016).

With the present study, we develop a cost-effective method of sexing Scops Owl, based on discriminant analysis of morphometrics. To have a correct attribution of sex, all samples collected were tested using a DNA molecular method, based on the analysis of two conserved CHD (chromo-helicase-DNA-binding) genes, namely CHD-W and CHD-Z, located on the sex chromosomes of all non-ratite species (Sacchi *et al*. 2004). A simple, non-invasive way to obtain a source of DNA in wild birds is feather collection (Griffiths & Tiwari 1995, Bello *et al*. 2001, Dai *et al*. 2015). Plucking feathers does not require special training and their easy storage allows biological sample preservation under most field conditions.

METHODS

Study area and field protocols

The study areas were located in Northern and Central Italy, near the northern limits of the Mediterranean climate zone, not far from the northern limits of the Scops Owl breeding range in Western Europe.

The northern one, the Special Area of Conservation and Special Protection Area "IT1180004 – Greto dello Scrivia" located in NW Italy, was in a riparian habitat along the Scrivia river between Villalvernia and Novi Ligure (Piemonte, Alessandria, 44º49'N, 8º50'E; 100–110 m a.s.l.). The habitat is an old gravel bed of the Scrivia which has been invaded by shrubby vegetation and scattered woodlands, and single trees with much dead wood (mainly poplars *Populus nigra* and oaks *Quercus robur*) on a well-drained soil.

The second area in Central Italy was located within the protected WWF Italy reserve "Laguna di Orbetello" (Tuscany, Orbetello, 42°28'N, 11°11'E; 0-2 m a.s.l.). It was located on an 8-km-long isthmus connecting the mainland with Mt. Argentario. Near the ringing area, there was an old Stone Pine (*Pinus pinea*) plantation, hay meadows with hedgerows and single trees such as Cork Oaks (*Quercus suber*) and Black Poplars, scattered buildings and a strip of Mediterranean scrubland along the coastline.

All the Scops Owls were captured as part of a national program established to ringing birds for population and migration monitoring in Italy (Spina & Volponi 2008).

Birds were captured in mist-nets scattered opportunistically in the habitat, all newly captured birds were ringed with metal rings, and aged, as adult or young (first calendar year), according to the criteria summarized by Demongin (2016). Five females captured during the breeding period (mid-May–June) were sexed according to the presence of a brood patch (Cramp 1985, Martinez *et al*., 2002).

For each of 105 sampled birds, we recorded the maximum length of the wing chord, eighth primary, bill from skull, tarsus and weight. From the same birds, feathers were collected as a source of genomic DNA. The feathers were stored at room temperature in 90% ethanol until analysis.

Genetic analysis

For all the feathers, genomic DNA extraction and molecular sexing method were performed as described in Sacchi *et al*. (2004). Besides, seven known-sex (three males and four females) Scops Owl samples of skeletal muscle from the tissue collection of the Museo Civico di Storia Naturale di Carmagnola were used as control specimens for the sexing protocol.

Biometric analysis

Descriptive analysis was carried out to derive indicators for each biometric measurement and their differences between sexes were analyzed using *t*-test.

Finally, a Generalized Linear Model (GLM) with binomial distribution was applied to select the biometric measures (predictors) significantly associated with sex (dependent variable). Model selection was done using a backward stepwise approach, and the best model was selected using the Akaike's Information Criteria AIC criteria (Akaike 1973).

The probability of correct classification for male and female and the model scores were derived and plotted together, to evaluate model performance in discriminating sex based on the selected biometric measures.

Descriptive analysis was carried out with the package "psych" (Revelle, 2019). GLM and t-test was carried out using the functionalities contained in the basic R package (R Core Team, 2018). Significant level was considered for $p < 0.05$.

Jackknife procedure was applied to evaluate the accuracy of each coefficient.
Average value of the coefficients (average), their confidence intervals (IC95), and standard error of the estimates (SE) are provided.

RESULTS

Genetic analysis

DNA was successfully amplified for all the reference samples. The amplicon analysis on agarose gel electrophoresis showed a unique band of about 380 bp in male and female (Fig. 1); after Asp700I enzymatic digestion, as suggested by Sacchi *et al*. (2004), males showed the band of 380 bp whereas the female amplicon was resolved into three bands (380, 280, and 100 bp) (Fig. 2). The restriction enzyme cuts CHD-W but not CHD-Z fragment. Results in reference samples validated the molecular sexing test in *Otus scops* species.

We collected feathers from 105 birds (33 juveniles, 68 adults and four not aged). Fifty-one birds resulted males and 54 females (five of which showed an evident brood patch). Among the juveniles, 19 were males and 14 were females. Among the adults, 30 were males and 38 females. Two males and two females not aged were discarded from the biometric analysis.

Biometric analysis

The descriptive indexes for the biometric measures, divided by male and female are presented in Table 1 for juveniles and in Table 2 for adults.

We did not find any significant differences in juvenile's biometric measures by sex (*t*-test). Instead, strong significant differences in adult's biometric measures by sex were found for the wing, eight primary, tail and weight (t-test).

Considering that biometric differences were not significant in juveniles, statistical analysis focused on adult subjects only.

A generalized linear model (GLM) was used to select the variables significantly associated with sex. The best model (AIC=49.211) selected three biometric measures: wing, tail and weight. The results of the GLM are presented in Table 3a.

Average value of the coefficients (average), derive from the jackknife procedure, their confidence intervals, and standard error of the estimates are provided in Table 3b.

The results of the model were used to derive model scores, and the predictive probability of male and female to be correctly identified was estimated. The model scores and the related predictive probabilities were plotted, to evaluate model performance and cut-off values to discriminate sex based on biometric measures (Fig. 3). A discriminant equation is provided below:

$$\text{Score} = 86.61138 + (-0.27561*\text{Wing}) + (-0.37167*\text{Tail}) + (-0.20153*\text{Weight})$$

A 90% probability to correctly classify females and males is reached respectively for a score value below -2.19 and above 2.0. Setting these cut-offs, 100% of males and females having a model score in this range were correctly classified. This corresponds to 53% of all the females in the sample and 42% of all the males. Using less strict parameters and using 80% probability of correct classification, 59% of the females are correctly classified with no mistakes, and 50% of the males but including a wrong classification of 1 subject (8% of the animals selected).

DISCUSSION

Our work is the first to show with genetically sexed birds that some biometric measures are certainly linked with sex and a discriminant function analysis (DFA) of wing, tail and weight can consistently sex, using an 80% cut-off probability, 55% of Scops Owls. In general, the model has better performance in classifying the females rather than the males.

The DNA technique of Sacchi *et al*. (2004) was applied to Scops Owl for the first time and unambiguously sexed individual birds, confirming its usefulness in different avian species.

The discriminant functions produced through morphometry provided an accurate method of sexing Scops Owl in the hand.

Our study highlighted that sex discrimination based on the biometric approach according to measurements of wing, eight primary, tail and weight is possible and accurate only for adults, analyzed during spring and summer, as we only tested birds trapped from April to August. Birds captured just before or during migration could be heavier due to the important fat reserves, so including weight in the DFA needs to be controlled in other seasons. Contrary to the expectations, we did not find yet any significant sexual difference in our population in bill length observed in other samples (Cramp 1985).

It is worth noting that our sample is from a limited area of the Scops Owl breeding range and mainly from the northern boundary of the western part of the range. Although the biometric variations among Scops Owl European populations seem very little or negligible (Cramp 1985), it is known that also in species with relatively small geographic variation the discriminant function calculated for birds of different geographical origin could mis-sex different percentages of individuals (Palomares *et al.* 1997). So, we would suggest that applications of similar studies to other Scops Owl populations or in other periods of the year would be useful to better define the applicability of this sexing method.

The ability to determine the sex will be valuable in future studies addressing inter-sexual differences in migration patterns and winter distribution.

In conclusion, we propose a simple system to sex Scops Owls to be tested in other regions and seasons. The discriminant analysis described here uses only three variables, which are easily measured in the field, and provides classification with a high level of accuracy.

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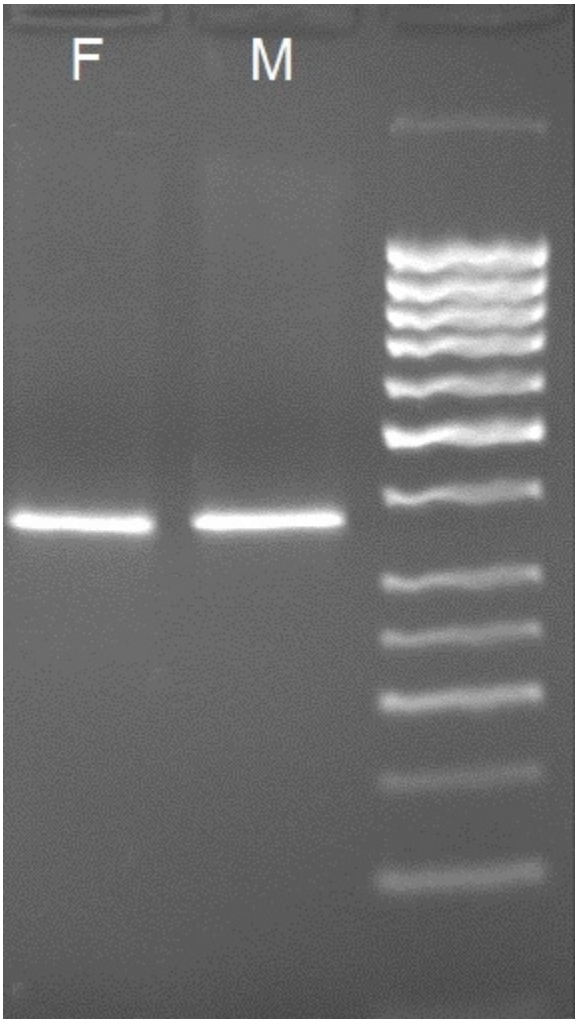

Figure 1. PCR product with P2-P8 PCR products were analysed by agarose gel electrophoresis 2% in TBE and visualised under UV light after ethidium bromide staining.: F, female; M, male; the last line is GeneRuler 50 bpDNA Ladder size standard, a unique band of about 380 bp results in all samples.

75x134mm (96 x 96 DPI)

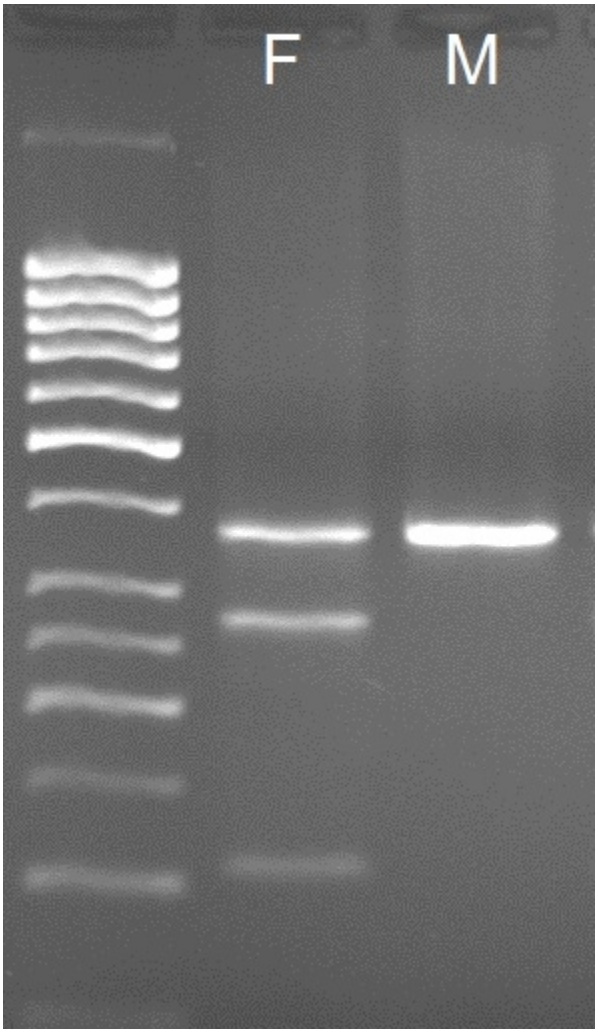

Figure 2. RFLP Asp700I Digestion products. Digestion products were analysed by agarose gel electrophoresis 2% in TBE and visualised under UV light after ethidium bromide staining.: F, female; M, male; the last line is GeneRuler 50 bpDNA Ladder size standard, an unique band results in male and three bands in female (380bp,280bp,100bp approximately).

78x135mm (96 x 96 DPI)

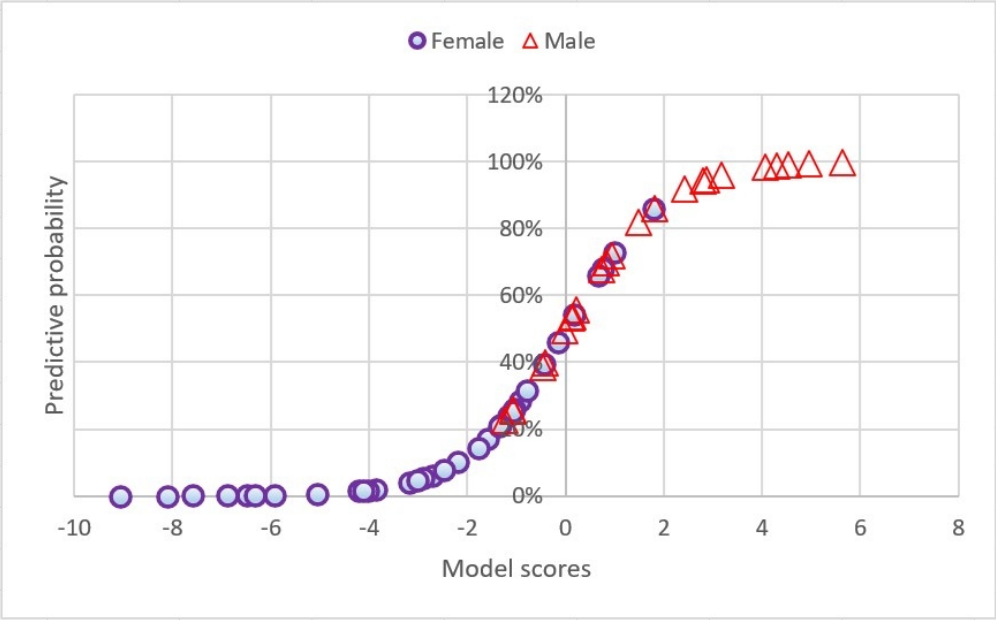

Figure 3. Model scores and probability of correct classification by sex.

229x143mm (96 x 96 DPI)

Variable	Sex	Mean±sd	Range	N	*t*-test
Wing	F	157.5±7.1	140.0-167.0	14	0.880 (P = 0.3909)
	M	155.7±3.4	148.0-162.0	19	
8th primary	F	117.6±7.4	94.0-125.0	14	1.319 (P = 0.2048)
	M	114.8±3.3	105.0-119.0	19	
Tail	F	72.1±5.7	61.0-81.5	14	1.332 (P = 0.2017)
	M	69.9±2.2	64.0-73.0	19	
Bill	F	16.4±0.6	15.5-18.0	14	1.625 (P = 0.1148)
	M	16.0±0.7	15.0-17.5	19	
Tarsus	F	27.9±1.5	25.6-31.3	14	0.478 (P = 0.6363)
	M	28.1±1.6	25.2-31.5	19	
Weight	F	85.9±6.6	76.0-99.8	14	1.838 (P = 0.0757)
	M	81.0±8.7	60.5-99.0	19	

Variable	Sex	Mean±sd	Range	N	*t*-test
Wing	F	161.9±3.6	155.0-169.5	38	**5.892 (P < 0.0001)**
	M	156.4±4,0	150.0-166.0	30	
8th primary	F	119.1±3.1	112.0-127.5	36	**4.467 (P < 0.0001)**
	M	115.5±3.2	110.0-121.0	29	
Tail	F	71.4±2.3	68.0-76.0	37	**3.407 (P = 0.0014)**
	M	68.8±3.4	60.0-76.0	27	
Bill	F	16.5±0.9	15.0-19.0	35	0.908 (P = 0.3680)
	M	16.4±0.7	15.0-17.7	23	
Tarsus	F	27.6±1.9	23.8-31.0	38	0.634 (P = 0.5286)
	M	27.3±2,0	23.7-32.0	30	
Weight	F	92.0±11.2	75.3-120.0	38	**6.208 (P < 0.0001)**
	M	79.2±5.5	68.0-92.0	30	

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	86.6	26.44	3.28	0.001
Wing	-0.3	0.12	-2.26	0.024
Tail	-0.4	0.20	-1.87	0.061
Weight	-0.2	0.08	-2.62	0.009

Coefficients	Average	95% confidence interval		SE
		Lower Bound	Upper Bound	
Intercept	86.91	86.41-	87.41	0.5014
Wing	-0.28	-0.28	-0.27	0.0024
Tail	-0.37	-0.38	-0.37	0.0041
Weight	-0.20	-0.20	-0.20	0.0013