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Discriminant Criteria for Field Sexing in the Eurasian Tree Sparrow by Combining Body Size and Plumage Features

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Simple Summary: We provided basic morphological information on Eurasian Tree Sparrow from the Iberian Peninsula, and sexed a sample of individuals using molecular methods, with the aim of deriving sexing criteria in the field. The use of two measurements, wing length and badge width, allows the correct sexing of more than 90% of the tree sparrows in the Iberian Peninsula using only the usual tools used in ringing programs.

Abstract: The Eurasian tree sparrow (*Passer montanus*) is a monomorphic passerine, for which it is impossible to differentiate between males and females based on external characteristics. Being a species frequently captured for ringing, having a reliable method to determine sex from conventional biometric measurements would facilitate its study and be very useful for the correct management and conservation of this declining species. In the present study, we used biometric measurements recorded in 66 individuals captured with mist nets in communal roosts in northern Spain during the winter and sexed them using molecular techniques. We conducted a discriminant function analysis (DFA) to derive equations that allowed us to determine the sex of the specimens from some of the measurements recorded in the field. Significant differences were found between males and females in wing length, third primary length, badge width and height and body weight. The DFA provided two functions that correctly classified the sex of 94.7% of the individuals using wing length and badge width, and 98.2% if weight was added to the analysis. Our results allow sexing from measurements that can be easily recorded in the field with the tools commonly used in banding sessions and without the need for additional training. Considerations of Bergmann's and Allen's rules on body size and the use of DFA in different populations are discussed.

Keywords: biometry; DFA; genetic; ringing; sexing

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1. Introduction

Sex determination is essential for the study of population dynamics in birds [1]. Discriminant functions capable of classifying the sex of monomorphic species have been used in studies of survival [2], breeding success [3], migratory patterns [4,5], roosting dynamics [6] and habitat use [7]. With this aim in mind, several morphological traits of captured birds are regularly measured during ringing activities.

Distinguishing between males and females is easy in species with clear size and plumage differences [8], but it is challenging in apparently monomorphic species. In these cases, other traits, such as the presence of an incubation patch, may be useful during the breeding season [9]. Recently, the use of universal molecular markers for sex determination has facilitated sexing in these species [10]. However, molecular sexing first requires specific training, ethical permissions and legal authorizations for blood sample collection that not all ringers have [11]. Molecular methods require analyses in specialized laboratories, and analyses involve the costs of consumable materials in the field and laboratory consumables, analytical equipment and personnel costs that are not negligible, especially when large numbers of samples are taken and analyzed.

Plumage features are being increasingly considered for age and sex determination in studies of monomorphic species [4,5,12–14]. Combining morphometric traits with the size, shape and color of plumage patches can be useful for sex determination in studies of apparently monomorphic species [13,15,16]. This requires previous sexing by means of molecular methods, and validation with discriminant functions that can subsequently be applied to individuals without the need for additional molecular sexing.

The Eurasian tree sparrow (*Passer montanus*) is considered a monomorphic species, for which it is impossible to determine the individual sex by external traits outside of the breeding season [9]. This species has a very wide distribution area, ranging from the Iberian Peninsula to Japan [17], where seven subspecies are accepted based partially on morphometries [17–19]. In recent decades, this species has suffered an important population decline at the European level [20] associated with changes in agricultural practices [18,21]. However, there are regions where populations have remained stable [22] and others where they have not declined, but have increased both in numbers and range [23]. This suggests that future studies may require practical field sexing tools to deepen the understanding of the factors responsible for these regional differences in population dynamics. Although geographic variation hampers universal sexing by morphometry, previous studies found some biometric differences between the sexes in each particular subspecies. In general, males of this species show a bigger wing length [15,16,24,25], badge area [15,16,25–27], tarsus length and body weight than females [15,16]. However, only two of these studies [15,16] performed a discriminant function analysis (DFA) for sexing.

In this paper, we provided basic morphological information on the Eurasian tree sparrow from the Iberian Peninsula, corresponding to the nominal subspecies (*P.m. montanus*), and sexed a sample of individuals using molecular methods, with the aim of deriving sexing criteria in the field. We explore whether combining body size traits with features of plumage badges can improve the discriminatory ability of the derived sexing functions.

2. Materials and Methods

2.1. Study Area

Fieldwork was carried out in "La Roza", an area of 40 ha mainly devoted to pear orchards (65%) and poplar plantations (25%) located next to the Ebro River and 3 km from the village of Alfaro, northeastern Spain (42°11′ N, 1°43′ W; Figure 1). This area is inhabited by an abundant tree sparrow population from the nominal subspecies [17] nesting in nest boxes (n = 70) installed in 2015 as part of a pear pest control program. During winter, these birds roost in small reed beds (*Phragmites australis*) that are interspersed between pear orchard fields.

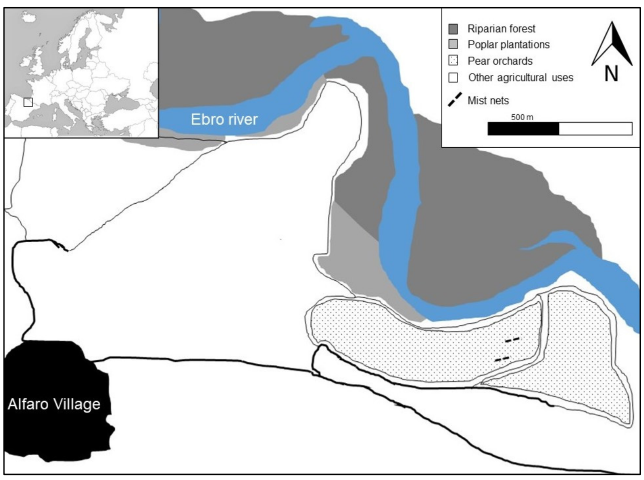

Figure 1. Study area and main land uses in the environment.

2.2. Field Data Collection

During the winter of 2019-20, tree sparrows (n = 66) were captured by mist netting [28] in communal roosts. Once captured, the birds were fitted with an official numbered metal ring and a colored plastic ring of the same diameter (http://www.cr-birding.org/sr/node/4586) (accessed on 1 January 2020). Morphometric variables recorded for each individual included weight (to the nearest 0.1 g), as measured with a digital precision balance (ProScale LC-300), maximum wing length (to the nearest 0.5 mm), length of the 3rd primary feather (to the nearest 0.5 mm) with a specific ruler and tarsus length (0.2 mm) with a digital caliper. All these measurements were taken as described in Svensson [9]. In addition, the width and height (0.2 mm) of the black throat badge were measured with a digital caliper (Figure 2). All measurements were made by the same specific ringer (SG), whose high repeatability in taking biometric measurements is certified by the Aranzadi Science Society, as described in Senar [29]. The degree of subcutaneous fat deposition was scored on a scale from 0 (total absence of fat) to 8 (inter-clavicular, abdominal and pectoral region completely covered by fat) [30]. Blood samples (approximately 0.1–0.2 mL) were obtained from jugular venipuncture under license from the relevant administrative body (Government of La Rioja; permit number 00860-2019/138984 O), and stored in absolute ethanol. The total time spent handling an individual bird never exceeded 15 min.

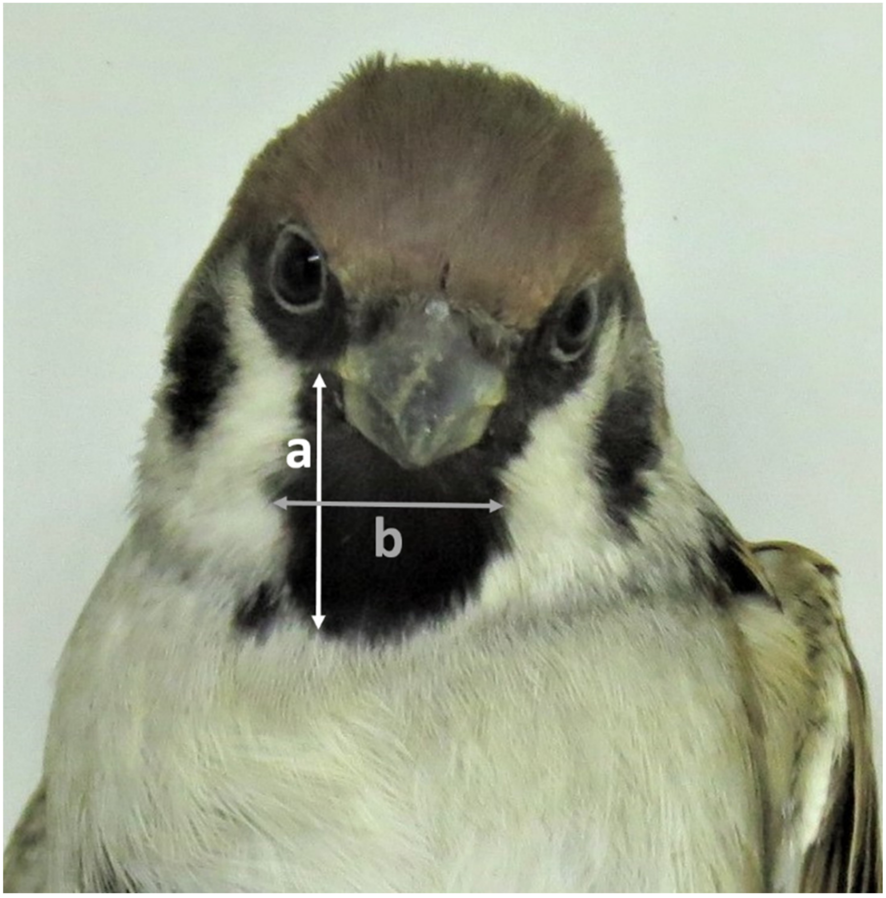

Figure 2. Measurement points for the height (**a**) and width (**b**) of the badge of the Eurasian Tree Sparrow.

2.3. Molecular Analyses

DNA was isolated from blood using the Quick-DNA Miniprep Kit (Zymo Research) according to the manufacturer's protocols. Molecular sexing was performed by polymerase chain reaction (PCR) using specific primers (P2 5′-TCTGCATCGCTAAATCCTTT-3′ and P8 5′-CTCCCAAGGATGAGRAAYTG-3′) for partial amplification of the chromodomain helicase DNA-binding protein 1 (CHD1) gene [31]. PCR was performed in 10 μL of reaction mixture containing 5 µL of 2 × MyTaq HS Mix (Bioline, Memphis, Tennessee 38134-5611 USA), 2.5 µM of each primer and ~20 ng of template DNA. The amplification protocol was composed of the following steps: 95 °C for 5 min followed by 35 cycles at 95 °C for 30 s, annealing at 50 °C for 1 min, 72 °C for 30 s and a final extension at 60 °C for 10 min.

2.4. Statistical Analyses

Prior to analysis, data distribution was tested according to a Shapiro–Wilks test for normality. As the values of all biometric measurements followed a normal data distribution ($p > 0.05$), we used one-way ANOVA to detect sex differences in morphological traits. A forward stepwise discriminant function analysis (DFA) was conducted to derive functions with the lowest percentage of misclassification of known-sex individuals. A jackknife procedure was used to test the discriminant functions derived from the total sample by excluding the individual being classified. Statistical analyses and checking of model assumptions were performed using SPSS software v. 26 (IBM SPSS Statistics, Portsmouth, UK).

Finally, we tested the validity of the formula proposed in a previous study with specimens from Hungary (D = 0.65 × WL + 0.32 × BW − 53.10) [15] to determine the sex of the birds in our population.

3. Results

A total of 66 tree sparrows were measured and sexed by molecular methods (34 females and 32 males). Significant differences between males and females were found in wing length, length of the third primary, badge width and height and body weight (Table 1). No specimen showed subcutaneous fat deposits above a 1 on the Kaiser scale [30], so this parameter was not taken into account in the analyses.

Table 1. Values (mean ± SD) of the morphometric variables registered in male and female Eurasian Tree Sparrows, and one-way ANOVA statistics of the comparison between sexes.

Traits	Males (n = 32)	Females (n = 34)	Statistics
Wing length (mm)	69.19 (±1.52)	66.41 (±1.49)	F = 67.6, df = 1, $p < 0.001$
3rd primary (mm)	53.22 (±1.22)	51.26 (±1.54)	F = 32.24, df = 1, $p < 0.001$
Tarsus length (mm)	17.35 (±0.69)	17.18 (±0.81)	F = 0.64, df = 1, $p = 0.41$
Badge width (mm)	10.53 (±0.76)	8.96 (±0.49)	F = 85.91, df = 1, $p < 0.05$
Badge height (mm)	12.82 (±1.93)	11.04 (±1.83)	F = 13.44, df = 1, $p < 0.001$
Weight (g)	21.05 (±1.26)	19.96 (±0.99)	F = 13.51, df = 1, $p < 0.001$

The DFA provided two functions that correctly classified more than 90% of the individuals (Figure 3).

The first, which includes wing length and badge width, correctly classified 94.7% of the birds from the following function, where D is the discriminant score:

D = 0.381 (wing length) + 1.160 (badge width) − 37.203.

Sparrows with positive scores were classified as males (group centroid = 1.397), and those with negative scores were classified as females (group centroid = −1.447).

An additional function adding weight correctly classified 98.2% of the individuals from the following function:

D = 0.289 (wing length) + 1.219 (badge width) + 0.315 (weight) − 37.977.

Sparrows with positive scores were classified as males (group centroid = 1.502), and those with negative scores were classified as females (group centroid = −1.556).

The formula proposed in a previous study from Hungary [15] correctly classified all our females, but only 6.25% of our males (2 out of 32).

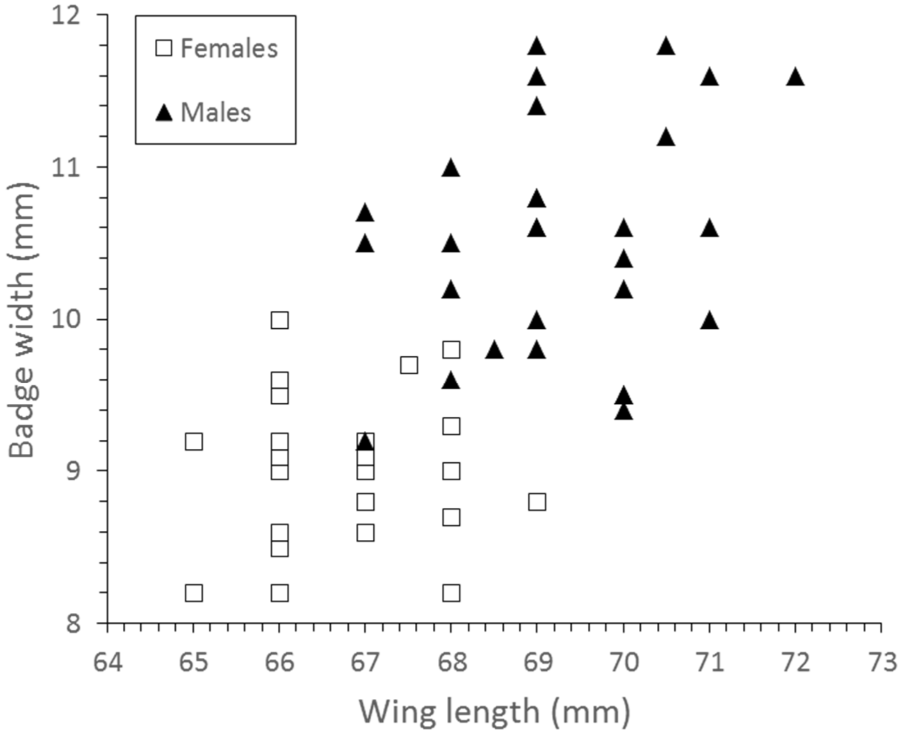

Figure 3. Morphological distribution of female and male Eurasian Tree Sparrows defined by the wing length and badge width.

4. Discussion

Our results confirm that male Eurasian tree sparrows in northern Spain have bigger wings and badges than females, in agreement with previous studies [15,16,24,29]. The biometry of the population under study was very similar to that described in Korea [16], despite corresponding to two different subspecies, *P. m. montanus* and *P. m. saturatus* [16]. In fact, the two formulas resulting from our DFA correctly classified sex when applying the mean values recorded in Korea [16] (wing length = 69.62 for males and 67.07 for females, badge width of 10.98 mm in males and 9.79 mm in females and weight of 21.43 g in males and 20.72 g in females). However, individuals from our population appeared to be smaller regarding wing length than those studied in Hungary [15] and Finland [24], where the values found for the same parameter were 70.1 and 70.3 mm in females and 73.0 and 73.3 mm in males, respectively. Due to these differences, the sexing criteria for Hungarian specimens are not applicable to our population in the Iberian Peninsula, since practically all Iberian individuals would be classified as females.

This smaller size of specimens from southern populations living in more temperate areas compared to those from northern ones, known as Bergmann's rule, is common in widely distributed species, including sparrows [32], and prevents the translation of reference values from one population to another [33]. A similar situation could occur when comparing populations from natural environments with others from peri-urban environments since, according to Allen's rule, specimens from urban environments could have shorter wings, as a consequence of a higher ambient temperature [34]. Given that the population studied in this work inhabits a natural environment, it could be that its biometry differs from that existing in other populations located in gardens in towns and cities, where the species is also present [35]. In these situations, simultaneous analysis of more than one measure using DFA can allow sex distinction even in species where there are marked biometric differences between populations [36]. In our case, the two DFAs performed allowed us to correctly classify 94.7% of the birds using wing length and badge width, and 98.2% if we add weight to the analysis. Both models provide an acceptable means of determining sex, but the first option may be more applicable even though the percentage of correctly sexed birds was slightly lower. This suggestion is based on the fact that weight can vary markedly throughout the year or even the day [37] or depending on the degree of urbanization of the occupied habitat [38], and therefore could lead to incorrect classifications without standardization and consideration of these sources of variation, whereas wing length and badge width are more stable over time. Moreover, these two measurements are easy to take in the field, show high repeatability and are uncorrelated, which makes them suitable for DFA [39].

The fact that it is precisely these two variables that allow tree sparrows to be sexed is not surprising, as it is common for males of passerine species to have longer wings than females [39], while the bib is a typical secondary sexual trait [40], which appears only in males in most species [9]. Previous studies measured bib size from digitized photographs [15,16,41,42] or transparent celluloid [28], which can provide more information about shape and color, but require the use of instruments not common in conventional ringing programs [42] and image processing software that requires prior training in its use [43]. Furthermore, in order to analyze the photographs correctly, they must have been taken with the bird in a standardized posture [41] and with fixed exposure and illumination values [43], which is not always possible in the field. In contrast, badge width can be measured with a conventional caliper and does not require extra training. In fact, our study correctly sexed birds with 94.7% accuracy, which is slightly higher than the value obtained previously [16] (86.9%), when the bib surface was calculated from photographic images.

In conclusion, the use of these two measurements, wing length and badge width, is a suitable method that allows the correct sexing of more than 90% of the tree sparrows in the Iberian Peninsula using only the usual tools used in ringing programs. Future work with other populations will allow us to check whether the formula obtained in this study can be used in populations in other latitudes or in urban environments.

Author Contributions: S.G., L.G. and D.V. formulated the research question and carried out the fieldwork. The laboratory work was carried out by F.M. Statistical analyses were performed by G.B. D.V. wrote the initial draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The capture and handling of the birds, as well as the acquisition of the blood samples, were carried out following the legislation in force in Spain and with the authorization of the Government of La Rioja (file number 00860-2019/138984 O).

Data Availability Statement: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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