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INTERSEXUAL SIZE AND PLUMAGE DIFFERENCES IN TREE SPARROWS (*PASSER MONTANUS*) – A MORPHOLOGICAL STUDY BASED ON MOLECULAR SEX DETERMINATION

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We investigated intersexual morphological differences in tree sparrows (*Passer montanus*), a species being considered as sexually monomorphic. Molecular sexing of the birds was performed by PCR amplification of the sex chromosome-linked CHD1 gene introns. All measured traits (body weight, wing, tail and tarsus length, bill size and the size of the black throat patch, i.e. badge hereafter) were greater in males than in females and the sex of about 90% of the individuals was correctly categorized by means of a discriminant analysis based on the morphological measurements. Nevertheless, wing length alone was equally good predictor of the sex. Other measured traits had only moderate discriminant value. Our results do not support that tree sparrows can be sexed based on the size of their badge alone. However, some of our results suggest intersexual differences in the function of the badge.

Keywords: intersexual differences, morphology, sex determination, tree sparrow, *Passer montanus*

INTRODUCTION

European tree sparrow (*Passer montanus*) is typically considered as a sexually monomorphic species. Sexes may only be distinguished by behavioural traits (CRAMP & PERRINS 1994, SUMMERS-SMITH 1995) or in breeding season by the shape of the cloacal region and the presence or absence of the incubation patch (SVENSSON 1992). No other differences are referred in handbooks (SVENSSON 1992, SUMMERS-SMITH 1995), except for CRAMP and PERRINS (1994) that refers to slight differences in coloration. However, several authors mention differences in morphological traits between the sexes (Table 1). Some studies (CORDERO 1992, PINOWSKA *et al.* 1998) also claim that sexes may be identified based on plumage characteristics only. In this study we determined the sex of birds caught outside the breeding season by means of analysing genes on the sex chromosomes; and inves-

Table 1. Traits found differing between sexes of tree sparrow in the literature.

Sampled population	Traits differing between sexes	Reference
P. m. montanus		
Germany	wing length, tail length, tarsus length, body weight	CLAUSING & CLAUSING (1976)
Germany	sternum length, keel length, ulna length	ST. LOUIS & BARLOW (1991)
North America	sternum depth	ST. LOUIS & BARLOW (1991)
Spain	badge shape, badge size, malar stripe brightness	CORDERO (1992)
Switzerland	tarsus length, body weight	HEEB (2001)
Hungary	wing length, tail length, tarsus length, bill size, body weight, badge size	this study
P. m. saturatus		
South Korea	wing length, badge size	PINOWSKA *et al.* (1998)

tigated intersexual differences in several morphological traits. We also investigated the potential use of morphological traits in sex identification in this species.

Furthermore, we determined the morphological correlates of the size of the badge, i.e. the black throat patch that is present in both male and female tree sparrows. Black and other melanin based colour patches frequently have status signal role in birds (SENAR 1999). While the black throat patch in the males of the closely related house sparrow (*Passer domesticus*) was extensively investigated (e.g. MØLLER 1988, LIKER & BARTA 2001, MCGRAW *et al.* 2003, RINGSBY *et al.* 2009), we have scarce knowledge of the function of badge size in tree sparrow. The size and the correlates of the badge of the tree sparrow have rarely been investigated (CORDERO 1992, PINOWSKA *et al.* 1998), and the only study investigating its potential signal role also found controversial results (TORDA *et al.* 2004).

MATERIALS AND METHODS

Tree sparrows were captured by mist netting in the Botanical Garden of the University of Debrecen (Hungary), in October and November 1999, after the post-breeding moult. Measurements were taken as follows: body weight by Pesola spring balance to the nearest 0.5 g, tarsus length by calliper to the nearest 0.1 mm, length and width of badge in a standardised holding position to the nearest 1 mm. The badge of birds was also photographed from the front view; a calliper was used as a size standard. Before release badge measurements and photographing was repeated. Badge size was characterised with four measures: length, width, length*width (badge of tree sparrows is quasi-rectangular) and the area measured from the digitalized photographs using Image J 1.24 for Linux software (see Fig. 1). Results for all four variables were similar, and throughout this study we will use badge area measured from photos, the most repeatable one (r = 0.79, $F_{72,73}$ = 8.33, p < 0.001; following LESSELLS & BOAG 1984). Wing length, tail length, length, height and width of bill were also measured to explore morphological differences between sexes. Repeatabilities for all measurements were significant and ranges from 0.51 to 0.76 (p < 0.014 for all cases; based on 34 recaptures). Z.B. made all measurements after capture; F.M. made badge area measurements from the photos.

In order to sex individuals, blood samples (10–50 µl) were taken by brachial venipuncture. Blood was transferred and stored as in BRUFORD and SACCHERI (1998), total DNA was isolated as in MANIATIS *et al.* (1982). The sex of birds was determined by PCR-based amplification of the sex-chromosome linked chromodomain helicase DNA binding protein 1 (CHD1; GRIFFITH & TIWARI 1995), using the PCR conditions (with minor alterations) and primers (2550F and 2718R) developed by FRIDOLFSSON and ELLEGREN (1999). These primers are specific for the CHD1 gene, amplifying a larger intron sequence (CHD1-Z, 670bp) in both sexes and a smaller one (CHD1-W, 450bp) in females only. PCR products were separated in 1% agarose gels and after visualisation with ethidium bromide, the samples were evaluated for sex determination. Sex identifying procedure was repeated in 32 individuals. 3 individuals were recaptured in the breeding season and sexed based on morphological traits (SVENSSON 1992). No inconsistent results were found in sex identification.

Standard statistical tests (t-test, F-test) were used to analyse differences in means and variances between sexes. Linear discriminant analysis with 1:1 a priori sex ratio and leave-one-out classification was used in order to classify the sex of individuals based on morphological measurements following BOSCH (1996). Linear covariance analysis (ANCOVA) was used to investigate covariates of badge size. Both in discriminant analyses and ANCOVA all measured variables were entered in the first step and backward stepwise procedure with p = 0.1 removal criteria was used to identify relevant variables. All measured variables reached or approached normal distribution. Statistical analyses were performed using PASW Statistics 18.0 for Windows.

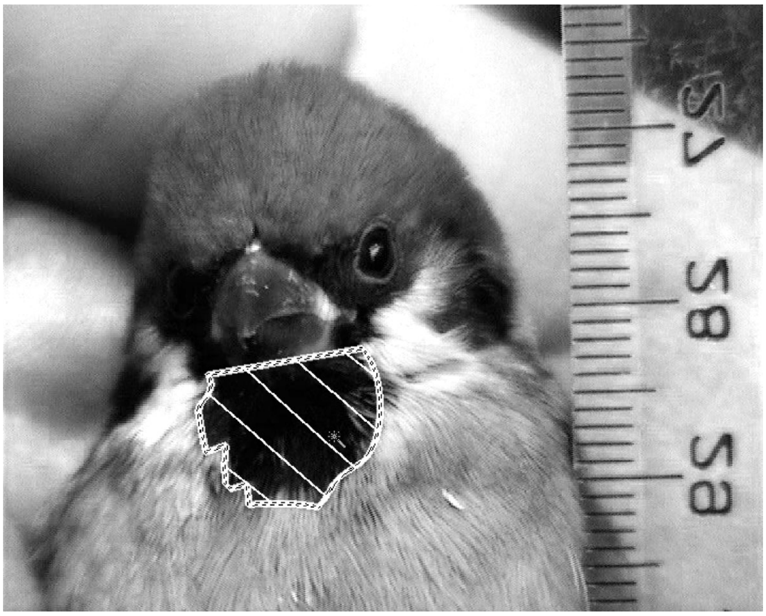

Fig. 1. A photography of the badge used for measurements; hatched area shows the badge area as measured with the image analyzing software (see text)

RESULTS

The study is based on measurements of 54 male and 58 female adult tree sparrows. All measured traits differed significantly between sexes, males were heavier, had longer tarsus, wing chord and tail, larger bill and badge (Table 2). Although, there was an important overlap in the ranges of traits between sexes (Table 2), a stepwise discriminant analysis based on the measured morphological traits correctly classified 87.0% of males and 89.7% of females (88.4% in total). Wing length alone was the best predictor of sex and allowed to correctly classify 90.2% of individuals; other measurements did not contribute to better sex identification based on morphology (Table 3). Badge area alone was sufficient to classify only 64.3% of the individuals (Table 3).

Table 2. Means and coefficient of variation (CV%) of morphological characteristics of tree sparrows (*Passer m. montanus*) in a Central European population in 1999, and statistics of comparison of range, mean and variance of the measured traits between sexes.

	wing length	tail length	tarsus length	bill length	bill width	bill height	body weight	badge size
Mean[a]								
Males	73.0	58.3	18.94	8.16	5.74	6.17	21.3	102.5
Females	70.1	56.5	18.66	8.01	5.61	6.09	20.4	86.8
Total	71.5	57.4	18.79	8.08	5.67	6.13	20.8	94.4
CV %								
Males	1.76	2.76	2.96	3.99	4.47	4.38	5.07	23.74
Females	2.21	2.87	2.92	4.15	4.35	3.84	5.69	19.89
Total	2.82	3.20	3.02	4.16	4.55	4.14	5.83	23.65
Comparison between sexes								
Ranges								
Overlap (%)[b]	21.4	62.5	78.6	86.7	85.7	73.3	66.7	55.7
Means								
t statistic	10.29	5.981	2.801	2.383	2.795	1.986	4.762	3.961
p-value[c]	**<0.001**	**<0.001**	**0.006**	0.019	**0.006**	0.050	**<0.001**	**< 0.001**
Variances								
F statistic	0.683	0.981	1.059	1.041	1.105	1.255	0.870	1.987
p-value[c]	0.923	0.528	0.415	0.442	0.354	0.198	0.700	**0.005**

Sample size equals 54, 58 and 112 for males, females and for all birds, respectively.
[a]Body weight is shown in g, badge size in mm^2, all other measurements in mm.
[b]100 × overlap of ranges of sexes / total range.
[c]P-values with bold font remain significant at $p = 0.05$ level after Bonferroni correction (for the eight comparison: $\alpha = 0.0063$)

Table 3. Accuracy of sexing tree sparrows by means of discriminant analysis using single measurements or combined functions.

measured trait	Wilks' λ	p-value[a]	cases correctly categorized	
			males	females
wing length (WL)	0.494	<0.001	90.7% (49/54)	89.7% (52/58)
tail length	0.755	<0.001	74.1% (40/54)	74.1% (43/58)
body weight (BW)	0.838	<0.001	74.1% (40/54)	60.3% (35/58)
badge area (BA)	0.878	<0.001	61.1% (33/54)	67.2% (39/58)
tarsus length	0.933	0.006	63.0% (34/54)	72.4% (42/58)
bill width	0.934	0.006	68.5% (37/54)	58.6% (34/58)
bill length	0.951	0.019	64.8% (35/54)	65.5% (35/58)
bill height	0.965	0.050	55.6% (30/54)	60.3% (35/58)
Combined functions				
D_1=0.65×WL+0.32×BW–53.10	0.461	0.006	85.2% (46/54)	84.5% (49/58)
D_2=0.63×WL+0.26×BW+1.11×BA–51.41	0.448	0.078	87.0% (47/54)	89.7% (52/58)

[a]Significance of F-test to remove one variable (in the cases of combined functions the last variable during the stepwise procedure)

The variance of badge area was greater in males than in females, while the variance of no other measured variable differed between sexes (Table 2). Among the measured morphological traits, tail length and body weight positively, while bill length negatively correlated with the badge size (Table 4).

DISCUSSION

The principal aim of this study was to investigate the intersexual morphological differences in the European Tree sparrow. We found significant difference in all measured traits between the sexes. The smallest overlap in the ranges of trait

Table 4. Morphological traits correlating with badge size in Tree Sparrows (ANCOVA; sex, each measured traits and its interactions with sex included in the 1st step, then effects that did not reach p = 0.1 level were excluded stepwise; minimal model is shown).

	df	Mean square	F-value	p	ß±SE
tail length	1	0.246	6.522	0.012	0.245±0.012
bill length	1	0.316	8.372	0.005	–0.248±0.057
body weight	1	0.272	7.210	0.008	0.247±0.017
sex	1	0.126	3.343	0.070	–0.184± 0.045
error	107	0.038			

size occurred in the case of wing length, while other traits highly overlapped between sexes. By means of discriminant analysis, we were able to correctly categorize the sex of about 90% of the individuals. Wing length had a primary role in the categorization as expected from the traits size overlap between sexes. Entering additional morphological traits in the discriminant analysis (others than wing length, including badge size) did not increase the number of correctly categorized individuals.

Our results contradict some previous studies (CORDERO 1992, PINOWSKA *et al.* 1998), in which the authors mention the possibility of sexing individuals based on plumage characteristics, e.g. badge size. In our study, the sex of only 64% of individuals could be correctly predicted based on badge size alone. However, one of the above mentioned studies (PINOWSKA *et al.* 1998) investigate the *Passer m. saturatus* subspecies.

In the literature, all studies comparing the size of traits among sexes refer to some differences (Table 1), however, CLAUSING and CLAUSING (1976) investigating three German population found no difference between sexes in tail length, tarsus length, bill length and body weight in some cases. All these results suggest that tree sparrow males are usually larger than females, however, differences may be small in some population (see CLAUSING & CLAUSING 1976).

We also found that the variance of badge size was greater in males than in females, which may indicate intersexual differences in the function of badge size. More importantly, the variance of the other traits did not differ between the two sexes. Similarly, MØLLER (1991) found greater mean size and variance in males that in females in the case of the tail streamer size in Barn Swallows (*Hirundo rustica*), where the size of tail streamers acts as a quality signal towards females during mate choice. MØLLER concludes that the main raison for these differences between males and females in the case of a signal may be sexual selection caused by female preference.

Based on our results, we cannot conclude that badge size may have a signal role in tree sparrows. The only study to date that investigated the potential signal role of badge size in tree sparrows (TORDA *et al.* 2004) did not identify the sex of experimental birds. They found significant positive correlation between badge size and dominance in one of the three experimental flocks. Any intersexual difference in the function of badge size – our results suggest that some may exist – might hide the correlation between badge size and dominance in the study of TORDA *et al.* (2004). In accordance, our results emphasize the importance of working with individuals of known sex in behavioural studies investigating this species.

In the case of the house sparrow, a close relative, only males possess and use the black throat patch as a signal (MØLLER 1988, LIKER & BARTA 2001). Although, in the tree sparrow both sexes possess the black badge, house and tree sparrow were hypothesised to originate in a common ancestor having black throat patch in males and having no throat patch in females (SUMMERS-SMITH 1995). The two species are also known to be very similar ecologically and behaviourally and the close genetic relationship is supported by several cases when mixed pairs of tree and house sparrows produced and fledged hybrids (SUMMERS-SMITH 1995). Furthermore, CORDERO *et al.* (2002) found that the sexually monomorphic tree sparrow and the dimorphic house sparrow did not differ in the frequency of extra-pair fertilisation (EPF), although, EPF have been hypothesised to differ between sexually monomorphic and dimorphic species (MØLLER & BIRKHEAD 1994, OWENS & HARTLEY 1998). We need further knowledge whether in the case of the sexually monomorphic tree sparrow which sex, if any, use the black badge as a signal of quality toward conspecific competitors or potential mates.

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