Predicting the sex of the Sedge Warbler 
(*Acrocephalus schoenobaenus*) by discriminant analysis

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Sexual size dimorphism was examined in 273 immature Sedge Warblers (*Acrocephalus schoenobaenus*) caught in northern Poland during autumn migration in 2008 and 2009. Each individual was measured (wing, tail, head to bill, bill length, bill width, bill height, two measurements of tarsus width and hind claw length) and a blood sample was taken for molecular sexing. A forward stepwise discriminant function analysis was used and three discriminant functions were proposed for sexing Sedge Warblers. The first function, applied to the nine measurements, identified wing and bill length as the best measurements for sexing and correctly classified 82% of individuals. The second and third functions, applied to only wing length and tail length, identified the former as the best single measurement, and these two correctly classified 83% and 82% individuals, respectively. These functions may be used as a tool for sexing Sedge Warblers when blood sampling is impossible, such as in historical ringing records. However, the functions may be valid only for young birds in north and central Europe measured by experienced ringers.

1. Introduction

In many passerines the sexes are monomorphic in plumage (60% of species; Price & Birch 1996) though they might differ in size. This sexual size dimorphism is male-biased in most avian species and families, and sexual selection is probably responsible for some, but not all, of the differences in body size (Szekely et al. 2007). Sexual size dimorphism might have a functional significance in parental roles (Andersson 1994, Selander 1972), partitioning of foraging niches (Shine 1989) and/or sensitivity to parasitism (Potti & Merino 1996, Zuk 1990), even when differences between the sexes are slight (e.g. Great Tits *Parus major* and Blue Tits *Parus caeruleus*; Blondel et al. 2002, Przybylo 1995, Przybylo & Merilä 2000). Sex should therefore be considered in the studies of biology and ecology of most species.

The Sedge Warbler (*Acrocephalus schoenobaenus*) breeds over a vast area of Europe and winters in sub-Saharan Africa (Cramp 1998). Its breeding ecology and migration have been extensively studied (e.g., Spina & Bezzi 1990, Gyurácz & Bank 1995, 1997, Buchanan & Catchpole 1997, Kullberg et al. 2000, Yosef & Chernetsov 2004, Marshall et al. 2007, Nicholson et al. 2007, Jakubas & Wojczulanis-Jakubas 2010). Though the species’ plumage is sexually monomorphic, sexes can be distinguished during the breeding season by behavioural traits or, when caught, by the presence of a brood patch or cloacal protuberance (Svens-
son 1992). However, these traits cannot be used outside the breeding season, so most studies conducted during these stages have ignored sex. Significant differences in wing length between adult males and females have been found in several populations (reviewed in Cramp 1998), which suggests that it may be possible to sex birds by body size. However, prior to this study, these sex differences have never been studied in detail. Here we used external measurements of molecularly sexed individuals migrating through northern Poland to derive a discriminant function (including standard measurements) for sexing Sedge Warblers out of the breeding period.

2. Material and methods

Data were collected during the Sedge Warbler autumn migration in 2008 (27 July–4 September) and 2009 (25–31 August). Sedge Warblers were caught using 20 mist nets set in reed beds in the southern part of the Drużno Lake reserve, northern Poland (54°05′ N, 19°27′ E). Drużno Lake is a big, shallow lake with many reed beds, making it an attractive breeding and stop-over site for Sedge Warblers. Caught birds were ringed and aged by external characters (Svensson 1992) and then randomly selected for the purpose of this study. Only immature birds were considered in the study because the sample size for adults (20 individuals) was not sufficient for statistical analyses.

From the 99 birds sampled in 2009, the following measurements were taken: wing length (maximum flattened cord), tail length, head to bill length, bill length, bill width and height (behind the proximate edge of the nostrils), two measurements of leg width (wide and narrow diameters of the tarsus bone just above the upper ring edge when the ring was resting on the tarsus normally) and hind claw length.

All measurements were taken following Svensson (1992). A wing-rule was used for wing length (with 1 mm accuracy) and dial calipers were used for all other measurements (with 0.1 mm accuracy). The birds were weighed with an electronic scale (Ohaus, with 0.1 g accuracy), but this trait was excluded from the analyses because fat reserves have a large effect on birds’ body weight, especially during migration. All measurements of the birds handled in 2009 were taken by the author KW-J.

From the 174 immature Sedge Warblers caught in 2008, only wing- and tail-length measurements were taken by five experienced ringers, including the author KW-J, with each ringer measuring different birds. This data set is more representative of conditions at a standard ringing station, with several different ringers taking a limited number of measurements.

A small amount of blood (10–15 μl) was taken from the underwing vein of all birds in both years for molecular sexing. The blood was preserved in 70% or 96% ethanol before laboratory analysis, i.e., 1–2 months after completion of field work.

Ethanol was evaporated from the blood and DNA was extracted using the Blood Mini kit (A&A Biotechnology, Gdynia, Poland). All birds with the primer pair P2 and P8 were successfully sexed according to the protocol described by Griffiths et al. (1998), using a 50°C annealing temperature for the PCR reaction. These primers amplify a 390-bp fragment on the W chromosome (in females only) and a 370-bp fragment on the Z chromosome (in both sexes) (Griffiths et al. 1998).

The size difference is clearly visible when the fragments are separated on a 3% agarose gel. Initially, an additional pair of primers that also amplifies fragments of different lengths on the Z and W chromosomes was tried (F2550 and R2718, Fridolfsson & Ellegren 1999). However, this set of primers failed to sex the birds—all of the 77 samples analyzed with these primers had only one PCR product, indicating that they were males, but 43 of these samples were known to be females. The DNA sexing technique was validated with P2 and P8 pair of primers (recommended by Dawson et al. 2001) using 55 other Sedge Warblers of known sex (43 males and 12 females; sex determined on the base of cloacal protuberance and brood patch presence; Svensson 1992), captured in the breeding season in the same location.

A forward stepwise discriminant function analysis (DFA) was used to determine the best measurements for sexing Sedge Warblers. Three different approaches to DFA were applied using three different sets of data. In the first approach, DFA was conducted on all measurements taken in 2009 to determine whether sex can be determined from size under ideal conditions, i.e., with many
measurements taken by a single ringer. In the second approach, from 2009, only wing and tail length were used. The two measurements are considered as a standard in the South-East European Bird Migration Network (Busse 2000). To evaluate the robustness of the DFA procedure when measurements are taken by multiple ringers, DFA was performed with a dataset from 2008 (measured by five ringers, and only wing and tail length measured).

The effectiveness of the DFA was assessed, first, in terms of the proportion of birds of known-sex that were classified correctly using all individuals in the analysis (self-test) and, second, by cross-validation (each case is classified by the functions derived from all cases other than that case; SPSS Inc., Chicago). Due to unequal sample sizes for males and females, chance-corrected procedure (Cohen’s kappa statistic) was used to determine if the classification was better than random (Titus et al. 1984). The covariance matrices did not differ between groups for the three sets of data with different sample sizes: data from 2009 with all nine measurements (Box’s M test = 3.331, $F_{3,176489} = 1.086, P = 0.354$), data from 2009 with only two measurements (Box’s M test = 2.879, $F_{1,28219} = 2.849, P = 0.091$), or data from 2008 with two measurements (Box’s M test = 0.007, $F_{1,81737} = 0.007, P = 0.932$).

Data did not exhibit multicollinearity ($r < 0.52$ for all pair-wise correlations). Thresholds were calculated for each DFA so as to later assign sex to individuals in the predictive group. Thresholds were calculated as the weighted average of values of discriminant scores (i.e., the value calculated by summing up the constant and all coefficients once the data are entered) for each sex (means were weighted by number of males and females; Hair et al. 1995). Individuals with discriminant scores greater than the threshold were classified as male and those with lower scores as female, with probability computed from group sizes (Table 2).

The sizes of males and females measured in 2009 were compared using Students t-tests for each measurement. In order to examine the effect of ringer on measurements of wing and tail in the 2008 data, factorial ANOVA was applied with sex and ringer as predictors (Zar 1999). To describe a measure of the sexual dimorphism three frequently-used indices were calculated: the ratio of the mean male value to mean female value for a given trait ($m/f$); decimal logarithm of the $m/f$ ratio and Storer’s index (Storer 1966; the relative percentage difference in a given value): $DI = 100[(f-m)/(m+f)0.5]$, where $m$ and $f$ are the mean values of a measurement in males and females, respectively.

To test for homogeneity of wing-length measurements collected through a month of autumn migration in 2008, Kruskal-Wallis tests comparing wing length (separately for males and females) among pentads (ranges after Busse 2000) of the study period were performed. Wing length of both sexes was similar throughout the whole study period (males: $H_{6,102} = 10.42, P = 0.11$; females: $H_{6,22} = 9.39, P = 0.15$).

All data met the assumption of homogeneity of variance and normality (Levene and Shapiro-Wilk tests; $P > 0.05$), except for tail length both for males and females ($W = 0.94, P = 0.02$ and $W = 0.95, P = 0.03$, respectively). Those data were not transformed because the deviations were minor and discriminant analysis is robust with respect to this assumption (Tabachnick & Fidell 1996). All statistical analyses were performed in SPSS 14.0 for Windows (SPSS Inc. Chicago).

### 3. Results

Of the nine measurements taken in 2009, wing, tail, head to bill and claw lengths differed significantly between the sexes, males being on average bigger than females (Table 1). However, there was considerable overlap in values of all four traits for males and females, within a range of 67–94%. When the stepwise discriminant function was applied to the nine morphological measurements, the one equation that best separated the sexes included only wing- and bill-length measurements:

$$D_i = \text{Wing} \times (0.78) – \text{Bill length} \times (0.82) – 43.01 \ (1)$$

The function $D_i$ assumes a priori probability of being male of 0.51 and female of 0.49 (computed from group sizes) and results in the cut-off point of $D = -0.01$ (Table 2). This function correctly classified 81.8% of the 99 individuals of known sex (78.0% of 50 males and 85.7% of 49 females). The cross-validation test produced similar classifica-
Table 1. Body measurements (in mm; mean ± SD) of the immature Sedge Warbler males (N = 50) and females (N = 49) caught in northern Poland in autumn migration 2009. Student's t-test results for intersexual comparison of the measurements, and overlap (%) of the measurements between the sexes are also shown. DI = dimorphism index.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Males</th>
<th>Females</th>
<th>Similarity (t_w)</th>
<th>Similarity (P)</th>
<th>Overlap (%)</th>
<th>DI (Storer’s) (m/f)</th>
<th>DI (log10[m/f])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wing length</td>
<td>66.88 ± 1.51</td>
<td>64.33 ± 1.18</td>
<td>9.38</td>
<td>&lt; 0.001</td>
<td>67</td>
<td>3.89</td>
<td>1.04</td>
</tr>
<tr>
<td>Tail length</td>
<td>52.88 ± 1.79</td>
<td>51.27 ± 1.91</td>
<td>4.34</td>
<td>&lt; 0.001</td>
<td>94</td>
<td>3.09</td>
<td>1.03</td>
</tr>
<tr>
<td>Head-bill length</td>
<td>31.04 ± 0.58</td>
<td>30.58 ± 0.59</td>
<td>3.93</td>
<td>&lt; 0.001</td>
<td>93</td>
<td>1.49</td>
<td>1.02</td>
</tr>
<tr>
<td>Bill length</td>
<td>10.15 ± 0.45</td>
<td>10.16 ± 0.44</td>
<td>-0.15</td>
<td>0.88</td>
<td>98</td>
<td>0.1</td>
<td>1.00</td>
</tr>
<tr>
<td>Bill width</td>
<td>3.75 ± 0.21</td>
<td>3.74 ± 0.21</td>
<td>0.17</td>
<td>0.86</td>
<td>97</td>
<td>0.27</td>
<td>1.00</td>
</tr>
<tr>
<td>Bill height</td>
<td>2.98 ± 0.22</td>
<td>2.96 ± 0.21</td>
<td>0.16</td>
<td>0.88</td>
<td>99</td>
<td>0.67</td>
<td>1.01</td>
</tr>
<tr>
<td>Tarsus 1 width</td>
<td>1.22 ± 0.08</td>
<td>1.21 ± 0.10</td>
<td>0.43</td>
<td>0.67</td>
<td>97</td>
<td>0.82</td>
<td>1.01</td>
</tr>
<tr>
<td>Tarsus 2 width</td>
<td>1.88 ± 0.15</td>
<td>1.87 ± 0.14</td>
<td>0.58</td>
<td>0.56</td>
<td>97</td>
<td>0.53</td>
<td>1.01</td>
</tr>
<tr>
<td>Hind claw length</td>
<td>7.11 ± 0.32</td>
<td>6.93 ± 0.30</td>
<td>2.76</td>
<td>0.007</td>
<td>90</td>
<td>2.56</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Table 2. Parameters of discriminant functions developed for sexing the immature Sedge Warblers caught in northern Poland during autumn migration in 2009 (D_1 and D_2) and in 2008 (D_3). SCC = squared canonical correlation, Wilks = Wilks Lambda, TSD = threshold score D.

<table>
<thead>
<tr>
<th>Function</th>
<th>SCC</th>
<th>Eigenvalue</th>
<th>Wilks</th>
<th>F</th>
<th>df</th>
<th>P</th>
<th>TSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>D_1, in 2009</td>
<td>0.71</td>
<td>1.03</td>
<td>0.49</td>
<td>49.31</td>
<td>2, 96</td>
<td>&lt; 0.001</td>
<td>&gt; – 0.01 = male</td>
</tr>
<tr>
<td>D_2, in 2009</td>
<td>0.69</td>
<td>0.91</td>
<td>0.53</td>
<td>87.90</td>
<td>1, 97</td>
<td>&lt; 0.001</td>
<td>&gt; – 0.01 = male</td>
</tr>
<tr>
<td>D_3, in 2008</td>
<td>0.67</td>
<td>0.82</td>
<td>0.55</td>
<td>140.62</td>
<td>1, 172</td>
<td>&lt; 0.001</td>
<td>&gt; – 0.16 = male</td>
</tr>
</tbody>
</table>

The function D_2, when applied to data from 2008 (only wing- and tail-length data were available and several different ringers involved in measuring), correctly classified 81.6% of the 174 individuals of known sex (91.2% of 102 males and 68.1% of 72 females). Chance-corrected procedure showed that classification was 61% better than chance (kappa = 0.610 ± 0.080 SE, P < 0.001).

Two-factor ANOVA of wing length versus sex and ringer, performed on data from 2008 (different ringers involved in measuring), revealed significant effect of sex (F_{1,163} = 34.8, P < 0.001) but not significant effect of ringer (F_{1,163} = 1.30, P = 0.27) and interaction (F_{1,163} = 0.80, P = 0.53). In the same procedure performed for tail length versus sex and ringer, significant effects of both sex and ringer, but no significant interaction, were revealed (F_{1,163} = 4.65, P = 0.03, F_{1,163} = 4.99, P < 0.001 and F_{1,163} = 0.16, P = 0.96, respectively). When the stepwise discriminant function was applied to data from 2008, the wing turned out to be the single best predictor of sex, and the equation was:

\[ D_2 = \text{Wing} \times (0.74) - 48.43 \]  

This equation assumes similar values of a priori probability of being male and female, and results in the same cut-off as the function D_1 (Tables 2–3). The second function correctly classified 82.8% of the 99 birds with known sex (80.0% of 50 males and 85.7% of 49 females). The cross-validation test also correctly classified 82.8% of the sample. The function explained 69% of the variability of data (Table 2). Chance-corrected procedure showed that classification was 66% better than chance (kappa = 0.657 ± 0.100 SE, P < 0.001).

In the second approach to this set of data, when the stepwise discriminant function was applied to the length of wing and tail, wing length was the single best predictor of sex:

\[ D_2 = \text{Wing} \times (0.74) - 48.43 \]  

The function explained 71% of the variation in the data (Table 2). Chance-corrected procedure showed that classification was 64% better than chance (kappa = 0.637 ± 0.100 SE, P < 0.001).
\[ D_3 = \text{Wing} \times (0.74) - 49.08 \]  

This equation assumes similar values of a priori probability of being male (0.59) and female (0.41) (computed from group sizes) and results in the cutoff point of \( D = -0.16 \) (Table 2). The function \( D_3 \) correctly classified 81.6% of the 174 individuals with known sex (91.2% of 102 males and 68.1% of 72 females). The cross-validation test correctly classified 81.3% of the sample. Chance-corrected procedure showed that classification was 61% better than chance (kappa = 0.610 ± 0.080 SE, \( P < 0.001 \)). The function explained 67% of the variability in the data (Table 2).

4. Discussion

Sedge Warbler males were bigger than females in four of nine body measurements (wing-, tail- and head-bill lengths, and hind-claw length), providing yet another example of subtle size dimorphism in passerines. Differences in body size in passerines are usually not as pronounced as in other bird taxa. The sexual dimorphism indices of Sedge Warblers were up to six times lower than respective values reported for raptors (e.g., Storer 1966) and shorebirds (e.g., Meissner & Pilacka 2008). Nevertheless, the consistent pattern of males being larger then females suggests a direct selective force. The causality and significance of the sex differences in body size might be related to sex differences in parental behaviour, habitat use, niche partitioning and migration. Therefore, the sex should be considered in future works, and data for both sexes should be pooled only after a careful consideration of the possible influence of sex on results.

The discriminant function analysis showed that sexes can be distinguished with over 80% efficiency based only on wing-length measures, even when wing length is measured by different ringers. Ellrich et al. (2010), applying a forward logistic regression for testing several morphometric features of five other passerines, found similar efficiency of sex discrimination for four species (78–87%). Of the three functions derived in the present study, the second one \( (D_2) \), based on wing length measured by one ringer), had the highest power of discrimination between sexes, with 82.8% of individuals being correctly classified along with a comparable number of correctly classified males and females.

The validation of the function \( D_2 \), with data collected by various ringers, also proved its applicability to function with another set of data. The discrimination power of other derived functions was only marginally lower. The function \( D_3 \) showed that a valid function can be generated even if data from different ringers are involved. The functions \( D_2 \) and \( D_3 \) with wing length only had comparably high discrimination efficiency as the function \( D_1 \) that employed both measurements.

This result indicates that the basic measurement of wing quite efficiently sexes these birds. That, together with the above 80% efficiency of discrimination, may promote the two functions \( (D_2 \text{ and } D_3) \) as a tool for sexing Sedge Warblers, when molecular methods cannot be applied. To decrease the probability of incorrect sexing, individuals with wing length falling within the range of highest overlap – 65–67 mm – should be left unsexed (Table 3). The proposed technique may be particularly useful for analyzing archival data (e.g., ringing records collected during autumn migration) or as a rough classification of individuals for certain study purposes, such as when parameters of interest are compared between sexes and a sample size for each sex is desired.

<table>
<thead>
<tr>
<th>Wing length</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>0.007</td>
<td>0.993</td>
</tr>
<tr>
<td>63</td>
<td>0.027</td>
<td>0.973</td>
</tr>
<tr>
<td>64</td>
<td>0.099</td>
<td>0.901</td>
</tr>
<tr>
<td>65</td>
<td>0.306</td>
<td>0.694</td>
</tr>
<tr>
<td>66</td>
<td>0.639</td>
<td>0.361</td>
</tr>
<tr>
<td>67</td>
<td>0.877</td>
<td>0.123</td>
</tr>
<tr>
<td>68</td>
<td>0.966</td>
<td>0.034</td>
</tr>
<tr>
<td>69</td>
<td>0.991</td>
<td>0.009</td>
</tr>
<tr>
<td>70</td>
<td>0.998</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 3. Probabilities of being male or female in relation to wing length (in mm), calculated from the function \( D_3 \) for immature Sedge Warblers (see text for details). Shaded areas indicate the probabilities of being male or female with \( P > 0.90 \) (dark grey) or \( P > 0.60 \) (light grey). Bolded values of wing length indicate the range of wing length in which the probability of incorrect sexing is less than 10%.
ments commonly occurs (e.g., Nisbet et al. 1970, Berthold & Friedrich 1979, Gosler et al. 1998, Pitzer et al. 2008). In the present study a significant effect of ringers on tail length was revealed. However, wing length, which was the only variable included in two of the three discriminative equations (1–3; see Results), was less susceptible to human error. The similarity of mathematical formulae and their discriminative ability, based on measurements taken by single or multiple ringers ($D_2$ and $D_3$) suggest good applicability to data gathered by different ringers.

It can not be ruled out that the magnitude of sexual size dimorphism varies geographically, as shown for some passerines (Ellrich et al. 2010). Birds from different latitudes may have different wing morphologies because of adaptations to different migration strategies and/or local conditions (Leisler & Winkler 2003). Chernetsov (2004) found that among Sedge Warblers migrating through Courish Spit, those captured in July had less pointed wings than those captured in August and September. This finding suggests that various Sedge Warbler populations migrate through the Courish Spit; this is also likely to be the case at the Drużno Lake, considering the proximity of the two areas (ca. 140 km). Given the time of data collection in the present study, it is likely that many immature Sedge Warblers were local birds sensu lato, but some birds might have been also transit migrants from northern populations, especially those captured at the end of August.

On the other hand, there was no temporal variation in wing length, which may indicate that the captured birds originated from the same population or from different populations with no or minor differences in wing length. Perhaps a difference in migratory distance between the Sedge Warblers from local and north-eastern populations is not sufficient for the detectable difference in wing length to evolve. In any context, caution should be used in applying the presented discriminant functions for distinguishing local individuals from other, such as more southern, regions. Body size in Sedge Warblers varies clinally so that wing and bill lengths tend to increase slightly from Britain towards Kazakhstan, and from eastern Europe towards northern Europe (Cramp 1998). However, a thorough evaluation of Sedge Warbler body-size variation has not yet been performed. An analogous investigation of sexual size dimorphism in other parts of the range of this species would provide information on possible geographical size variation. The present functions should only be used for the same age cohort for which they were developed, i.e., immatures; in many passerine species, first-year birds have shorter wings than older birds (e.g., Alatalo et al. 1984, Norman 1997, Tiainen & Hanski 1985).

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**Sukupuolen määrittäminen ruokokerttusilla erotteluanalyysillä**

Sukupulimidorsia tutki 273 esiäikuisella ruokokertussella (Acrocephalus schoenobaenus) Pohjois-Puolassa syysmuuton aikana 2008–2009. Yksilöiltä mitattiin siiven, pyrstön, pään + nakan ja nakan pituudet, nakan leveys ja korkeus, kaksi nilkkamittaa ja keskivarpaan kynnyn pituus, sekä otettiin veriniyty molekyylimenetelmällä tapahtuvaa sukupuolen määrittystä varten. Askeltaa erotteluanalyysi johti kolmeen sukupuolien erottelevaan funktioon lajilla. Ensimmäinen, jonka muodostamiseen käytettiin kaikkia mitattuja annokuja, osoitti siiven ja nakan pituudet tärkeimmäksi erottavaksi tekijäksi ja luokitti oikein 82 % yksilöistä. Toinen ja kolmas funktio käyttivät amoastaan siiven ja pyrstön pituuksia; ensin mainitti osoittautui tärkeimmäksi yksittäiseksi sukupuolila 1mrottavaksi mittasaureeksi, ja nämä tekijät yksittäisissä tunnistivat sukupuolet oikein vastaavasti 83 ja 82 % yksilöistä. Tuotettuja funktioita voidaan käyttää ruokokerttusen sukupuolen määrittelyyn silloin, kun veriniytyttä ei ole saatavissa, kuten historiallisissa rengastusaitouistoissa. Funktioit voivat kuitenkin olla luotetta-
via vain Pohjois- ja Keski-Euroopan nuorille linnuille sovellettuina ja silloinkin vain, jos aineisto on kokeneiden rengastajien keräämä.

References


