



**HAL**  
open science

## Morphological sexing of passerines: not valid over larger geographical scales

Heike Ellrich, Volker Salewski, Wolfgang Fiedler

► **To cite this version:**

Heike Ellrich, Volker Salewski, Wolfgang Fiedler. Morphological sexing of passerines: not valid over larger geographical scales. *Journal für Ornithologie = Journal of Ornithology*, 2009, 151 (2), pp.449-458. 10.1007/s10336-009-0478-z . hal-00568361

**HAL Id: hal-00568361**

**<https://hal.science/hal-00568361>**

Submitted on 23 Feb 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1           **Morphological sexing of passerines: not valid over larger**  
2                                   **geographical scales**

3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23

Heike Ellrich, Volker Salewski & Wolfgang Fiedler

Vogelwarte Radolfzell at the Max-Planck Institute of Ornithology, Schlossallee 2, 78315  
Radolfzell, Germany.

Address for correspondence: Volker Salewski, Max-Planck Institute for Ornithology,  
Schlossallee 2, 78315 Radolfzell, Germany.  
Email: [salewski@orn.mpg.de](mailto:salewski@orn.mpg.de)

Phone: ++ (0) 7732 150119  
Fax: ++ (0) 7732 150169

24 **Abstract**

25 Sex determination of birds is important for many ecological studies but is often difficult in  
26 species with monomorphic plumage. Morphology often provides a possibility for sex  
27 determination, but the characters need to be verified. We tested whether five passerine species  
28 can be sexed according to standard morphological measurements applying a forward logistic  
29 regression with sex determined by molecular analysis as the dependent variable. Furthermore,  
30 we tested whether the results can be used on a larger geographic scale by applying  
31 morphological sexing methods gained by similar studies from other regions to our data set. Of  
32 the five species of this study only garden warblers could not be sexed morphologically. In the  
33 robin 87.2% of all individuals were sexed correctly. For reed warblers, willow warblers and  
34 reed buntings the respective values were 77.6%, 89.4% and 86.4%. When the logistic  
35 regression functions from similar studies on robins and reed buntings in Denmark and  
36 Scotland were applied to the birds from south-western Germany they performed less well  
37 compared to the original data set of these studies and compared to the logistic regression  
38 function of our own study. The same was the case for willow warblers when a wing length  
39 criterion used in Great Britain was applied to the birds of our study. These discrepancies may  
40 have several explanations: (1) the models are optimised for the data set from which they were  
41 extracted, (2) inter ringer variation in measurements, (3) the use of different age cohorts, (4)  
42 different morphology due to different habitat availability around the study site, or, most  
43 likely, (5) different morphology due to different migratory behaviour. We recommend that  
44 morphological sex differentiation methods similar to this study (1) be only used population  
45 specific, (2) only with one age cohort and (3) to adjust the extracted equations from time to  
46 time.

47

48 **Keywords:** passerines, morphology, sex determination, PCR

49

50 Sex determination of birds is important in many ecological studies but sex differentiation is  
51 difficult in species with monomorphic plumage even when the concerned individuals are  
52 captured. During the breeding season most individuals of many species can be sexed either  
53 due to the presence of an incubation patch, most pronounced in females, or the cloacal  
54 protuberance in males (Drost 1938, Svensson 1992). However, during the non-breeding  
55 season these characters are invalid. Nevertheless, sexing birds in the non-breeding season is  
56 desirable especially for migration studies which analyse differential migration phenomena,  
57 i.e. sex specific different migration phenologies, migration routes, wintering areas and  
58 wintering ecology with respect to differential habitat use or territorial behaviour.

59

60 Many passerine species which are monomorphic in plumage characters show a distinct size  
61 dimorphism with males usually being larger compared to females. This size dimorphism is  
62 often expressed by longer wings, longer tarsi or a higher body mass (Svensson 1992). These  
63 measurements are taken routinely at most ringing stations collecting data for bird migration  
64 studies on a large scale (Bairlein 1995). However, although there is often a statistically  
65 significant difference in the mean values of a morphological character between the sexes there  
66 is also an overlap of varying degrees in the measurements which leads to uncertainties when  
67 relying on morphological sex determination alone. Furthermore, to establish morphology as a  
68 reliable means for sex determination the respective characters have to be verified by testing  
69 them on a sufficiently high number of individuals of explicitly known sex.

70

71 In former times researchers sometimes sacrificed a number of individuals of the species under  
72 study for sex determination (Kalchreuter 1971). An ethically sounder, but nevertheless  
73 invasive method is laparotomy when the gonads are checked through a cut into the body  
74 cavity of the living bird. The cut heals within several days but care has to be taken to avoid  
75 injuries of the liver, the kidney or the digestive tract and there is always the risk of infections

76 (Berthold 1969). Morgan (2005) discussed the possibility to analyse multi-modal distributions  
77 of morphological characters to differentiate sexes accordingly. This approach was used e.g. by  
78 Catry et al. (2005) who separated male and female chiffchaff *Phylloscopus collybita* based on  
79 the bi-modal distribution of wing length. However, although this method is based on sound  
80 assumptions there is no independent verification that the individuals classified as males or  
81 females do in fact belong to the respective sex cohort. Relatively unproblematic and reliable  
82 is taking a blood sample for later molecular sex determination in the laboratory (Griffiths et  
83 al. 1998), but this method is not practicable for all mist-netted birds at field stations where  
84 sometimes hundreds of birds are captured daily. Furthermore, in many countries blood  
85 sampling is more restricted by animal welfare legislation than trapping and ringing of birds.  
86

87 However, many recent authors who wanted to verify sex determination by morphological  
88 measurements used molecular methods for sex determination (Griffiths et al. 1998) of a sub-  
89 sample of the species under study (Madsen 1997, Hipkiss 2007, Ottvall and Gunnarsson  
90 2007). The sex is then used as the dependent variable in either a discriminant analysis or a  
91 logistic regression with a number of morphological measurements as explaining variables to  
92 identify the variables associated with the sex of the species under study. In recent years these  
93 methods have been used to investigate morphological variables which can be used for sex  
94 determination in a number of non-passerine and passerine bird species (Madsen 1997, Walton  
95 and Walton 1999, Bertellotti et al. 2002, Campos et al. 2005, Hipkiss 2007, Ottvall and  
96 Gunnarsson 2007, Shealer and Cleary 2007). Although these methods can lead to reliable sex  
97 determination of the population under study its general application for the species concerned  
98 has two caveats: First, the statistical models are selected to fit a particular sample well. It will  
99 therefore fit the sample better than the entire population from which it is drawn or a sample  
100 from another population. Second, there are intra-specific differences in morphology due to  
101 different migration distances or other ecological factors (temperature, habitat) mainly varying

102 across latitudes. In migratory species those populations with a relatively longer migration  
103 distance usually have longer wings compared to populations with relatively shorter migration  
104 distance as shown e.g. for the willow warbler (Lindström et al. 1996) or the blackcap *Sylvia*  
105 *atricapilla* (Fiedler 2005).

106

107 In the present study we want to test first whether the sex of first year individuals of five  
108 common passerine species which are captured regularly at a constant effort mist-netting site  
109 in south-western Germany can be sexed reliably with standard morphological measurements.  
110 For this purpose we used a set of morphological variables in connection with molecular sex  
111 determination to verify the validity of morphological sexing. Second, we compare our results  
112 with similar studies to test whether morphological sexing is reliable with the same criteria  
113 over a larger geographical range. We therefore use only standard measurements which are  
114 taken within many studies and thus can be compared with the results of other stations.

115

116

## 117 **MATERIAL AND METHODS**

### 118 Target species and morphological data

119 Birds were captured as part of a monitoring programme on the peninsula Mettnau (47.729°N,  
120 8.998°E), Lake Constance, near Radolfzell in south-western Germany. Since 1972 between  
121 30 June to 6 November mist-netting follows standardised methods (details see Berthold and  
122 Schlenker 1975). The early start of the autumn netting season enables the capture of local  
123 breeding birds as well as migrating individuals of most species during the entire autumn  
124 migration period. The proportions of local birds and of birds on passage are, however,  
125 unknown. All captured birds are identified to species, ringed and aged following the criteria  
126 given in Svensson (1992) and Jenni and Winkler (1994). Standard measurements taken are:  
127 (1) feather length i.e. the length of the third outermost primary feather (hereafter: feather)

128 following Berthold and Friedrich (1979); (2) wing length (hereafter: wing) according to  
129 method “maximum length” in Svensson (1992); and (3) tarsus length (hereafter: tarsus)  
130 according to the “alternative method” in Svensson (1992, Fig. 18B). Feather and wing length  
131 were measured with a precision of 0.5 mm and tarsus with a precision of 0.1 mm. (4) Body  
132 mass is taken with an electronic balance with a precision of 0.1 g. For every bird the fat in the  
133 furcula pit was scored with on an ordinal nine digit scale from 0 (no fat) to 8 (entire flight  
134 muscle covered with fat) according to Kaiser (1993). Furthermore, the thickness of the flight  
135 muscle was estimated on an ordinal four digit scale according to Bairlein (1995). As both  
136 estimates showed low variation between individuals (fat scored 0, 1 or 2 in about 87%;  
137 muscle scored 1 or 2 in about 88% in all birds respectively) we did not consider fat and  
138 muscle scores in the further analyses. Therefore, our study remains comparable to other  
139 studies which did also not consider fat and muscle scores (e.g. Walton & Walton 1999). All  
140 birds in this study were measured between 30<sup>th</sup> June and 27<sup>th</sup> September 2007 by H. Ellrich  
141 and only those individuals for which all four measurements were available were considered.

142

143 Target species were selected according to the *a priori* expectation of sufficient numbers  
144 mistnetted for the analyses based on experience from previous years. Furthermore, only those  
145 species were selected in which sex of first year birds before juvenile moult cannot be  
146 determined in the hand according to plumage characters. The selected species were (sample  
147 size in brackets): robin *Erithacus rubecula* (94), reed warbler *Acrocephalus scirpaceus* (147),  
148 garden warbler *Sylvia borin* (79), willow warbler *Phylloscopus trochilus* (47) and reed  
149 bunting *Emberiza schoeniclus* (44). Morphology of the flight apparatus and body mass of  
150 passerines varies with age, i.e. first year birds in many species have shorter wings and a lower  
151 body mass compared to older birds (e.g. Alatalo et al. 1984, Schmitz and Steiner 2006,  
152 Markovets et al. 2008). For some passerine species it has been shown that variation in wing  
153 length does also occur between different age cohorts after the first primary moult, i.e. wing

154 length increases with age (Merom et al. 1999, Dale et al. 2002). Furthermore, the  
155 consideration of first year birds only excludes birds in primary moult. The consideration of  
156 birds being more than one year old could therefore lead to erroneous results. It is in general  
157 not possible to age birds of the species considered in this study when they are more than one  
158 year of age, i.e. after their first primary moult. Therefore, only fully grown first year birds  
159 were included in the analyses. Every bird was considered only once.

160

### 161 Molecular sexing

162 Molecular sexing followed Griffiths et al. (1998) using a modified PCR amplification of the  
163 CHD genes. For the amplification two different primer pairs were chosen: the P2 (5'-TCT  
164 GCA TCG CTA AAT CCT TT-3') and the P8 (5'-CTC CCA AGG ATG AG (AG) AA (CT)  
165 TG-3') primers (Griffiths et al. 1998) were used for DNA amplification in the garden warbler,  
166 reed warbler, willow warbler and reed bunting and the primer pair 3007 (5'-TAC ATA CAG  
167 GCT CTA CTC CT-3') and 3112 (5'-CCC CTT CAG GTT TAA AA-3') was used for  
168 amplification in the robin (Ellegren and Fridolfsson 1997). PCR amplification was carried out  
169 in a total volume of 10 µl. The PCR was performed in an Applied Biosystems 2720 Thermal  
170 cycler (Applied Biosystems). An initial denaturation step at 94°C for 3 min was followed by  
171 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 45 s and 72°C for 90 s for both primer  
172 pairs. A final cooling period of 4°C completed the program. PCR products were separated by  
173 electrophoresis on 2% agarose gels. The gel was stained with SybrGold gel stain (Molecular  
174 Probes).

175

### 176 Statistics

177 All morphological variables were tested for a normal distribution with a one-sample  
178 Kolmogorov-Smirnov-test. Then a forward conditional logistic regression was used to extract  
179 morphological variables which could be used to discriminate between the sexes. In forward



180 conditional regressions removal testing is based on the probability of likelihood-ratio statistics  
181 and a variable is entered in the regression when  $p < 0.05$  and a variable is removed when  $p >$   
182 0.1. In the logistic regression sex revealed by the molecular analysis was used as the  
183 dependent variable and the morphological measurements feather, wing and tarsus as well as  
184 body mass were covariates. The sexes were coded “0” for male and “1” for female for the  
185 regression. The goodness-of-fit of the logistic regression was tested with a Hosmer-  
186 Lemeshov-test (Hosmer and Lemeshov 1989).

187

188 With the linear logistic function retrieved from the model

189

$$190 \quad d = \alpha + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_i x_i \quad \text{equation 1}$$

191

192 where  $\alpha$  is a constant and  $\beta_{1, 2, \dots, i}$  regression coefficients of the predictor variables  $x_{1, 2, \dots, i}$ ,  
193 an individual bird with certain predictor variables will be classified as being male ( $d < 0$ ) or  
194 female ( $d > 0$ ). The probability that a bird with a given morphology is female can be  
195 estimated according to:

196

$$197 \quad p_{\text{female}} = \frac{e^d}{1+e^d} \quad \text{equation 2}$$

198

199 where  $d$  is the logistic function and  $e$  is the base of natural logarithms, approximately 2.718.

200 The probability of being male is:  $p_{\text{male}} = 1 - p_{\text{female}}$ .

201

202 Birds migrating through the study area from more northern latitudes may have a different  
203 wing morphology compared to resident conspecifics because of adaptation to different  
204 migration distance (Leisler and Winkler 2003, Fiedler 2005). Therefore, we tested whether  
205 day of the season is associated with the morphological measurements with a one-way

206 ANOVA with feather, wing, tarsus and body mass as dependent variables and day of the  
207 season (30 June = 1, 1<sup>st</sup> July = 2 etc) as a factor.

208

209 Due to the low sample size for some species we refrained from splitting the sample to do the  
210 analyses with one part of the sample and test its accuracy with the second half of the sample.

211 SPSS 12.0 was used for all statistical analysis. The accepted significance level was  $p < 0.05$ .

212

213

## 214 **RESULTS**

### 215 Molecular sexing

216 Molecular sexing revealed that the sample included 54 male and 40 female robins, 68/79 reed

217 warblers, 47/33 garden warblers, 24/23 willow warblers and 24/20 reed buntings. In all

218 species the proportion of the two sexes did not differ significantly from an even ratio

219 (binomial test,  $p > 0.1$ ).

220

### 221 Morphological sexing

222 All morphological variables were normally distributed for both sexes in all species

223 (Kolmogorov-Smirnoff test,  $p > 0.05$ ). In all measurements with significant intra-specific

224 inter-sexual differences male birds showed the higher values, i.e. were the larger sex (Table

225 1).

226

227 A two step forward conditional logistic regression with sex as revealed by molecular sexing

228 as the dependent variable and the morphological variables wing, feather and tarsus as well as

229 body mass as covariates included wing length and feather length (Nagelkerke-R<sup>2</sup>: 0.669,

230 Hosmer-Lemeshow GOF:  $p = 0.588$ ) for the robin. With these two variables 87.2% (males:

231 88.9%, females: 85.0%) of the robins could be sexed correctly with the equation  $d = 120.585 -$

232 1.161\*wing – 0.655\*feather (Table 2, Figure 1a). In reed warblers wing and tarsus were  
233 included in a two step logistic regression (Nagelkerke-R<sup>2</sup>: 0.389; Hosmer - Lemeshow GOF:  
234 p = 0.145). With the function  $d = 72.765 - 1.367*tarsus - 0.638*wing$  the sex could be  
235 predicted correctly for 77.6% (males: 73.5%, females: 81.0%) of all individuals (Table 2,  
236 Figure 1b). In willow warblers only wing was included in the analysis (Nagelkerke-R<sup>2</sup>: 0.803;  
237 Hosmer-Lemeshow GOF: p = 0.416) and for 89.4% (males: 91.7%, females: 87.0%) of the  
238 birds the sex could be predicted correctly with the regression equation  $d = 97.233 -$   
239  $1.486*wing$  (Table 2, Figure 1c). For the reed bunting 86.4% (males: 87.5%, females:  
240 85.0%) of all individuals could be sexed correctly according to a one step logistic regression  
241 (Nagelkerke-R<sup>2</sup>: 0.721; Hosmer-Lemeshow GOF: p = 0.981) with the function  $d = 75.423 -$   
242  $0.98*wing$  (Table 2, Figure 1d). The garden warbler was the only species for which none of  
243 the variables met the conditions for the inclusion in a conditional forward regression.  
244 Therefore, it is not possible to sex first year garden warblers using the measurements and the  
245 statistical methods applied in this study.

246

247 None of the selected variables was significantly associated with capture date (ANOVA: p >  
248 0.05; power on the 0.05 significance level for the robin: wing = 0.855, feather = 0.797; reed  
249 warbler: wing = 0.507, tarsus = 0.292; willow warbler: wing = 0.804; reed bunting: wing =  
250 0.595). Therefore, the extracted variables could be used throughout the respective trapping  
251 periods.

252

253 In addition to the mere classification of a bird as being male or female according to the d  
254 values calculated from equation 1, the probability that a bird with a certain combination of  
255 morphological measurements is female was estimated according to equation 2. In the robin  
256 the morphological criteria for a > 95% probability of belonging to the correct sex was found  
257 in 11 (27.5%) of females and in 20 (37%) of males. The respective values for a > 90%

258 probability were 16 (40.0%) for females and 29 (53.7%) for males (Table 3a). In the other  
259 species (Table 3b-d) the respective values were for reed warblers: > 95% correct probability  
260 for eight females (10.1%) and 3 males (4.4%) and for > 90% correct probability for 14  
261 females (17.7%) and eight males (11.8%), for willow warblers: > 95% correct probability for  
262 13 females (56.5%) and 17 males (70.8%) and for > 90% correct probability for 14 females  
263 (60.9%) and 17 males (70.8%) and for reed buntings: > 95% correct probability for four  
264 females (20%) and 15 males (62.5%) and for > 90% correct probability for eight females  
265 (40%) and 17 males (70.8%). All birds with a > 90% probability to be sexed correctly were in  
266 fact sexed correctly.

267

#### 268 Comparison with studies from other regions

269 There are two comparable studies (Madsen 1997, Walton and Walton 1999) about the sexing  
270 of some of our target species from other regions. Additionally, there are several other hints  
271 about sexing of these species where the exact methods of verification were not always known.  
272 Below we apply the characters mentioned in former studies to verify their validity for the  
273 sample considered in this study.

274

275 Madsen (1997) studied robins during the breeding season and during autumn migration in  
276 Denmark and used molecular sexing of 138 birds of mixed age to verify morphological sex  
277 determination. The author was able to sex 80% of all birds correctly according to wing length  
278 based on the results of a logistic regression. According to this regression analysis birds with a  
279 wing length  $\geq 71$ mm would be male and birds with a wing length of  $< 71$ mm female. When  
280 this split was applied the data of this study only 60.6 % of all robins were sexed correctly.  
281 Whereas all males were sexed correctly, 37 (92.5%) out of 40 females were classified as  
282 males.

283

284 J. Pettersson (cited in Svensson 1992) found that about 60% of “non-adult” “Fenno-Scandian  
285 migrant” robins could be sexed at Ottenby, Sweden, by using wing length as the sole  
286 separation criterion. Birds with a wing length > 74mm would be males and birds with a wing  
287 length < 71mm females although details for the verification of this character are not given.  
288 Applying these characters to the robins considered here revealed that only 34 out of 94 birds  
289 fell within the criteria of Pettersson (in Svensson 1992) including 31 males which were all  
290 sexed correctly and four females of which one was sexed as a male (wing 75 mm). In total  
291 only 35% of all robins could be sexed correctly according to the criteria of Pettersson (in  
292 Svensson 1992).

293

294 Norman (1983) stated to be able to sex 96% of first year willow warblers caught during April  
295 to May in northeast England correctly when assuming that males have a wing length of  $\geq 65$   
296 mm and females a wing length of < 64 mm. These criteria could be applied to 42 out of 47  
297 willow warblers considered in this study of which 90.5% were sexed correctly. All males (24)  
298 were sexed correctly, but four out of 23 females were sexed as males and 5 females could not  
299 be sexed. When applied to all birds of the study 80.9% of all birds could be sexed correctly.

300

301 Walton and Walton (1999) studied reed buntings between July and December from south east  
302 Scotland using a logistic regression. With the variables wing and mass 95.4% of all first year  
303 birds could be sexed correctly according to the equation:

$$304 \quad d = 132.31 - 1.33 * \text{wing} - 1.58 * \text{mass}$$

305 If  $d < 0$  the sex was assigned to be male and if  $d > 0$  the sex was assigned to be female.

306 Applying this equation to the data set of this study 81.8% of all birds were sexed correctly.

307 This included 70.8% of correctly sexed males and 95.0% of correctly sexed females.

308 Comparing the results of Walton and Walton (1999) with the results of the logistic regression  
309 of our analysis revealed that 4.6% more birds could be classified correctly.

310

311

312 **DISCUSSION**

313 In this study we investigated whether the sex of five passerine species can be determined with  
314 morphological characters. We restricted our analyses to standard measurements because our  
315 intention was to apply potential sex-separating characters to the long time series of this study  
316 site and possibly to a wide range of ringing stations working with similar standard  
317 methodology. The sex of the measured birds was verified with molecular analyses. In the  
318 garden warbler sex determination was not possible with the morphological variables applied  
319 in this study and the statistical criteria for the inclusion in a logistic regression. In the other  
320 four species a varying number of variables showed significant difference between the sexes.  
321 The sex of the latter four species could be determined correctly with a varying proportion  
322 from 77.6% for reed warblers to 89.4% for willow warblers with one (willow warbler, reed  
323 bunting) or two (robin, reed warbler) morphological measurements. Therefore, between about  
324 11% and 22% of the measured birds could not be sexed correctly. It is difficult to assess  
325 whether this is accurate enough to be used in further analyses. This question will depend  
326 mainly on the questions addressed and on the species considered.

327

328 There are several possibilities which may improve the potential application of studies using  
329 an approach like the one presented here. First, a repetition of a similar analysis, but applied to  
330 a higher number of birds may improve its accuracy. However, on an inter-specific level a  
331 larger number of individuals analysed is not related to a higher proportion of correctly sexed  
332 birds. This study included 147 reed warblers but only 47 willow warblers, but the proportions  
333 of correctly sexed birds was 77.6% and 89.4% respectively. Second, the proportion of  
334 correctly sexed birds can be increased when only a subset of the sample is used according to  
335 the most discriminating values of the respective morphological variables. When the

336 probability that a bird with certain character values belongs to a certain sex was >90% all  
337 respective individuals were in fact sexed correctly. Therefore, these combinations of character  
338 values could be used to select a subsample of individuals for studies that need to analyse sex  
339 specific traits. This approach may, however, include the caveat that only individuals at the  
340 extreme range of certain variables are considered. For example, body size, amongst other  
341 factors, may be an indicator of the condition of an individual. In size dimorphic species with  
342 e.g. larger males, an inter sexual overlap of characters may lead to the mere consideration of  
343 'large' males and 'small' females, i.e. a preference for the individuals with high condition in  
344 one sex over those individuals with low condition in the other sex with consequently spurious  
345 results.

346

347 The study showed that morphological sex determination according to logistic regression  
348 functions could only be applied with care between study sites and generally performs with  
349 less accuracy between sites. If all birds of the target species were considered then the  
350 application of morphological characters from other studies performed less well than testing  
351 our own data. This may have been for several not mutually exclusive reasons. First, the  
352 statistical analysis itself influences the results. In a logistic regression model variables are  
353 selected to fit the particular data set well. The fit will therefore be less good when a different  
354 data set, either from the same or a different population, is used. This may be the reason for  
355 smaller differences like in the application of the criteria of Walton and Walton (1999) to our  
356 data set of reed buntings, but we assume that differences in model fit are unlikely to cause the  
357 difference in explanatory power of approximately 20% of the models as in the robin. Second,  
358 inter-ringer variation in measurements may influence the results. The occurrence of inter-  
359 ringer variances in standard measurements has been shown in a number of studies (Nisbet et  
360 al. 1970, Berthold and Friedrich 1979, Gosler et al. 1998). Nevertheless, assuming that the  
361 ringers in all studies were experienced, the differences between the means of the

362 measurements according to internationally standardized methods should be low (Nisbet et al.  
363 1970). Therefore, it seems unlikely that inter-ringer variation in measurements can account  
364 for the magnitude of some of the differences of the explanatory power of certain variables  
365 between this study and those from other sites (possible exception: see below). Third, the  
366 inclusion of different age cohorts in the analyses may lead to biased results. It has been shown  
367 for many passerine species that first year birds have shorter wings than older birds (e.g.  
368 Alatalo et al. 1984, Tiainen and Hanski 1985, Norman 1997). Additionally, it has been  
369 discussed that wing length of passerines increases with age (Smith et al. 1986, Dale et al.  
370 2002) and may decrease again in old age (Møller and de Lope 1999). The inclusion of  
371 different age cohorts as in the study of Madsen (1997) on robins could be the reason for the  
372 strong sex bias when the discriminating criteria were applied to the birds of our study.  
373 However, due to the inclusion of adult robins into the sample the mean wing lengths should  
374 be longer compared to a sample where only first year birds are considered. Additionally, the  
375 same effect should appear if robins from northern Europe are expected to have longer wings  
376 than birds from Central Europe. However, Madsen (1997) noted a distinctly shorter mean  
377 wing lengths for both sexes (males:  $72.2 \pm 1.32$  sd; females:  $69.8 \pm 1.54 \pm$  sd) than we did in  
378 south-western Germany (Table 1). Madsen's wing length data is also lower than comparable  
379 data from Scandinavia (Pettersson 1983). Due to these differences all males of our study are  
380 sexed correctly according the criteria of Madsen (1997), but the great majority of females are  
381 also sexed as males. Although the same measurement technique was used in both studies  
382 (method "3" according to Svensson 1992), a ringer bias cannot be excluded. Fourth, birds  
383 considered at different sites may differ in their morphology (see e.g. Hanski and Tiainen 1991  
384 for willow warblers). This may either be due to different habitats surrounding the study site as  
385 morphology of a number of passerine species has been found to differ between habitats  
386 (Lundberg et al. 1981, Michalak 1995, Blondel 2007), or to different migratory behaviour.  
387 There are no data on habitat types of the study sites considered, thus this influence cannot be



388 excluded, but Cuadrado (1991) explained the low probability of sexing wintering robins  
389 correctly in Spain with the inclusion of birds with different migratory behaviour into the  
390 study. It has been discussed that migration distance is linked with wing length (Leisler and  
391 Winkler 2003) and Fiedler (2005) showed on an intra-specific level that blackcaps *Sylvia*  
392 *atricapilla* with increasing migratory distances have longer wings. Therefore, site specific  
393 morphology as an adaptation to different migratory behaviour is presumably the main reason  
394 why morphological sexing can not be applied over larger geographic scales. Assuming longer  
395 wings in populations from more northerly latitudes because of longer migration distances the  
396 wing length which splits the sexes should be higher than in more southern populations and  
397 consequently more shorter winged females should be sexed correctly and more males  
398 incorrectly. However the opposite is the case when applying the criteria of Pettersson (in  
399 Svensson 1992) and of Norman (1983) to the robins and the willow warblers of this study  
400 respectively. We have no information how the sex of the robins studied by Pettersson (in  
401 Svensson 1992) was verified which makes it difficult to discuss the differences compared to  
402 our study. With respect to willow warblers British birds spend the non-breeding season in  
403 West Africa (Wernham et al. 2002) and recoveries of willow warblers ringed in southern  
404 Germany indicate similar non-breeding areas (Zink 1973). Therefore, the distance of south  
405 German willow warblers to their non-breeding areas may be even longer compared to British  
406 willow warblers although the latter breed at higher latitudes. Furthermore, the birds of this  
407 study could include some migrants from more north-eastern breeding areas with an even  
408 longer migration distance.

409

410 In conclusion, we showed that in four out of five investigated passerine species the sex could  
411 be determined with varying accuracy. For some species the proportion of correctly sexed  
412 individuals was low and may not be sufficient to draw sound inferences when applied to  
413 further analyses e.g. for the investigation of differential migration patterns. Furthermore, it is

414 shown that results from one study site will cause spurious results when applied at another  
415 study site for a variety of mutually non-exclusive reasons. As morphology of species at a  
416 given site may also vary with time (Nowakowski 2000, Salewski et al. in press) we  
417 recommend the use of morphological sex differentiation methods similar to this study only on  
418 a population specific basis, only with one age cohort, only birds at the same stage of feather  
419 wear (feathers in spring may be more worn compared to feathers in autumn or vice versa) and  
420 to adjust the extracted equations from time to time.

421

422

## 423 **ZUSAMMENFASSUNG**

### 424 **Kriterien zur morphologischen Geschlechtsbestimmung bei Singvögeln sind nicht über** 425 **größere geographische Räume hinweg anwendbar**

426 Die korrekte Geschlechtsbestimmung von Singvögeln ist für viele Studien erwünscht, aber bei  
427 monomorphen Arten oft schwierig. Die Geschlechtsbestimmung anhand morphologischer  
428 Merkmale bildet einen guten Ansatz, muss aber anhand anderer Methoden verifiziert werden.  
429 Wir prüften bei fünf Singvogelarten mit Hilfe einer logistischen Regression, in der das durch  
430 molekulare Methoden bestimmte Geschlecht der untersuchten Vögel als unabhängige  
431 Variable einging, ob eine morphologische Geschlechtsbestimmung anhand von  
432 Standardmaßen (Flügel-, Feder- und Tarsuslänge, Gewicht) durchgeführt werden kann.  
433 Darüber hinaus untersuchten wir, ob durch ähnliche Untersuchungen ermittelte  
434 Geschlechtsbestimmungskriterien auch auf die von uns untersuchten Vögel mit gleicher  
435 Genauigkeit angewandt werden können. Von den untersuchten Arten ließen sich nur bei der  
436 Gartengrasmücke keine morphologischen Variablen ermitteln, die der  
437 Geschlechtsbestimmung dienen können. Beim Rotkehlchen konnte bei 87,2% der Vögel das  
438 Geschlecht korrekt bestimmt werden. Für Teichrohrsänger, Fitis und Rohrammer waren die  
439 entsprechenden Werte 77,6%, 89,4% und 86,4%. Wenn die Funktion einer logistischen

440 Regression von ähnlichen Studien aus Dänemark und Schottland auf den vorliegenden  
441 Datensatz von Rotkehlchen und Rohrhammern angewandt wurde, war die  
442 Geschlechtsbestimmung in weniger Fällen korrekt als mit der Funktion der eigenen Studie.  
443 Das gleiche traf zu, wenn beim Fitis die Flügellänge analog zu einer Studie in Großbritannien  
444 zur Geschlechtsbestimmung herangezogen wurde. Für diese Unterschiede gibt es mehrere  
445 mögliche Erklärungen: (1) die Regressionsmodelle sind für den Datensatz optimiert, aus dem  
446 sie ermittelt wurden, (2) es bestehen Unterschiede in den Messungen verschiedener Beringer,  
447 (3) es wurden verschiedene Altersklassen als Grundlage zur Ermittlung morphologischer  
448 Unterschiede herangezogen, (4) das Habitat um die jeweiligen Untersuchungsgebiete ist nicht  
449 vergleichbar, oder, am wahrscheinlichsten, (5) es bestehen Unterschiede in der Morphologie  
450 aufgrund von unterschiedlichem Zugverhalten. Wir empfehlen, dass morphologische  
451 Geschlechtsbestimmungen, wie sie in dieser Studie durchgeführt wurden, (1) nur  
452 populationspezifisch angewandt werden, (2) nur eine Altersklasse berücksichtigen sollten  
453 und dass (3) die ermittelten Kriterien von Zeit zu Zeit auf fortbestehende Gültigkeit überprüft  
454 werden sollten.

455

456

#### 457 **ACKNOWLEDGEMENTS**

458 We thank all volunteers at the Mettnau ringing station in 2007 that made this study possible.  
459 HE thanks J. Martens, Johannes-Gutenberg-University, Mainz, for his support and H. van  
460 Noordwijk for the help in the laboratory. We thank G. Segelbacher, Greg Conway and an  
461 unknown referee for discussing our manuscript. R. Holland kindly improved our English. All  
462 work related to the handling of birds (current permit No. 56-8853.17/01, Regierungspräsidium  
463 Freiburg) and blood sampling (permit O-05/39a, Regierungspräsidium Freiburg) was done in  
464 compliance with German law.

465

466

467 **REFERENCES**

468 Alatalo RV, Gustafsson L, Lundberg A (1984) Why do young passerine birds have shorter  
469 wings than older birds. *Ibis* 126:410-415

470 Bairlein F (1995) *Manual of Field Methods*. Wilhelmshaven

471 Bertellotti M, Tella JL, Godoy JA, Blanco G, Forero MG, Donazar JA, Ceballos O (2002)

472 Determining sex of magellanic penguins using molecular procedures and discriminant  
473 functions. *Waterbirds* 25:479-484

474 Berthold P (1969) Die Laparotomie bei Vögeln. Ein Hilfsmittel zur Geschlechtsbestimmung  
475 und zur Beobachtung des Gonadenzyklus. *Der Zoologische Garten* 37:271-279

476 Berthold P, Friedrich W (1979) Die Federlänge: Ein neues nützliches Flügelmaß. *Vogelwarte*  
477 30:11-21

478 Berthold P, Schlenker R (1975) Das "Mettnau-Reit-Ilmitz-Programm" - ein langfristiges  
479 Vogelfangprogramm der Vogelwarte Radolfzell mit vielfältiger Fragestellung. *Vogelwarte*  
480 28:97-123

481 Blondel J (2007) Coping with habitat heterogeneity: the story of Mediterranean blue tits. *J*  
482 *Ornithol* 148(Suppl 1):S3-S15

483 Campos F, Hernández M, Arizaga J, Mirando R, Amezcua A (2005) Sex differentiation of  
484 Corn Buntings *Miliaria calandra* wintering in northern Spain. *Ringling & Migration*  
485 22:159-162

486 Catry P, Lecoq M, Araújo A, Conway G, Felgueiras M, King JMB, Rumsey S, Salima H,  
487 Tenreiro P (2005) Differential migration of chiffchaffs *Phylloscopus collybita* and *P.*  
488 *ibericus* in Europe and Africa. *J Avian Biol* 36:184-190

489 Cuadrado M (1991) Wing length criteria for sex determination of Robins *Erithacus rubecula*  
490 wintering in southern Spain. *Ornis Svecica* 1:55-57

491 Dale S, Slagsvold T, Lampe HM, Lifjeld JT (2002) Age-related changes in morphological  
492 characters in the pied flycatcher *Ficedula hypoleuca*. *Avian Science* 2:153-166

493 Drost R (1938) Geschlechtsbestimmung lebender Vögel nach der Form der Kloakengegend.  
494 *Vogelzug* 9:102-105

495 Ellegren H, Fridolfsson A-K (1997) Male-driven evolution of DNA sequences in birds.  
496 *Nature Genetics* 17:182-184

497 Fiedler W (2005) Ecomorphology of the external flight apparatus of blackcaps (*Sylvia*  
498 *atricapilla*) with different migration behaviour. *Ann N Y Acad Sci* 1046:253-263

499 Gosler AG, Greenwood JJD, Baker JK, Davidson NC (1998) The field determination of body  
500 size and condition in passerines: a report to the British Ringing Committee. *Bird Study*  
501 45:92-103

502 Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds.  
503 *Molecular Ecol* 7:1071-1075

504 Hanski IK, Tiainen J (1991) Morphological variation in sympatric and allopatric populations  
505 of European Willow Warblers and Chiffchaffs. *Ornis Fennica* 68:1-16

506 Hipkiss T (2007) Can migrating Tengmalm's Owls *Aegolius funereus* be reliably sexed in  
507 autumn using simple morphometric measurements? *Ringing & Migration* 23:210-204

508 Hosmer D, Lemeshov S (1989) *Applied Logistic Regression*. John Wiley and Sons, New  
509 York

510 Jenni L, Winkler R (1994) *Moult and Ageing of European Passerines*. Academic Press,  
511 London

512 Kaiser A (1993) A new multi-category classification of subcutaneous fat deposits of  
513 songbirds. *J Field Ornithol* 64:246-255

514 Kalchreuter H (1971) Alters- und Geschlechtsbestimmung bei der Rabenkrähe (*Corvus*  
515 *corone corone*). *Vogelwarte* 26:106-112

516 Leisler B, Winkler H (2003) Morphological Consequences of Migration in Passerines. In:  
517 Berthold P, Gwinner E, Sonnenschein E (eds) Avian Migration. Springer, Berlin  
518 Heidelberg, pp 175-186

519 Lindström Å, Hedenström A, Pettersson J (1996) The autumn migration of Willow Warblers  
520 *Phylloscopus trochilus* in Sweden: results from a nation-wide co-operative project. *Ornis*  
521 *Svecica* 6:145-172

522 Lundberg A, Alatalo RH, Carlson A, Ulfstrand S (1981) Biometry, habitat distribution and  
523 breeding success in the pied flycatcher *Ficedula hypoleuca*. *Ornis Scandinavica* 12:68-79

524 Madsen V (1997) Sex-determination of Continental European Robins *Erithacus r. rubecula*.  
525 *Bird Study* 44:239-244

526 Markovets ML, Zduniak P, Reuven Y (2008) Differential sex- and age-related migration of  
527 Bluethroats *Luscinia svecica* at Eilat, Israel. *Naturwissenschaften* 95:655-661

528 Merom K, McCleery RH, Yom-Tov Y (1999) Age related changes in wing-length and body  
529 mass in the reed warbler *Acrocephalus scirpaceus* and clamorous reed warbler *A.*  
530 *stentoreus*. *Bird Study* 46:249-255

531 Michalak P (1995) Inter-habitat morphometric differentiation of male Willow Warblers  
532 *Phylloscopus trochilus*. *Ornis Fennica* 72:138-139

533 Morgan JH (2005) A computer method for resolving mixed normal distributions. *Ringling &*  
534 *Migration* 22:145-153

535 Nisbet ICT, Baird J, Howard DV, Anderson KS (1970) Statistical comparison of wing-lengths  
536 measured by four observers. *Bird Banding* 41:307-308

537 Norman SC (1983) Variations in wing-lengths of Willow Warblers in relation to age, sex and  
538 season. *Ringling & Migration* 4:269-274

539 Norman SC (1997) Juvenile wing shape, wing moult and weight in the family sylviiidae. *Ibis*  
540 139:617-630

541 Nowakowski JJ (2000) Long-term variability of wing length in a population of the Reed  
542 Warbler *Acrocephalus scirpaceus*. *Acta Ornithologica* 35:173-182

543 Møller AP, de Lope F (1999) Senescence in a short-lived migratory bird: age-dependent  
544 morphology, migration, reproduction and parasitism. *J Anim Ecol* 68:163-171

545 Ottvall R, Gunnarsson G (2007) Morphological and molecular sex identification of  
546 Redshanks *Tringa totanus*. *Bird Study* 54:127-129

547 Pettersson J (1983) Rödhakens *Erithacus rubecula* höstflyttning vid Ottenby. *Vår Fågelvärld*  
548 42:333-342

549 Schmitz P, Steiner F (2006) Autumn migration of Reed Buntings *Emberiza schoeniclus* in  
550 Switzerland. *Ringing & Migration* 23:33-38

551 Shealer DA, Clearey CM (2007) Sex determination of adult Black Terns by DNA and  
552 morphometrics: tests of sample size, temporal stability and geographic specificity in the  
553 classification accuracy of discriminant function models. *Waterbirds* 30:180-188

554 Smith JNM, Arcese P, Schluter D (1986) Song sparrows grow and shrink with age. *Auk*  
555 103:210-212

556 Svensson L (1992) Identification Guide to European Passerines. Stockholm

557 Tiainen J, Hanski IK (1985) Wing shape variation of Finnish and Central European Willow  
558 Warblers *Phylloscopus trochilus* and Chiffchaffs *P. collybita*. *Ibis* 127:365-371

559 Walton C, Walton P (1999) Sexing first year reed buntings *Emberiza schoeniclus* using  
560 biometrics. *Ringing & Migration* 19:327-331

561 Wernham C, Toms M, Marchant J, Clark J, Sirirwardena G, Baillie S (2002) The migration  
562 atlas – movements of the birds of Britain and Ireland. T & AD Poyser, London

563 Zink G (1973) Der Zug europäischer Singvögel I,1. Vogelwarte Radolfzell, Radolfzell

564 Table 1: Morphological measurements of five passerine species. Shown is the number of birds considered (male/female), mean (significant inter-  
 565 sexual differences according to a 2-tailed t-test with equal variances are indicated with \* when  $p < 0.05$  and \*\*\* when  $p < 0.001$ ) and standard  
 566 deviation (sd) of body mass, tarsus length, wing length and feather length of both sexes (determined by molecular sexing).

567

Species		Mass		Tarsus		Wing		Feather	
		male	female	male	female	male	female	male	female
<b>Robin</b>	Mean	16.31	15.98	25.16	25.18	74.68***	72.24***	55.69***	53.48***
<b>(54/40)</b>	Sd	0.93	0.78	0.68	0.74	1.44	1.12	1.27	1.02
<b>Reed Warbler</b>	Mean	11.16	11.24	22.63***	22.02***	66.58***	65.27***	50.48***	49.40***
<b>(68/79)</b>	Sd	0.63	0.62	0.65	0.56	1.36	1.32	1.383	1.32
<b>Garden Warbler</b>	Mean	18.76	18.82	19.78	19.63	78.16	77.76	58.53	58.24
<b>(47/33/)</b>	Sd	1.67	2.08	0.70	0.70	1.86	2.58	1.50	2.00
<b>Willow Warbler</b>	Mean	8.88*	8.13*	19.46***	18.51***	67.69***	63.39***	50.90***	47.76***
<b>(24/23)</b>	Sd	0.69	0.85	0.58	0.55	1.55	1.55	1.50	1.48
<b>Reed Bunting</b>	Mean	18.20*	16.55*	19.62*	18.96*	79.56***	74.90***	60.35***	56.20***
<b>(24/20)</b>	Sd	1.58	1.29	0.64	0.71	1.97	1.96	1.69	1.94



568 Table 2: Results of a logistic regression with sex as the dependent variable and all  
569 morphological characters as covariates. Shown are the B values ( $\pm$  SE) and the results of  
570 Wald statistics of the characters and the constant of the model for each species.

<b>Species</b>		<b>B</b>	<b>SE</b>	<b>Wald</b>	<b>p</b>
<b>Robin</b>	Mass	-0.214	0.355	0.364	0.546
	Tarsus	0.760	0.462	2.709	0.100
	Wing	-1.376	0.488	7.952	0.005
	Feather	-0.588	0.421	1.951	0.162
	Constant	117.070	26.317	19.789	<0.001
<b>Reed Warbler</b>	Mass	0.115	0.334	0.119	0.731
	Tarsus	-1.362	0.345	15.629	<0.001
	Wing	-0.513	0.222	5.344	0.021
	Feather	-0.165	0.199	0.685	0.408
	Constant	71.331	13.868	26.457	<0.001
<b>Garden Warbler</b>	Mass	0.086	0.138	0.394	0.530
	Tarsus	-0.324	0.376	0.740	0.390
	Wing	-0.070	0.207	0.114	0.735
	Feather	0.002	0.261	<0.001	0.995
	Constant	9.775	9.374	1.087	0.297
<b>Willow Warbler</b>	Mass	-0.118	0.703	0.028	0.867
	Tarsus	-0.618	1.015	0.371	0.543
	Wing	-1.389	0.626	4.917	0.027
	Feather	-0.034	0.542	0.004	0.956
	Constant	105.207	35.368	8.849	0.003
<b>Reed Bunting</b>	Mass	0.152	0.406	0.139	0.709
	Tarsus	-0.786	0.831	0.894	0.344
	Wing	-0.389	0.460	0.713	0.399
	Feather	-0.776	0.568	1.871	0.171
	Constant	87.646	26.341	11.071	0.001

571

Table 3: Probability of a bird with a certain combination of morphological variables being female. Bold line separates probabilities indicating that a bird is female ( $p > 0.5$ ) or male ( $p < 0.5$ ). Dark grey shading: indicates  $> 95\%$  probability of being female ( $p > 0.95$ ) or male ( $p < 0.05$ ). Light grey shading: indicates  $\geq 90\%$  probability of being female ( $p > 0.900$ ) or male ( $p \leq 0.100$ ).

a) Robin

		Feather-length [mm]													
		51.5	52.0	52.5	53.0	53.5	54.0	54.5	55.0	55.5	56.0	56.5	57.0	57.5	58.0
Wing-length [mm]	70.0	0.996	0.995	0.993	0.990	0.986	0.981	0.974	0.964	0.951	0.933	0.909	0.879	0.839	0.790
	70.5	0.993	0.991	0.987	0.982	0.976	0.967	0.954	0.938	0.915	0.886	0.849	0.802	0.745	0.678
	71.0	0.988	0.984	0.977	0.969	0.957	0.942	0.921	0.894	0.858	0.814	0.759	0.694	0.620	0.541
	71.5	0.979	0.971	0.960	0.946	0.926	0.901	0.867	0.825	0.772	0.710	0.638	0.559	0.478	0.397
	72.0	0.963	0.949	0.931	0.907	0.876	0.835	0.785	0.725	0.655	0.578	0.496	0.415	0.339	0.270
	72.5	0.936	0.913	0.883	0.845	0.797	0.739	0.672	0.596	0.515	0.434	0.355	0.284	0.223	0.171
	73.0	0.891	0.855	0.809	0.753	0.688	0.613	0.534	0.452	0.373	0.300	0.236	0.182	0.138	0.104
	73.5	0.820	0.767	0.703	0.631	0.552	0.470	0.390	0.316	0.250	0.193	0.147	0.111	0.082	0.061
	74.0	0.719	0.648	0.570	0.489	0.408	0.332	0.264	0.205	0.157	0.118	0.088	0.065	0.048	0.035
	74.5	0.589	0.508	0.426	0.349	0.278	0.218	0.167	0.126	0.094	0.070	0.051	0.038	0.027	0.020
	75.0	0.445	0.366	0.294	0.231	0.178	0.135	0.101	0.075	0.055	0.040	0.029	0.021	0.015	0.011
	75.5	0.309	0.244	0.189	0.144	0.108	0.080	0.059	0.043	0.032	0.023	0.017	0.012	0.009	0.006
	76.0	0.200	0.153	0.115	0.086	0.063	0.046	0.034	0.025	0.018	0.013	0.009	0.007	0.005	0.004
	76.5	0.123	0.092	0.068	0.050	0.036	0.027	0.019	0.014	0.010	0.007	0.005	0.004	0.003	0.002
	77.0	0.073	0.054	0.039	0.029	0.021	0.015	0.011	0.008	0.006	0.004	0.003	0.002	0.002	0.001
	77.5	0.042	0.031	0.022	0.016	0.012	0.008	0.006	0.004	0.003	0.002	0.002	0.001	0.001	0.001
	78.0	0.024	0.017	0.013	0.009	0.007	0.005	0.003	0.002	0.002	0.001	0.001	0.001	0.000	0.000
	78.5	0.014	0.010	0.007	0.005	0.004	0.003	0.002	0.001	0.001	0.001	0.001	0.000	0.000	0.000
	79.0	0.008	0.006	0.004	0.003	0.002	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000

b) Reed warbler

		Wing-length [mm]															
		62.5	63.0	63.5	64.0	64.5	65.0	65.5	66.0	66.5	67.0	67.5	68.0	68.5	69.0	69.5	70.0
Tarsus-length [mm]	20.4	0.993	0.991	0.987	0.983	0.977	0.968	0.956	0.941	0.921	0.894	0.860	0.817	0.764	0.702	0.631	0.554
	20.6	0.991	0.988	0.984	0.978	0.969	0.958	0.944	0.924	0.898	0.865	0.823	0.772	0.711	0.642	0.566	0.486
	20.8	0.989	0.984	0.979	0.971	0.960	0.946	0.927	0.902	0.870	0.830	0.780	0.721	0.652	0.577	0.498	0.419
	21.0	0.985	0.979	0.972	0.962	0.948	0.930	0.906	0.875	0.836	0.788	0.730	0.662	0.588	0.509	0.430	0.354
	21.2	0.980	0.973	0.963	0.950	0.933	0.910	0.880	0.842	0.795	0.739	0.673	0.599	0.520	0.441	0.364	0.294
	21.4	0.974	0.965	0.952	0.936	0.914	0.885	0.848	0.803	0.747	0.682	0.610	0.532	0.452	0.375	0.304	0.241
	21.6	0.967	0.955	0.938	0.917	0.890	0.854	0.810	0.756	0.692	0.621	0.543	0.464	0.386	0.313	0.249	0.194
	21.8	0.956	0.941	0.921	0.894	0.860	0.817	0.764	0.702	0.631	0.554	0.475	0.397	0.323	0.258	0.202	0.155
	22.0	0.944	0.924	0.898	0.865	0.823	0.772	0.711	0.642	0.566	0.486	0.408	0.333	0.267	0.209	0.161	0.122
	22.2	0.927	0.902	0.870	0.830	0.780	0.721	0.652	0.577	0.498	0.419	0.344	0.276	0.217	0.167	0.127	0.096
	22.4	0.906	0.875	0.836	0.788	0.730	0.662	0.588	0.509	0.430	0.354	0.285	0.224	0.174	0.133	0.100	0.075
	22.6	0.880	0.842	0.795	0.739	0.673	0.599	0.520	0.441	0.364	0.294	0.233	0.180	0.138	0.104	0.078	0.058
	22.8	0.848	0.803	0.747	0.683	0.610	0.532	0.452	0.375	0.304	0.241	0.187	0.143	0.109	0.081	0.060	0.045
	23.0	0.810	0.756	0.692	0.621	0.543	0.464	0.386	0.313	0.249	0.194	0.149	0.113	0.085	0.063	0.047	0.034
	23.2	0.764	0.702	0.631	0.554	0.475	0.397	0.323	0.258	0.202	0.155	0.118	0.088	0.066	0.049	0.036	0.026
	23.4	0.711	0.642	0.566	0.486	0.408	0.333	0.267	0.209	0.161	0.123	0.092	0.069	0.051	0.038	0.028	0.020
	23.6	0.652	0.577	0.498	0.419	0.344	0.276	0.217	0.167	0.128	0.096	0.072	0.053	0.039	0.029	0.021	0.015
23.8	0.588	0.509	0.430	0.354	0.285	0.225	0.174	0.133	0.100	0.075	0.055	0.041	0.030	0.022	0.016	0.012	
24.0	0.520	0.441	0.364	0.294	0.233	0.180	0.138	0.104	0.078	0.058	0.043	0.031	0.023	0.017	0.012	0.009	

c) Willow Warbler

<b>61.0</b>	0.999
<b>61.5</b>	0.997
<b>62.0</b>	0.994
<b>62.5</b>	0.987
<b>63.0</b>	0.974
<b>63.5</b>	0.946
<b>64.0</b>	0.894
<b>64.5</b>	0.800
<b>65.0</b>	0.655
<b>65.5</b>	0.475
<b>66.0</b>	0.301
<b>66.5</b>	0.170
<b>67.0</b>	0.089
<b>67.5</b>	0.044
<b>68.0</b>	0.022
<b>68.5</b>	0.010
<b>69.0</b>	0.005
<b>69.5</b>	0.002
<b>70.0</b>	0.001

Wing-length [mm]

d) Reed Bunting

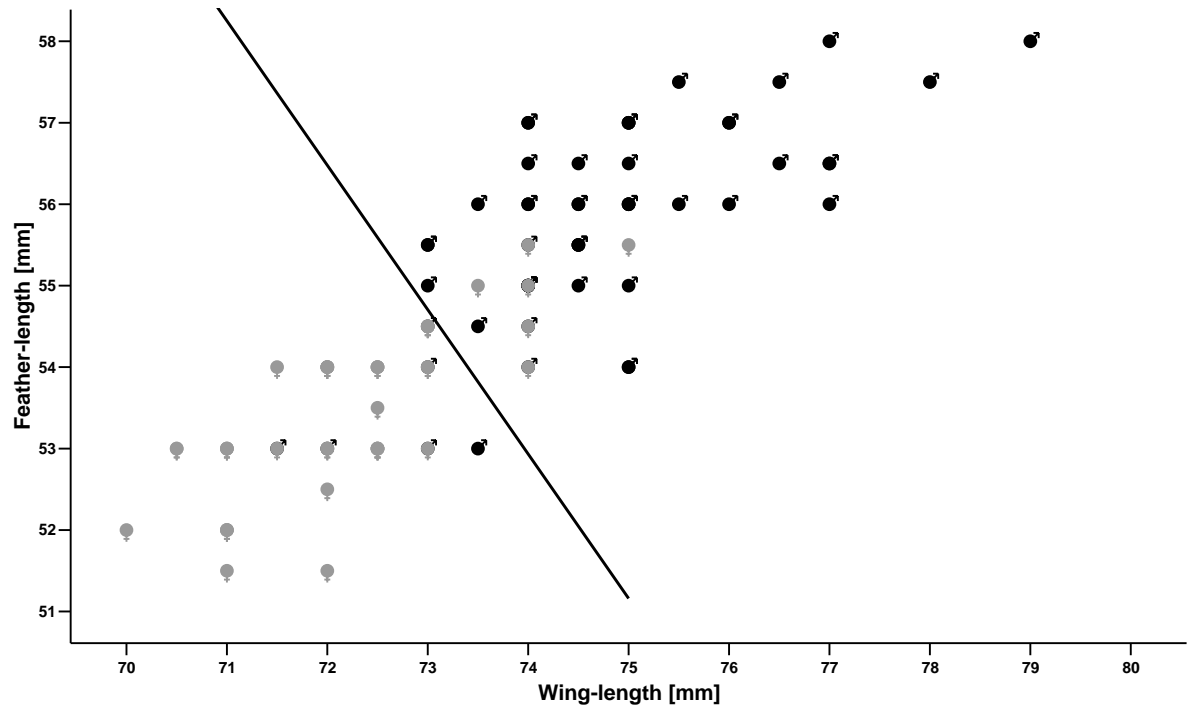
<b>71.5</b>	0.995
<b>72.0</b>	0.992
<b>72.5</b>	0.988
<b>73.0</b>	0.980
<b>73.5</b>	0.967
<b>74.0</b>	0.948
<b>74.5</b>	0.918
<b>75.0</b>	0.872
<b>75.5</b>	0.807
<b>76.0</b>	0.720
<b>76.5</b>	0.611
<b>77.0</b>	0.491
<b>77.5</b>	0.371
<b>78.0</b>	0.266
<b>78.5</b>	0.181
<b>79.0</b>	0.120
<b>79.5</b>	0.077
<b>80.0</b>	0.048
<b>80.5</b>	0.030
<b>81.0</b>	0.019
<b>81.5</b>	0.012
<b>82.0</b>	0.007
<b>82.5</b>	0.004
<b>83.0</b>	0.003

Wing-length [mm]

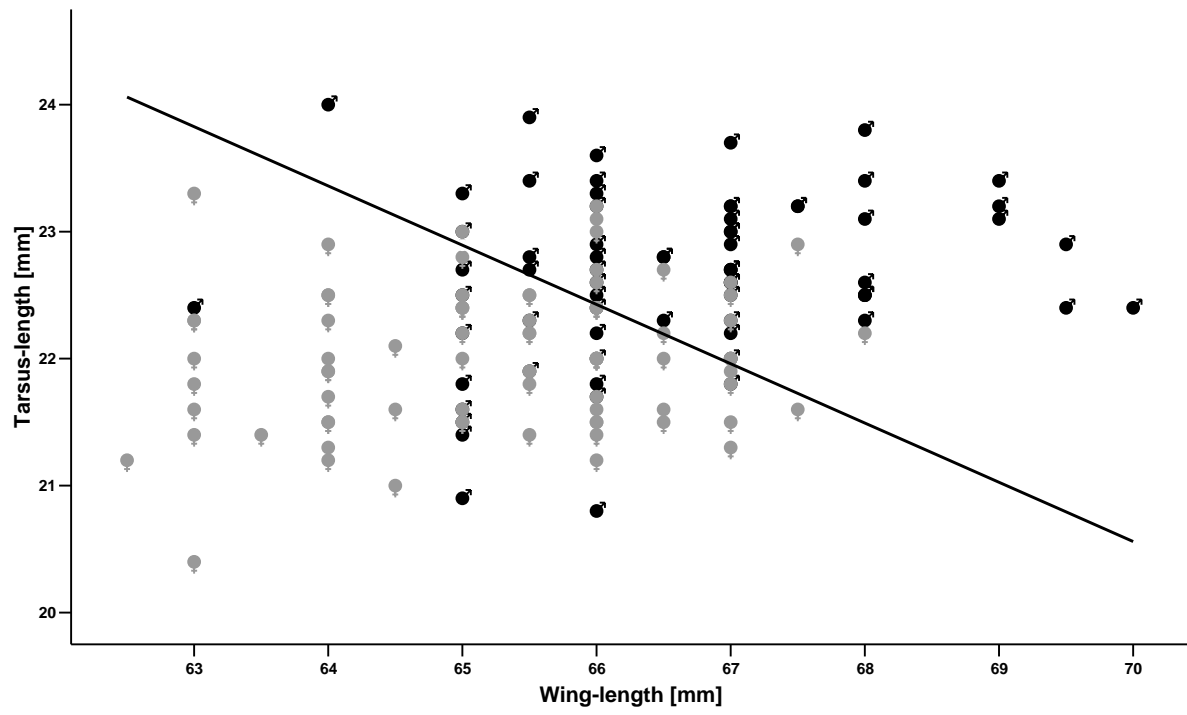
Figure legend:

Figure 1. Morphometric sex determination. Shown are the morphometric variables extracted by a forward conditional logistic regression and the distribution of the captured birds according to their sexes. ♀ and ♂ indicate the sex of the birds according to molecular sexing. Note that one symbol can indicate several individuals. The line shows the separation of the sexes according to the results of logistic regression. Birds below/to the left of the line were classified as females and birds above/to the right of the line were classified as males.

a) Robin

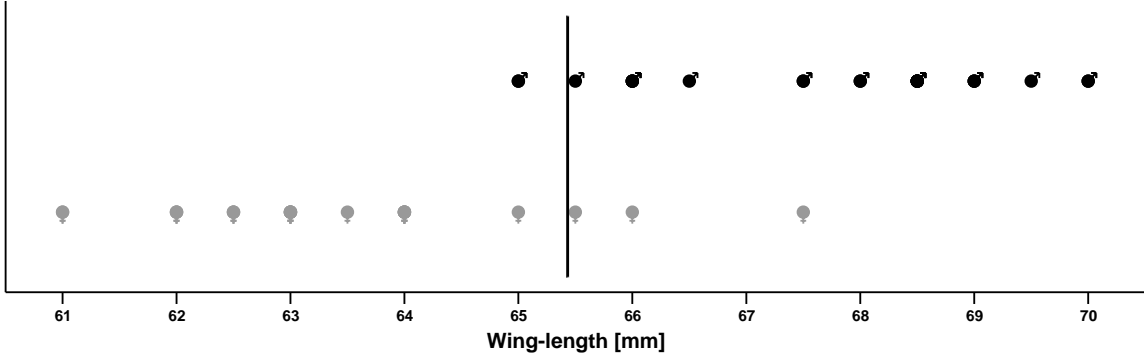


b) Reed warbler





c) Willow warbler



d) Reed bunting

