Sexing Common Snipe Gallinago gallinago in the field using biometric criteria

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The Common Snipe *Gallinago gallinago* is a common inland wader caught at many ringing stations across Central and Western Europe during autumn migration. But sex determination is not easy in live birds because the species has no clear sexual dimorphism. This is an impediment to studies of its ecology. During 2005–2009, we measured various external features of 1,343 Common Snipes caught at Jeziorsko Reservoir in central Poland (51°40'N, 18°40'E), to determine the utility of measurements in determining sex. We chose a random sample of 284 birds for DNA sexing from blood samples. Discriminant analysis of their measurements showed that the length of the outermost rectrix was the most useful trait in sexing adult Common Snipes. Bill-length, the length of the outermost rectrix and the distance between the tips of the two outermost rectrices were helpful in sexing first-year birds. Discriminant procedures allowed for the correct sexing of about 80% of adult and immature Common Snipes.

INTRODUCTION

Efficient ageing and sexing techniques are fundamental to studies of avian demography. These have been determined during a long history of bird hunting and catching in the course of which an array of methods have been developed based on birds' external features. Age-related plumage differences were first identified in passerines and these now allow effective recognition of several age and sex classes in many species (e.g. Svensson 1972). Similar ageing techniques were developed for waders during large ringing programmes in the 1960s in Western Europe (Piersma et al. 1987, Prater et al. 1977, van de Kam et al. 2004). However, easy sexing techniques applicable in the field are still lacking for many wader species that do not show clear sexual dimorphism. The development of molecular sexing of birds by analysis of DNA provides a means to obtain precise data for these species, but these methods require blood sampling and laboratory procedures (Dos Remedios 2010, Dubiec & Zagalska-Neubauer 2006). Combining the results of DNA sexing and several external traits, including biometrics, would enable the development of easier sexing techniques applicable in the field.

Plumage characteristics of Common Snipe Gallinago gallinago are complicated and individually variable (Włodarczyk et al. 2008). In consequence, ageing methods based on plumage patterns are difficult to apply on live birds caught for ringing (Kaczmarek et al. 2007, Schmitz 2007). Published information on sex-related differences in the external characteristics of Common Snipe, such as plumage or body size, are confusing. Some authors have emphasized problems with the efficient application of morphological traits for sexing (Meissner & Sciborski 2005, Włodarczyk *et al.* 2006). The main aim of this study was to develop an easy technique to sex Common Snipe using biometric data collected from live birds.

METHODS

This study was conducted at the Jeziorsko Reservoir in central Poland (51°40'N, 18°40'E). Common Snipes were caught during autumn migration between the end of July and mid-September in 2005–2009. The periods we worked in each year and the methods we used to catch waders are described in earlier publications (see Włodarczyk *et al.* 2005, 2007). During the 2005–2009 ringing seasons we caught 1,343 Common Snipes, including 1,056 first-year birds, 228 adults and 59 individuals whose age could not be determined due to their advanced stage of moult.

Blood samples were taken from 284 birds, 224 first-years (21% of our catch) and 60 adults (26% of the catch) randomly chosen from snipes that had not yet moulted their outermost rectrices. Birds with worn, missing or broken outermost rectrices were not sampled. Samples of $c.100 \ \mu$ l of blood were taken from the ulnar vein and stored in 96% ethanol. We used a sexing method based on the differences in the chromo-helicase-DNA-binding (CHD) gene between males and females (Griffiths *et al.* 1998). DNA was extracted using a Blood Mini kit (A&A Biotechnology). Specific amplification of CHD gene was performed, using primer pair 1237L/1272H

(Kahn et al. 1998). Polymerase chain reactions (PCR) were run with 9 µl of DNA template in 25 µl volume, containing 0.8 µl of each primer, 2 µl of 1.5 mM magnesium chloride and 12.5 µl of Sigma Ready Mix (Sigma-Aldrich) in a thermal cycler Mastercycler, Eppendorf. The thermal profile was: an initial denaturing step at 94°C for 2 min, followed by 40 cycles of 94°C for 30 sec, 53°C for 1 min 10 sec and 72°C for 2 min, with a last annealing at 48°C for 2 min and a final elongation at 72°C for 10 min. PCR products were developed on 3% agarose gel stained with ethidium bromide during 2.5hour long electrophoresis at 5 mV/cm, to ensure separation of two CHD gene alleles expected in females, which might differ in length by only up to 80 bp (Dubiec & Zagalska-Neubauer 2006). Under UV light we observed one band for males (only CHD-Z allele) and two bands for females (CHD-W and CHD-Z alleles). The sex-ratio was defined as the proportion of males in the whole sample.

Each bird sampled for molecular sexing was also measured in the field. The following measurements were collected: total wing length (maximum chord WL), head length (HL), tarsus length (TL), bill length (BL), length of bill from the tip to the proximal edge of the nostrils (Nalospi, NL), length of the outermost rectrix (R7), and the distance between the tips of the two outermost rectrices (R6-R7). Wing length and the length of the outermost rectrix were measured with a ruler to the nearest 1 mm. All the other measurements were taken with calipers to the nearest 0.1 mm. The distance between the tips of the two outermost rectrices was expressed as a negative number when R7 was longer than R6. Backward and forward stepwise discriminant analyses on all the measurements we took were used to identify a set of biometric traits suitable for sex determination. Results of discriminant analysis served to develop classification functions for both sexes and calculate classification scores for each bird. Each case was then classified into the sex group for which it had higher classification score. Deviation of the sex-ratio from parity was tested with the G-test. All analyses were performed using STATISTICA 6.0 software (StatSoft 2001).

RESULTS

The sex-ratio of the first-year Common Snipes was malebiased (0.73) and differed significantly from parity ($G_{1,224}$ = 50.19, p < 0.001). All the measurements differed significantly between the sexes, except for wing length, which was thus excluded from further analysis as being unlikely to contribute to sex recognition (Table 1). We performed forward stepwise discriminant analysis using all the other measurements. The following measurements significantly contributed to the sexing procedures and therefore were included in the classification functions 1 and 2 (Table 2): bill length (Wilks' Lambda: 0.77, p < 0.001), length of the outermost rectrix R7 (Wilks' Lambda: 0.79, p < 0.001), and the distance between the tips of the two outermost rectrices R6–R7 (Wilks' Lambda: 0.78, p < 0.001). A priori classification probabilities were the same for both sexes and the functions allowed for the correct sexing of 78.1% of the birds (83.3% of the females, 76.2% of the males). Backward stepwise discriminant analysis gave similar results.

As with the first-year birds, the sex-ratio of adults was also male biased (0.75) and significantly differed from parity $(G_{1.60} = 15.68, p < 0.001)$. All the measurements of adults differed significantly between the sexes, except for tarsus length, which was excluded from further analysis as being unlikely to contribute to sex recognition (Table 3). We performed forward stepwise discriminant analysis on all the other measurements. The following variables contributed significantly to the model and were included in the classification functions 3 and 5 (Table 2): wing length (Wilks' Lambda: 0.69, p = 0.025), bill length (Wilks' Lambda: 0.69, p = 0.022), and the length of the outermost rectrix R7 (Wilks' Lambda: 0.70, p = 0.014). A priori classification probabilities were the same for both sexes and the functions allowed correct sexing of 80.0% of the birds (93.3% of the females, 75.6% of the males). When we performed a backward stepwise discriminant analysis we left in the model only the length of the outermost rectrix (classification functions 4 and 6, Table 2), because it was the most

 Table 1.
 Measurements of male and female first-year Common Snipes caught on migration through Jeziorsko Reservoir, central Poland, between the end of July and mid-September, 2005–2009.

Measurement	Females (n = 60)		Males (n = 164)			
	\overline{x}	SE	\overline{x}	SE	t	р
Wing length (WL) [mm]	136.75	0.46	136.37	0.27	0.73	0.467
Head length (HL) [mm]	98.40	0.39	96.66	0.25	3.43	< 0.001
Bill length (BL) [mm]	69.90	0.36	68.01	0.23	3.95	< 0.001
Nalospi (NL) [mm]	62.56	0.34	60.93	0.23	3.50	< 0.001
Tarsus length (TL) [mm]	34.22	0.16	33.44	0.10	4.11	< 0.001
Outermost rectrix length (R7) [mm]	47.40	0.35	50.59	0.25	6.38	< 0.001
Distance between tips of rectrix 6 and rectrix 7 (R6–R7) [mm]	3.36	0.16	1.79	0.12	6.46	< 0.001

Table 2. Classification equations and percent of Common Snipes correctly sexed (BL = bill length, R7 = outermost rectrix length, R6-R7 = distance between the tips of the two outermost rectrices, WL = wing length). Sex determination is based on the equation that results in the highest classification score (see Methods).

Age group	roup Sex Classification equation		Percent correctly sexed		
First-year birds	Females Males	(1) $K_1 = 6.629*BL + 4.315*R7 + 4.488*(R6-R7) - 342.157$ (2) $K_2 = 6.405*BL + 4.558*R7 + 4.021*(R6-R7) - 337.396$	83.3% 76.2%		
Adults	Females	(3) K ₁ = 15.333*WL + 7.009*BL + 2.441*R7 - 1354.363 (4) K _{1a} = 5.311*R7 - 137.717	93.3% 80.0%		
	Males	(5) $K_2 = 15.650*WL + 6.697*BL + 2.754*R7 - 1391.807$ (6) $K_{2a} = 5.748*R7 - 161.186$	75.6% 80.0%		

Measurement	Females (n = 15)		Males (n = 45)			
	\overline{x}	SE	\overline{x}	SE	t	р
Wing length (WL) [mm]	135.80	0.66	138.73	0.43	3.49	< 0.001
Head length (HL) [mm]	99.59	0.79	97.01	0.47	2.76	0.008
Bill length (BL) [mm]	71.03	0.79	68.41	0.41	3.07	0.003
Nalospi (NL) [mm]	63.71	0.79	61.27	0.42	2.85	0.006
Tarsus length (TL) [mm]	34.06	0.43	33.58	0.15	1.36	0.179
Outermost rectrix length (R7) [mm]	51.60	0.65	55.84	0.49	4.57	< 0.001
Distance between tips of rectrix 6 and rectrix 7 (R6–R7) [mm]	0.46	0.39	-0.64	0.24	2.29	0.025

Table 3. Measurements of male and female adult Common Snipes caught on migration through Jeziorsko Reservoir, central Poland, between the end of July and mid-September, 2005–2009.

sexually dimorphic trait in adult Common Snipes (Wilk's Lambda = 1.00, p < 0.001). As with the previous functions, the length of the outermost rectrix allowed for the correct sexing of 80.0% of birds (80.0% of females and 80.0% males).

DISCUSSION

Sex determination in Common Snipe has been studied by several authors who used birds that had been hunted (Devort et al. 1986, Strandgaard 1986, Stronach 1979). Most studies have stressed the importance of two biometric traits: bill length and the length of tail feathers. Some authors have indicated that single measurements are adequate sexing criteria, for example the length of the outermost rectrix was suggested by Devort et al. (1986). Others proposed combinations of different external traits. Stronach (1979) suggested using the wing length in combination with the length and the width of the middle and the outermost tail feathers. Green (1991) used the outermost tail feather length and the bill length as sexing criteria, with an efficiency of about 81% of correctly sexed birds. Green (1991) focused on the morphometrics of adult snipes in Britain, so his method might have limited application to birds from continental populations. Another thorough analysis of biometric data was based on hunted specimens of Wilson's Snipe Gallinago delicata, a recently established Nearctic species, previously classified as a subspecies of the Common Snipe (McCloskey & Thompson 2000). Discriminant equations allowed the correct sexing of as many as 88% of birds using the following measurements: total length of outermost rectrix, length of the first secondary, length of the fifth and the tenth primaries. Equations developed by McCloskey & Thompson (2000) could not, however, be applied during field studies, because these calculations rely on the measurement of plucked rectrices and primaries. In contrast to some of the quoted papers, we used measurements collected from live birds and examined first-year and adult birds separately. The Common Snipes that migrate through Jeziorsko Reservoir originate from breeding populations across Central and Eastern Europe, as well as Asia (Minias et al. 2010). Therefore it seems likely that the equations we have developed can be used for the continental populations of Common Snipe from a wide geographical area.

Our results confirm the high utility of the length of the outermost rectrix in sex determination of Common Snipe. In this species the outer tail feathers play a major role during display flight and are therefore likely to be under strong sexual selection. Although some authors have suggested that both sexes perform drumming flights (Tuck 1972), recent observations have confirmed that this behaviour is confined to males (Green 1991, Rouxel 2000). Sexual selection is the primary factor responsible for the development of different traits in animals (Andersson 1994). In the Common Snipe, the

longer the tail feathers, the louder the sound that can be generated, which allows males to attract females more efficiently. The sound that is produced may also help males to establish and defend breeding territories. Protrusion of the outermost rectrices beyond the edge of the tail, commonly observed in adult males, may further strengthen drumming sound. In contrast, short outermost tail feathers are expected to increase the manoeuvrability of birds (Thomas 1993). In consequence, selective pressures acting on the outermost rectrices of male and female Common Snipes are likely to have opposite directions, which would produce a pronounced sexual dimorphism in this trait. Our results confirm the high efficiency of the combination of both the length of the outermost rectrix and the distance between the tips of the two adjacent outermost rectrices for sexing Common Snipes.

Another trait that contributed significantly to sexing efficiency was bill length, with females having longer bills than males. In many waders females are larger than males (Piersma 1996, van de Kam *et al.* 2004). Larger females can lay larger eggs, which provide more nutrient reserves for the developing embryos. Moreover, large females incubate large eggs more effectively than smaller females (Figuerola 1999). Sexual size-dimorphism may also have adaptive importance after the breeding season. Sexual dimorphism in bill length associated with similar differences in the body size allows the birds to exploit different types of prey and consequently reduces competition between the sexes.

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