Sexual Dimorphism in Plumage and Size in Black-Tailed Godwits Limosa Limosa Limosa

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Sexual dimorphism in plumage and size in Black-tailed Godwits *Limosa limosa limosa*

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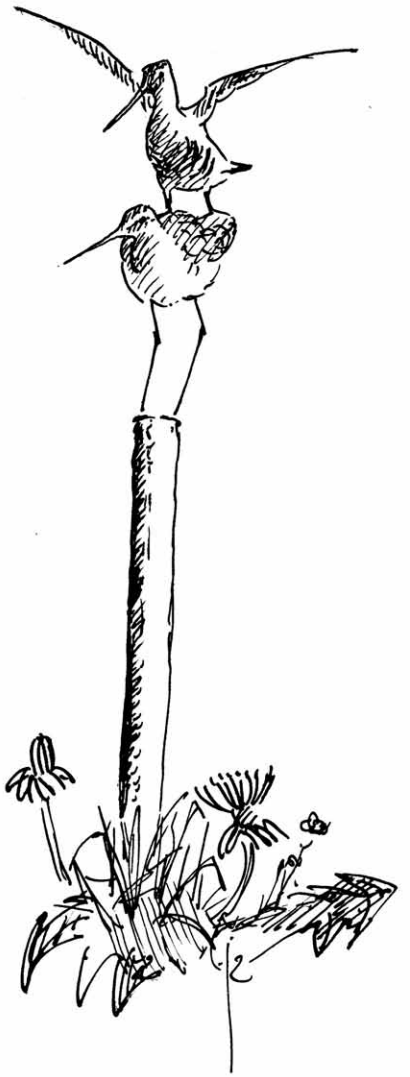

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Systematic sex-related differences in size and plumage are informative of sex-specific selection pressures. Here, we present an analysis of sexual dimorphism in body size and plumage of Black-tailed Godwits *Limosa limosa limosa* from a breeding population in The Netherlands. Molecular methods were used to unambiguously assign the sex of captured birds. To quantify breeding plumage, we developed nine plumage scores. These scores describe the intensity of orange in the breast plumage, the extent of black bars on the belly, the coverage and number of breeding feathers on the back, the conspicuousness of the white eye stripe, the extent of white plumage on the head, the percentage of orange colour in the bill and the percentage of white and black spots covering the neck. Most females were structurally bigger, and had a greater body mass. Nonetheless, we found a greater overlap in bill length between the sexes than expected on the basis of literature data: biometrics alone are not sufficient to correctly discriminate between the sexes. Black-tailed Godwits are sexually dimorphic mostly with respect to the amount of white spots on the neck, females being of lighter colour than males. In addition, females showed fewer black bars and less orange on the breast, had more white in the head and fewer and a smaller extent of breeding feathers on the back. Interestingly, we found a genotypic polymorphism on the sex-related CHD1 gene on the Z chromosome, commonly used for molecular sexing in birds. Males of the less frequent genotype had significantly more white in their plumage and had fewer black bars on their breast, while in females no differences between the two genotypes were found.

Key words: sexual size dimorphism, sexual plumage dimorphism, molecular sexing, meadowbirds, repeatability

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INTRODUCTION

Sexual dimorphism is the systematic phenotypic difference between females and males of the same species (Bennett & Owens 2002). In birds, the most obvious and abundant differences between the sexes are body size and plumage coloration. The extent and variation of sexual dimorphism is correlated with variations in mating systems, sex differences in parental care and the frequency of extra-pair paternity (Darwin 1871, Bateman 1948, Trivers 1972, Andersson 1994, Owens *et al.* 1995, Sandercock 2001). In waders with aerial display, small males are assumed to be better performers because small body size is thought to enhance agility, which in turn positively influences fitness (Jönsson & Alerstam 1990, Figuerola 1999). Together with a fecundity advantage for bigger females (Summers & Underhill 1991, Sandercock 1998), selection should lead to a female-biased dimorphism in size in these species (Jönsson & Alerstam 1990, Székely *et al.* 2004). It has been suggested that plumage quality informs potential mates about migratory condition, parasite resistance and age (Piersma & Jukema 1993, Piersma *et al.* 2001, Battley 2007). If these plumage traits have a genetic basis, it can lead to selection towards the more competitive sex being more ornate. Plumage may also function as camouflage in waders and other cryptic ground breeders (Heinsohn *et al.* 2005, Hill & McGraw 2006b). Thus, the sex that spends more time incubating often shows a more cryptic plumage. These mechanisms are expected to go hand in hand and can lead to a strong plumage dimorphism.

Black-tailed Godwits *Limosa limosa limosa* are one of the stronger sexually size-dimorphic wader species and show some degree of plumage dimorphism. Males are the smaller and the more colourful sex (Glutz von Blotzheim *et al.* 1985). Male nuptial display includes acrobatic and fast flights (Lind 1961). Pairs typically stay together for several years and both partners incubate the eggs and care for the young equally (Beintema *et al.* 1995). Two conditions are crucial to study sexual size and plumage dimorphism: (1) a reliable method to unambiguously identify sex independent of plumage and size characters; and (2) repeatable measurements describing size and plumage. When birds cannot be sacrificed and dissected, non-molecular sexing and studying sexual dimorphism always includes some degree of circular logic. Up to now, all studies reporting differences between sexes in Black-tailed Godwits of the nominate race *limosa* either used dissection and sex determination by gonads (Glutz von Blotzheim *et al.* 1985), discriminant function analysis (Groen & Yurlov 1999, Glutz von Blotzheim *et al.* 1985) or behavioural traits, size and plumage together (Groen 1993, Groen & Hemerik 2002) to sex adult birds. Gunnarsson *et al.* (2006) used molecular techniques to sex adult Icelandic Black-tailed Godwits *Limosa l. islandica*, a closely related subspecies.

In this paper, we report on the degree of sexual dimorphism in Black-tailed Godwits with respect to size measurements, body mass and plumage traits. In contrast to size measurements, the description and quantification of plumage is far from standardized in birds (Hill & McGraw 2006a). Here we defined nine plumage scores and test them for observer repeatability. To study sexual dimorphism, birds were sexed genetically and we then examined correlations between size and plumage variables and between the sexes. We tested for between year repeatability of all traits.

METHODS

Study area

Our study area (52°59'N, 5°24'E) in the province of Friesland, The Netherlands, consists of 300 ha extensively managed meadows and an adjacent wetland along the shore of the Lake IJsselmeer. This area, called Workumerwaard, is divided into two parts by a summer dike alongside Lake IJsselmeer. The inner part of about 215 hectares consists of 22 meadows separated by water-filled ditches. A paved road intersects the area. The meadows are managed according to agricultural nature management plans, which encompass mowing only after 8 or 15 June and no use of arti-

ficial fertilizer. Management is done by the provincial nature conservation organization 'It Fryske Gea' and by farmers. The outside part is a nature reserve and managed by It Fryske Gea. Entry is not allowed and mowing is limited to the summer dike. The area is not fertilized, but in summer cows and horses graze it in low density.

This study took place during the breeding season of godwits in March until June in the years 2004–2006. Every year, local volunteers of the meadowbird conservation society 'Fûgelwacht Warkum' searched the area thoroughly for godwit nests and reported approximate locations to us. We revisited the nests and determined exact positions with handheld Garmin GPS 12 to the nearest 2 m. Catching was scheduled only three days before the hatching day. The hatching date of a clutch was estimated by measuring the degree of buoyancy of the eggs which is related to the incubation stage (van Paassen *et al.* 1984, Beintema *et al.* 1995). When cracks were found in the eggs three days before the estimated hatching date, or when the chicks were audibly beeping from inside the eggs, catching attempts were undertaken with either a walk-in trap or an automatic fall-trap. Once a bird entered a walk-in trap and sat down on its nest, an observer started running towards it causing the bird to flee. The funnel shaped entrance prevented the bird from escape. The automatic fall-trap consists of two metal rings connected with mistnet fabric. Both rings rest on three metal poles that are placed around a nest. This construction allowed a bird to enter the trap from all sides. Once the bird sat down on its nest, the lower ring was released by a remote control and the bird was trapped. The two types of traps worked well though some individuals were easier to catch than others. We never observed nest abandonment after catching attempts.

Body size

Within 15 min after capture, birds were weighed to the nearest gram. The following body size dimensions were also measured: wing length (flattened and straightened, ±1 mm), bill length (exposed culmen, ±0.1 mm), total head length (±1 mm), tarsus length (±0.1 mm), and tarsus + toe length (tarsus plus mid-toe length without nail ±1 mm). We measured and weighed 70 female and 64 male Black-tailed Godwits.

Plumage

To quantify plumage, we took digital pictures of each bird with a resolution of 2272x1704 pixels. Photos were taken with Nikon Cool Pix 4500 cameras of the back, the breast and the head in profile (Fig. 1). This happened after the bird was colour-ringed so that it could be identified individually on the photo. For objective colour judgment, we added a grey card to every picture of the front part of the birds (Fig. 1).

We scored nine plumage variables (Table 1). The bars score describes the extent of black bars on the belly on a scale from one to five. Orange score is the intensity of orange a bird displays on the breast. Orange bill is the percentage of orange coloration in the bill in relation to the total bill length, with an accuracy of five percent. Eye stripe is the extent and intensity of the white eye stripe, on a scale from one to five. White in head is the percentage of white feathers covering the head in profile, with an accuracy of five percent. White spots is the percentage of the neck covered with white feathers, with an accuracy of ten percent. The black spots score is the percentage of the neck covered with black spots with ten percent accuracy.

Godwits, like many other waders, only partially moult into breeding plumage. This is most clearly visible on the back of a bird, where between few to all feathers can be moulted into breeding feathers. It is not known whether the remaining feathers are also moulted into a winter version, or not at all (Battley *et al.* 2004). Back score is the extent of breeding feathers covering the back of a bird, on a scale from one to five. Finally, the absolute number of breeding feathers on the back defines the variable breeding feathers; they were counted per single feather.

We had complete data on breeding plumage scores of 57 female and 53 male godwits. These numbers were lower than the ones used for size dimorphism analysis because we only used photos

Figure 1. Examples of the photos used to score breeding plumage of Black-tailed Godwits. Top row: ventral photo showing exemplary variation in bars score, orange score and white and black spots. Middle row: side views of godwits, used to score white head, eye stripe and orange bill score. Bottom row: back with extracted wing to score back score and count breeding feathers on back. Females: top left, middle right, bottom left.

Table 1. Descriptions and range of plumage scores for Black-tailed Godwits. See text for further explanations of these scores.

Plumage score	Range	Description
Bars	1–5	1= no black bars on breast and belly, 2= some, 3= normal extent of black bars, 4= until the legs, 5= black bars extend the legs and underneath the tail
Orange	1–5	1= winter plumage, 2= weak orange, 3= normal orange, 4 = dark orange-red, 5 = very dark red, like *L.l. islandica* on the breast and neck
Bill	0–100	percentage of orange in bill in relation to total bill
Eye stripe	1–5	1= no eye stripe, 2= barely visible, 3= normal extent from ear to bill, 4= broad, 5= very broad and long
White head	0–100	percentage of white in head
White spots	0–100	percentage of neck covered in white feathers
Black spots	0–100	percentage of neck covered in black feathers
Back	1–5	1= no breeding feathers on the back, 2= some breeding feathers, but less than 1/3 of the back covered, 3= 1/3 to 2/3 of the back covered, 4= more than 2/3 of the back covered, 5=total back covered
Breeding feathers	count	absolute number of breeding feathers on the back

on which birds could be scored unambiguously. To test for within-observer repeatability, JS scored photos twice with the identity of birds unknown to her. Repeatabilities were calculated following Lessells & Boag (1987), standard errors as described by Becker (1984). Observer repeatability was high; the lowest repeatabilities were 0.80 for orange score, 0.84 for eye stripe and 0.87 for orange in bill. All other plumage scores reached repeatabilities >0.90. Standard errors were low (≤ 0.08).

Molecular sexing

A blood sample of 20 μl was taken from the brachial wing vein before body size and plumage measurements were taken. The area around the vein was cleaned with a cotton ball dipped in ethanol. The blood was drawn from the puncture with a sterilized microcapillary tube. The sample was stored in 96% ethanol at –20°C for the first weeks and at –80°C thereafter. DNA was extracted in the laboratory using the chelex extraction method of Walsh *et al.* (1991). Birds were sexed following Griffiths *et al.* (1998). This method is based on the amplification of a supposedly neutral fragment of an intron on the conservative CHD1 gene located on the sex chromosomes. These fragments differ in base pair length between the Z and the W chromosome in most bird species. Males with ZZ genotype have two fragments of the same length, whereas females of the genotype ZW have two fragments of unequal length. Fluorescently labelled PCR products were separated on an ABI 377 automatic sequencer. Subsequently their length was determined using Genescan 3.1 software.

We observed a polymorphism on the Z chromosome; PCR products originating from this chromosome were either 374 or 378 basepairs in length. The PCR product of the W chromosome was 393 basepairs long. Birds with genotypes 374/378 basepairs and 378/378 basepairs were scored as males (genotype 374/374 basepairs was not observed) and birds with genotypes 374/393 basepairs and 378/393 basepairs were scored as females. To verify our results and as recommended by Dawson *et al.* (2001), we also used the method of Fridolfsson & Ellegren (1999), which consists of amplifying a different fragment of an intron on the same gene. These PCR products were separated on a 3.3% agarose gel. These fragments are also of different length on the Z and W chromosome. This confirmed our previous sex assignment and we did not find a polymorphism.

Statistics

To study how the different variables are correlated with each other with respect to sex, we entered standardized values of body mass and all size variables in a first analysis of principal components (PCA) and all plumage variables in a second PCA. The first two principle components with eigenvalues bigger than one were extracted. We plotted the score of the first (PC1) and second principal component (PC2) of each bird in a bicoordinate system with PC1 and PC2 as axes (Gabriel 1971, and see Battley *et al.* 2001 for an example). Additionally, the eigenvalue loadings of each variable were plotted in the same graph as a vector. The length and direction of these vectors reveal correlations between different variables. The smaller the angle between two vectors, the more both vectors correlate with each other. A longer vector indicates a

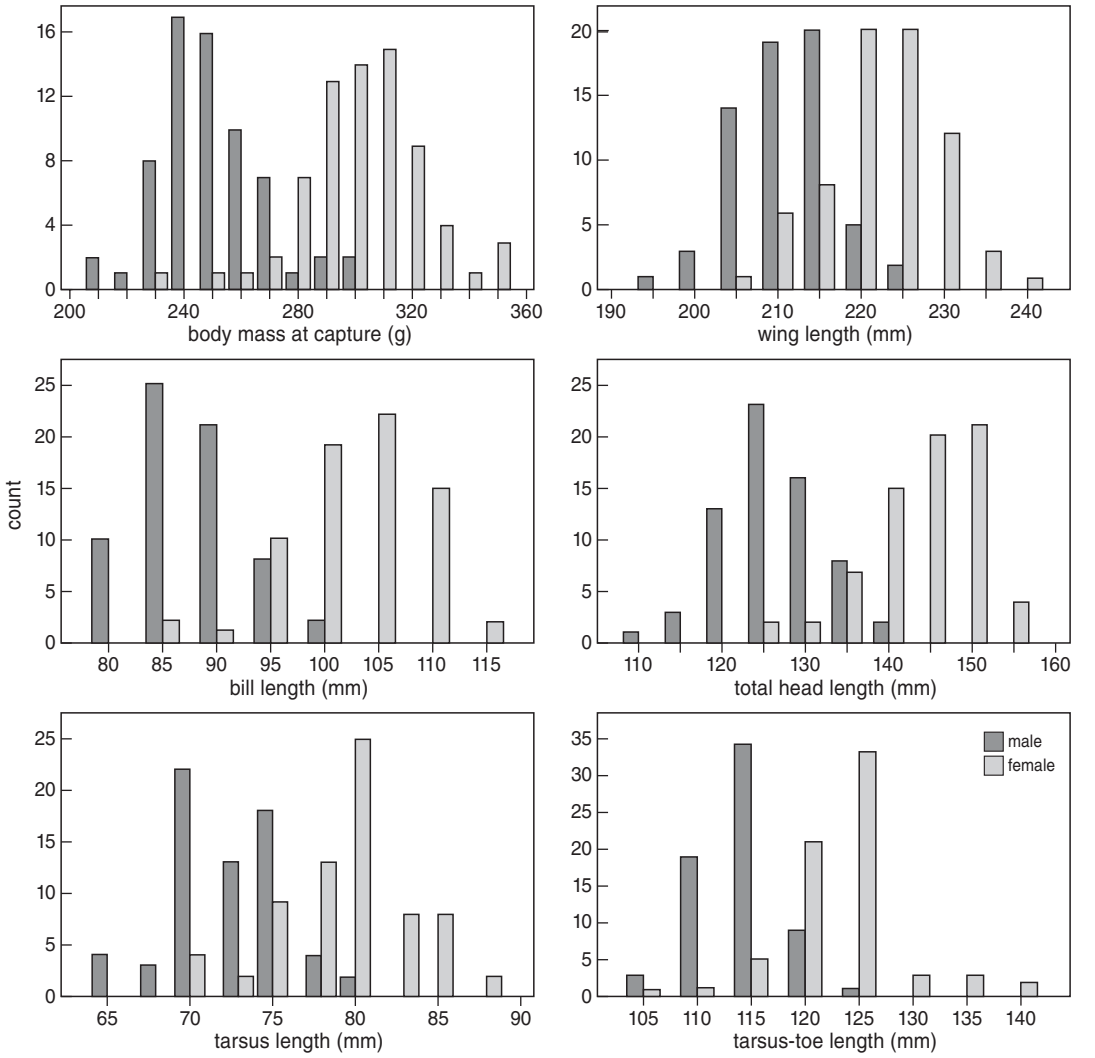

Figure 2. Histogram of body mass, wing length, bill length, total head length, tarsus length and tarsus-toe length in female and male.

better fit. The positions of males and females in this plot relative to the vectors indicate the strength and direction of the dimorphism for the respective variables. Individuals might show trait variation between years. We calculated individual repeatabilities between years separately for females and males. We additionally tested for differences in size and plumage in birds with the less frequent allele with 374 basepairs for both sexes. We used Statistica 7.0 for Microsoft Windows XP, SPSS 14.0 and R 1.14 for Mac OS X to calculate statistics.

RESULTS

Sexual dimorphism

All morphological variables, as well as body mass, showed a bimodal distribution (Fig. 2). Female godwits were bigger and heavier than males, with all variables significantly different between the sexes (Table 2). Wing length was the least dimorphic trait; the most dimorphic traits were body mass and bill length (Fig. 3). However, there was considerable overlap. The distributions of female body mass and size were skewed to the left; a few females were as small as males.

The distributions of the plumage scores were less distinctly bimodal. Female and male godwits differed significantly in orange score, white spots, back score, breeding feathers, bars score and white in head (Table 3). They did not differ with respect

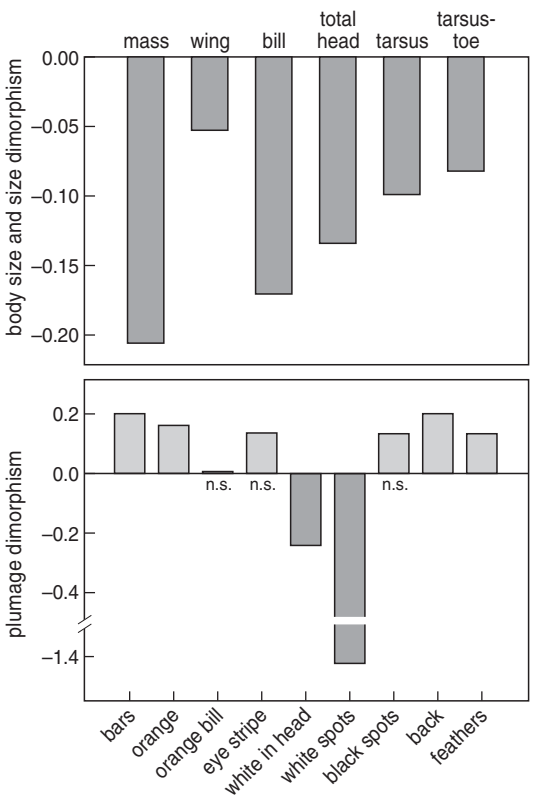

Figure 3. Sexual dimorphism of size and mass measurements (top) and plumage variables (bottom). Positive values indicate a male-biased dimorphism whereas negative values indicate females-biased dimorphism on the respective variable. N.s. indicates non-significant differences between females and males (statistics see Table 2 and 3).

Table 2. Sexual size dimorphism in the Black-tailed Godwit. Depicted are means with standard deviations and results of separate two-sided *t*-tests testing for differences between sexes, with *P*-values. Body mass in grams, lengths in mm.

Size variable	Females (n = 70)			Males (n = 64)			t	P
	Mean	SD	Range	Mean	SD	Range		
Body mass	305.50	21.15	231–354	254.88	17.70	219–304	14.95	<0.001
Wing	224.13	6.75	208–241	213.08	5.31	199–227	10.47	<0.001
Bill	105.04	6.09	86–117	89.77	5.51	81–102	15.17	<0.001
Total head	145.6	7.15	122–159	128.59	6.05	112–144	14.80	<0.001
Tarsus	80.38	3.93	72–89	73.33	3.35	66–81	11.14	<0.001
Tarsus toe	125.31	5.82	109–142	115.86	4.09	106–127	10.80	<0.001

to bill, black spots and eye stripe score. The biggest sexual dimorphism between males and females was found in white spots and in white head (Fig. 3). Males were more orange and were more ornate than females and had less white in their plumage. The dimorphism with respect to white spots was more than seven times as pronounced as that of any other variable (Fig. 3).

Table 3. Sexual plumage dimorphism in the Black-tailed Godwit. Depicted are means with standard deviations and results of separate Mann-Whitney-*U* tests testing for differences between sexes, with *P*-values.

Plumage variable	Females (n = 57)			Males (n = 53)			*U*	*P*
	Mean	SD	Range	Mean	SD	Range		
Bars	3.18	1.20	1–5	3.89	1.09	1–5	–3.11	0.02
Orange	3.36	0.78	2–5	4.06	0.86	2–5	–4.10	<0.001
Bill	66.00	9.17	50–80	66.20	7.97	50–80	–0.16	0.87
Eye stripe	2.88	1.20	1–5	3.23	1.36	1–5	-1.39	0.16
White head	46.23	24.66	5–90	35.75	26.26	0–90	–2.16	0.03
White spots	33.33	24.66	5–90	13.11	21.24	0–90	–5.03	<0.001
Black spots	9.47	11.98	0–50	8.83	12.11	0–50	–0.25	0.80
Back	2.66	0.97	1–5	3.34	1.04	1–5	–3.54	<0.001
Breeding feathers	23.40	11.48	2–52	28.4	12.30	2–54	–2.36	0.02

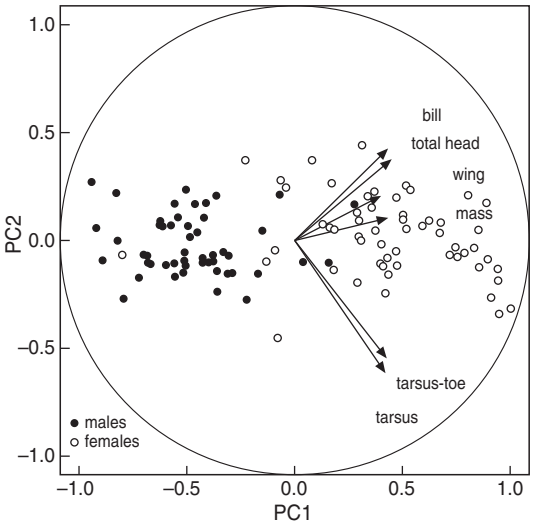

Figure 4. Relationships between biometric variables (wing length, tarsus length, tarsus-toe length, bill length, total head length) and body mass. The axes represent the first two principal components of the standardized data for all birds. Arrows represent the loadings of each variable on the first two components. Bill and total head length are strongly correlated with each other, as are tarsus and tarsus-toe length.

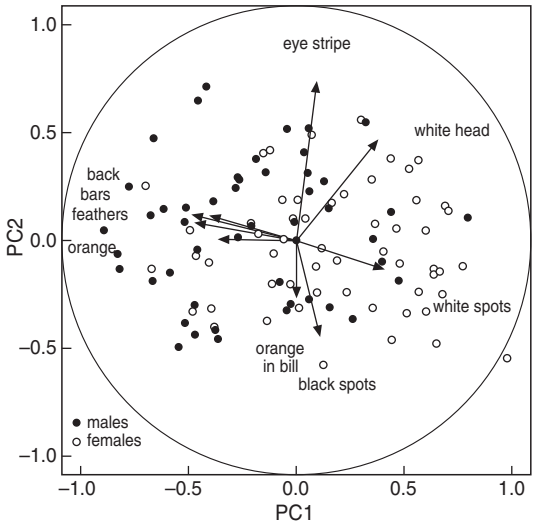

Figure 5. Relationships between the plumage variables (eye stripe, white in head, white spots, black spots, orange in bill, orange, bars and breeding feathers on back). The axes represent the first two principal components of a principal component analysis on the standardized data for all birds. Arrows represent the loadings of each variable on the first two components.

The first two principle components of a PCA explained 86% of the variation in morphological variables and body mass (PCA: $KMO = 0.85$, $\chi^2 = 873.94$, $P < 0.001$). To examine the correlation structure of the morphological variables, we produced a biplot figure (Fig. 4). Bill length and total head length were correlated with each other, as were tarsus length and tarsus-toe length. Wing length was slightly stronger correlated with bill length and total head length than body mass. However, body mass was least correlated with any of the size traits, confirming that body mass is best seen as an index of mass-corrected storage (van der Meer & Piersma 1994).

The first two principle components of a PCA of plumage traits explained 57% of the variation in the variables (PCA: $KMO = 0.75$, $\chi^2 = 355.35$, $P < 0.001$). A close inspection of the biplot figure for the plumage variables (Fig. 5) revealed a strong correlation between the plumage traits bars score, orange score, back score and breeding feathers. The amount of white spots was negatively correlated with these. Further, eye stripe score was negatively correlated with bill score, and to a lesser extent with black spots. The score white in head was not correlated with any of the other plumage traits.

Between-year variation

For both females and males that were recaptured in more than one year, size measurements were highly repeatable (Fig. 6). The only exception was tarsus length in females (0.41), which was due to one bird that had a tarsus length of 83.2 and 88.8 mm in two subsequent years, while tarsus-toe length stayed the same (128 mm), suggesting a measurement error. Body mass was highly repeatable in males between years (0.72), but not in females (–0.32). This was not due to a single outlier; the mean mass difference of a female between two consecutive years was 38 ± 23 g, whereas in males it was 7 ± 7 g (averages ± SD).

For females, orange in bill, white in head and white spots were most repeatable between years (0.82, 0.84, 0.79, respectively), whereas bars score, orange score and black spots were least repeatable (0.08, 0.34 and 0.37 respectively). In males, bars score, eye stripe, white head, back score and breeding feathers showed repeatabilities of >0.70, and bill >0.50. Orange score and black spots showed low repeatabilities, but in contrast to females, white spots was least repeatable.

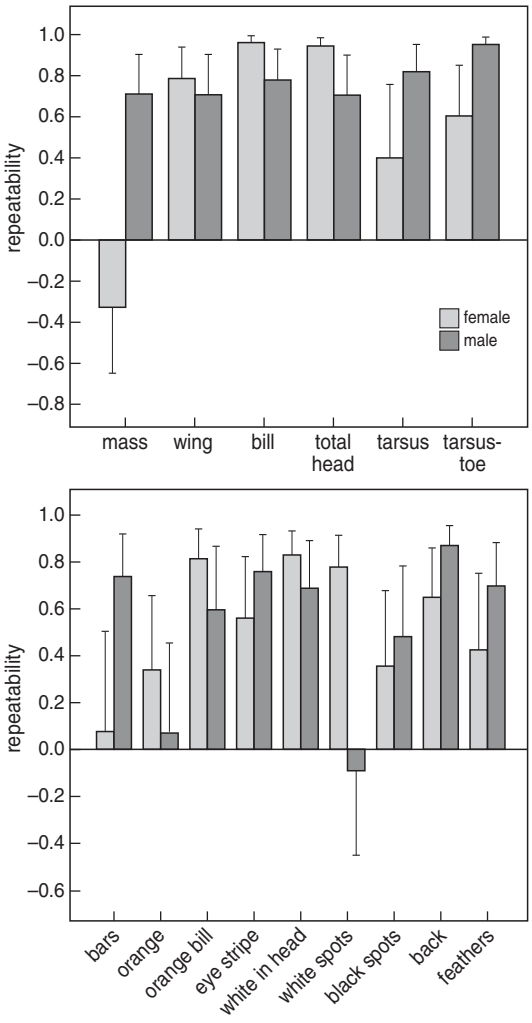

Figure 6. Repeatabilities within individual Black-tailed Godwits between years. Error bars indicate standard errors. Top: repeatability of mass and size measurements. $n = 6$ females and 7 males. Bottom: repeatability of plumage scores taken from pictures. $n = 6$ females and 7 males.

Genotype correlated plumage variation

We discovered a genotypic polymorphism on the Z chromosome when using P2P8 primers (Griffiths *et al.* 1998) for molecular sexing: 29% of all males had the less frequent 374/378 genotype. Nine percent of female godwits carried the 374/393 genotype, while all others had the 378/393 genotype. The frequencies did not deviate from Hardy-Weinberg equilibrium. We tested for differences in size and plumage between birds with and without the less frequent 374 basepair allele. We found no difference in size, body mass and plumage in females. In males, there was no difference in size and mass, but males with the genotype 374/378 scored higher on white spots (Mann-Whitney $U = 2.528$, $P = 0.012$, $n_{374/378} = 19$, $n_{378/378} = 34$) and lower on bars (Mann-Whitney $U = -2.69$, $P = 0.0072$, $n_{374/378} = 18$, $n_{378/378} = 34$) than males with the more common 378/378 genotype.

DISCUSSION

Sexual dimorphism

Male Black-tailed Godwits were confirmed to be smaller and lighter than females. Our mean values of body mass did not differ significantly from the averages presented by Glutz von Blotzheim *et al.* (1985) nor from Groen & Yurlow (1999) or Cramp & Simmons (1983), with data from godwits breeding in the same geographic range as our population, in Western Europe. However, in our population, we found three females with a bill smaller than the lowest given by Groen & Yurlow (1999), and even 10 females with a bill shorter than given in Glutz von Blotzheim *et al.* (1985). Several of our females had bills up to one cm shorter than the shortest bill length cited in this literature. Yet, sexing was unambiguous. The two female birds with the shortest bills were caught in two years and DNA-samples of both years confirmed female sex. Furthermore, both birds were paired to DNA-confirmed males. It is likely that the relatively small sample size ($n = 20$) of Glutz von Blotzheim *et al.* (1985) prevented them from detecting these females with a short bill length. Similarly, the discriminant function analysis used by Groen & Yurlow (1999) was probably not able to distinguish these females from males. All our variables, including the most dimorphic traits, showed large overlaps between males and females (Fig. 2, Table 2, Table 3). We therefore like to stress the importance of reliable molecular assays to unambiguously assign sex also in dimorphic species.

We established that female godwits have less black bars on the belly, a lower orange intensity, display more white in their head and neck and have fewer breeding feathers on their back. This is consistent with verbal descriptive reports in the literature (Cramp & Simmons 1983, Glutz von Blotzheim *et al.* 1985, Gunnarsson *et al.* 2006). We also found that the plumage traits back score, bars, breeding feathers, orange score and white spots were correlated with each other; a bird that scored high on one variable also scored high on the other, and low on white on neck. This indicates that colourful individuals are colourful with respect to all these traits. Further, the distinct sexual dimorphism on both size and plumage traits indicates some degree of sexual selection pressures on these traits.

Between-year variation

Between-year repeatability was relatively high for size measurements in males as well as in females. However, body mass was not repeatable in females, but it was in males. Female godwits may be able to vary their strategic nutrient energy stores responding to external variation and temporal needs.

For different plumage scores, individual repeatability between years differed considerably between the sexes. For both sexes, orange in bill, eye stripe, white in head, back score and breeding feathers were repeatable between years, whereas orange score and black spots were not. This may give hints as to the functions of the various plumage traits. In birds, multiple ornaments may communicate multiple messages (McGraw & Hill 2000, McGraw *et al.* 2002, Doucet & Montgomerie 2003). Traits that are not repeatable, but phenotypically plastic, may reflect a bird's state with

regard to variable condition, mediated by environmental variability. Such plumage traits may be a signal for body condition with respect to body mass, health status or parasite load (Piersma & Jukema 1993, Piersma *et al.* 2001). Senar and Quesada (2006) propose that these characters may play a role in sexual selection, too. In spite of a low repeatability of environmentally influenced traits, the relative ranks of the individuals could be maintained if the variation in the environment would be the same for all individuals.

There were marked differences between the sexes. The percentage of white spots on the neck was highly repeatable in females but not in males. It was the other way around in the bars score. These differences appear in the direction of the sexual dimorphism of the traits. These may be plumage traits that play an important role in sexual signalling or reflect differences in natural selection pressure between sexes. One sex might be able to afford plasticity in some traits but not in others (Piersma & Drent 2003). Sample size prevented us from testing for temporal trends within one season. However, as we caught all birds on their nest, plumages have consistently been scored at a time when moult must have been complete.

Genotype correlated plumage variation

We detected a polymorphism on the location used for unambiguous sexing, and found correlated variation in plumage traits of males. A similar polymorphism on the same gene occurs in four auklet species (*Aethia pygmaea, A. pusilla, A. cristatella, Cyclorrhynchus psittacula*, Dawson *et al.* 2001), Moorhens *Gallinula chloropus* (Lee *et al.* 2002), Red Knots *Calidris canutus* (A.J. Baker, pers. comm.) and Upland Sandpipers *Bartramia longicauda* (B.A. Sandercock, pers. comm.). Lee *et al.* (2002) found a reduced survival for Moorhen males with the rarer genotype, and B.A. Sandercock (pers. comm.) found a reduced reproduction in males with the rarer genotype. These finding are consistent with the idea that these plumage ornaments have a genetic basis. The primer set we used primes for a fragment of an intron of the CHD1 gene (Lee *et al.* 2002). CHD1 was proposed to be a regulatory gene and therefore thought to be conservative (Lee *et al.* 2002). Black-tailed Godwits are now the third species in which variation in this region is correlated to functional traits.

On agarose gels the genotypes 374/378, 374/393 & 378/393 all showed two distinct bands, but unfortunately it was impossible to discriminate between the three genotypes accurately. Consequently, males with the 374/378 genotype can be misinterpreted as females. In this study 29% of all males could have been missexed if only using agarose gels, as is widely practiced in molecular sexing of birds for studies in evolutionary ecology. Our study shows that especially in species with an overlap in body dimensions between both sexes, neither standard molecular protocols nor sex assignment based on body dimensions give constantly correct results. Molecular sex assignment should always be verified in future studies of topics related to sexual differences and sex ratios.

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SAMENVATTING

Dit artikel beschrijft de verschillen in lichaamsgrootte en verenkleed tussen mannetjes en vrouwtjes van de Grutto *Limosa limosa limosa*. De vogels werden tijdens de broedtijd gevangen in de Workumerwaard, Friesland. De sekse werd vastgesteld door bloed met moleculaire technieken te onderzoeken. Vrouwtjes waren groter en zwaarder dan mannetjes. Vooral in snavellengte bestond er echter een forse overlap tussen de seksen, die groter was dan op grond van literatuuronderzoek werd verwacht. Vermoed wordt dat in eerder onderzoek de kleinste vrouwtjes voor mannen zijn uitgemaakt. Geconcludeerd wordt daarom dat de seksen niet betrouwbaar te onderscheiden zijn door alleen naar lengtematen te kijken. Het verenkleed werd op grond van negen punten beoordeeld: de intensiteit van oranje op de borst, de hoeveelheid zwart op de buik, de bedekking en het aantal prachtveren op de rug, de duidelijkheid van de witte oogstreep, het wit op de kop, de hoeveelheid oranje op de snavel en de hoeveelheid witte en zwarte spikkels in de nek. De seksen verschilden het meest in de hoeveelheid witte spikkels in de nek, waardoor vrouwtjes lichter leken dan mannetjes. Bovendien hadden vrouwtjes minder zwarte strepen en minder oranje op de borst, en minder prachtveren op de rug, maar ze hadden juist meer wit op de kop. De onderzochte Grutto's bleken polymorf in een van de genen van het Z-chromosoom die vaak gebruikt worden om vogels moleculair te seksen. Mannetjes van het minst voorkomende genotype hadden meer witte spikkels in de nek en minder zwarte strepen op de borst dan mannetje van het andere type. Bij vrouwtjes was geen verschil in verenkleed aantoonbaar tussen beide genotypes. (DH)

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