No Assortative Mating Based on Size in Black Guillemots Breeding in the Canadian Arctic

LISHA L. BERZINS[1]*, H. GRANT GILCHRIST[2], AND GARY BURNESS[3]

[1]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, Ontario, K9J 7B8, Canada

[2]Environment Canada, Science and Technology Branch, National Wildlife Research Centre, Carleton University, Ottawa, Ontario, K1A 0H3, Canada

[3]Department of Biology, Trent University, Peterborough, Ontario, K9J 7B8, Canada

*Corresponding author; E-mail: lishaberzins@trentu.ca

Abstract.—The Black Guillemot (*Cepphus grylle*) is a monomorphic, socially monogamous member of the Alcidae. Although aspects of their breeding and foraging ecology have been extensively studied, less is known about possible sex-based differences in morphology, nor whether Black Guillemots mate assortatively based on body size. Using molecular techniques, we identified the sex of 26 male and 21 female Black Guillemots captured in the Canadian Arctic, and measured six external body measurements: outer tarsus length, wing length, culmen length, bill depth, head plus bill length and body mass to test for sexual size dimorphism (SSD) and assortative mating. Overall, males were 1.7% and 8% larger than females in outer tarsus length and bill depth, respectively. Within breeding pairs, bill depth was the most dimorphic trait. Despite these morphological differences no evidence of assortative mating based upon body size was found. Thus, mate choice for body size does not appear to be an underlying mechanism of SSD in bill depth in Black Guillemots. *Received 23 September 2008, accepted 23 January 2009.*

Key words.—Black Guillemot, discriminant analysis, mate choice, sexual size dimorphism.

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Among visually monomorphic birds, sexual size dimorphism (SSD) in morphological traits has been reported (see Casaux and Baroni 2000; Devlin *et al.* 2004 for examples). Often, the degree in SSD is greater in one morphological trait than in any other morphological variables (e.g. bill morphology, Genovart *et al.* 2003). Hypotheses concerning the evolution and maintenance of SSD (reviewed by Andersson 1994), suggests that SSD in bill morphology may reflect sex differences in feeding ecology, or sexual selection by either competition for mates or mate choice. Mate choice may indicate that individuals mate non-randomly, termed assortative mating, and this mating pattern for body size occurs most commonly among organisms (Crespi 1989).

Black Guillemots (*Cepphus grylle*) are monomorphic, socially monogamous, long-lived seabirds that occur in the Arctic (circumpolar distribution) and the Atlantic (sub-Arctic and boreal distribution) (Gaston and Jones 1998). Although aspects of their breeding and foraging ecology have been extensively studied (see Asbirk 1979; Cairns 1987; Petersen 1981; Frederiksen 1998 for examples), less is known about possible sex-based differences in morphology (but see Gaston and Jones 1998), or whether Black Guillemots mate assortatively based on some aspect of body size. Similarly, without morphological data from known-sex birds, there is no method to sex Black Guillemots based on external measurements (Frederiksen 1998).

In the current study, we captured male and female Black Guillemots breeding in the Canadian Arctic and measured six morphological traits, and subsequently confirmed their sex using molecular techniques. Our objectives were to test whether (1) SSD in Black Guillemots exists, (2) Black Guillemots mate assortatively based on some aspect of body size, and (3) also to develop a field method for sexing Black Guillemots using morphological measurements.

METHODS

Study Site and Field Methods

We studied Black Guillemots breeding on East Bay Island, Southampton Island, Nunavut (64°01'N, 81°47'W) during July-August 2007. We captured 47 in-

cubating individuals (26 males and 21 females) in nesting crevices at mid-incubation (day 15-16 post-laying). All individuals were weighed with a Pesola spring scale (±0.25 g), but we only report body mass for birds that incubated their natural clutch size and were not used in an ongoing experimental manipulation (N = 25). Also, we measured outer tarsus length (tibiotarsal articulation and tarsometatarsus bone), culmen length (length of unfeathered bill), bill depth (at unfeathered base of bill) and head plus bill length (distal tip of bill to back of skull). All measurements were taken using Vernier calipers (±0.1 mm). Flattened wing length (carpal joint to the tip of the longest primary feather) was measured with a steel wing ruler (±1 mm). All measurements were performed on the individual's right side by the same observer (LLB).

From all captured birds, a blood sample was collected from the brachial vein with a non-heparinized syringe, and approx. 25 µL of blood was applied on DNA FTA filter paper (Fisher Scientific, # 09923340) for subsequent molecular genetic sexing.

Molecular Sexing Techniques

Genomic DNA was extracted from the FTA filter paper using a Qiagen kit (DNeasy Blood & Tissue kit, # 69506) following the manufacturer's protocol, except that AE buffer was replaced with $TE_{0.1}$ buffer (10mM Tris and 0.1 mM EDTA [pH 8.0]). DNA was quantified by fluorescence using PicoGreen (in the Natural Resources DNA Profiling and Forensic Center, Trent University, ON, Canada).

Amplification of genomic DNA by polymerase chain reaction (PCR) was performed in 20 µL final volume containing 1x PCR buffer, 0.2 mM dNTPs, 0.4 µg/µL BSA (bovine serum albumin), 1.5 mM $MgCl_2$, 0.3 µM of each P8 (Invitrogen, # 58171579) and P2 (Invitrogen, # 58171577) primers, 0.05 U/µL Taq polymerase (Invitrogen, # 18038-042), and 10 ng (5 ng/2 µL) of genomic DNA. PCR was performed in a MJ Research thermal cycler (PTC-225) under the following PCR cycling protocol: an initial denaturing step at 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 50°C for 1 min, and 72°C for 1 min. A final extension cycle at 60°C for 45 min, followed by 4°C for 1 hr completed the PCR.

PCR products were separated by electrophoresis in 1.5% agarose gels stained with ethidium bromide and visualized under ultraviolet light. All negative controls (lysis, extraction and PCR) used in the study were negative for DNA contamination. We sexed both members of a breeding pair when possible, and in all cases we identified one male and one female (N = 42 birds).

Statistical Analysis

Data were approximately normally distributed, and the variances were approximately homogeneous. We performed independent t-tests to test for differences between known-sex males and females of the colony in all morphological traits. Within pairs where both members were captured (N = 20 pairs), we calculated an index of dimorphism (DI) for each morphological trait by dividing the mean value of a trait for males by the mean value for females (where a value of 1 indicates monomorphism).

To test whether Black Guillemots mated assortatively based on morphological traits, we tested correlations among all morphological traits between members of a pair using a Pearson correlation.

To test whether known-sex individuals could be discriminated as male or female, we used a forward stepwise discriminant function analysis (DFA) on five morphological traits: outer tarsus length, wing length, culmen length, bill depth and head plus bill length. Wilk's Lambda test statistic was calculated to test whether the sexes could be discriminated. Non-significant morphological traits (MANOVA: $P > 0.05$) were removed from the model by the DFA. A jackknife classification was then performed to obtain an unbiased classification of sex (see Chardine and Morris 1989). We calculated the cut-off discriminant score of the discriminant function as the midpoint between the mean discriminant scores for males and females (following van Franeker and ter Braak 1993).

All analyses were conducted using STATISTICA (v 7.0), with the exception of the DFA (including the jackknife classification), which used SYSTAT (v 11.0) (SYSTAT Software Inc. 2004). Comparisons were considered statistically significant at $P \leq 0.05$. In all tables, the mean is presented with the standard deviation (SD) and 95% confidence limits (CL).

RESULTS

Sexual Size Dimorphism

Overall, males were significantly larger than females for two morphological traits: outer tarsus length and bill depth (both $P < 0.05$, Table 1). Sexes did not differ in wing length, culmen length, head plus bill length or body mass (Table 1).

Table 1. Morphological measurements of male and female Black Guillemots.

	Males			Females			Difference	
Trait	N	Mean ± SD	95% CL	N	Mean ± SD	95% CL	t	P
Outer tarsus	26	36.4 ± 0.9	36.0-36.7	21	35.8 ± 0.8	35.4-36.1	2.4	0.02
Wing length	26	163.3 ± 3.1	162.0-164.5	21	164.7 ± 3.5	163.1-166.3	1.5	0.1
Culmen	26	26.0 ± 1.0	25.6-26.4	21	25.5 ± 1.2	25.0-26.1	1.4	0.2
Bill depth	26	10.1 ± 0.4	9.9-10.2	21	9.3 ± 0.4	9.1-9.5	36.1	<0.001
Head plus bill	26	76.3 ± 1.2	75.8-76.8	21	75.6 ± 1.7	74.7-76.2	1.9	0.06
Body mass[a]	14	380.7 ± 21.8	368.2-393.3	11	385.7 ± 20.8	371.7-399.7	0.6	0.6

[a]Body mass was used only for a subset of birds not used in experimental manipulation, see text for details.

Within pairs, the most dimorphic trait was bill depth, and the difference between the sexes was 0.4 mm (Table 2); although 26% of females were either the same size or larger than males in bill depth within a pair.

Assortative Mating

We found no evidence of assortative mating based on the six morphological traits measured; no morphological traits were correlated within pairs (Table 2).

Discriminant Function Analysis of Sex

The forward-stepwise DFA indicated that males and females could be reliably discriminated based on morphological traits (Wilk's Lambda = 0.4, $F_{2,44} = 33.3$, $P < 0.0001$, N = 47). Bill depth and wing length were selected by the DFA as the best morphological traits to predict sex, and the DFA correctly classified 91% of individuals whose sex had been determined genetically (100% of males, N = 26; 81% of females, N = 17; 19% of females were classified as male, N = 4). With the unbiased jackknife classification, the percent of correctly classified individuals remained at 91%. The discriminant function equation that correctly classified most individuals was:

$$D = (-2.80 \times \text{bill depth}) + (0.20 \times \text{wing length}) - 5.67. \quad (1)$$

The cut-off discriminant score for this function was: D = -0.04, where birds with a D > -0.04 are estimated to be female, whereas those with a D < -0.04 are estimated to be male. Male and female canonical scores of the discriminant function are presented in Fig. 1.

DISCUSSION

We found that male and female Black Guillemots differed in bill depth and outer tarsus length, with males being generally larger. Within pairs, bill depth was the most dimorphic trait. However, we found no evidence of assortative mating for any of the

Table 2. Descriptive statistics, dimorphism index (DI) and correlation coefficient for morphological traits between members of a breeding pair of Black Guillemots.

Trait	N	Mean ± SD	95% CL	DI m/f [a]	Correlation r	Correlation P
Outer tarsus						
Male	20	36.2 ± 08	35.7-36.5	1.01	-0.06	0.80
Female	20	35.7 ± 0.8	35.3-36.0			
Wing length						
Male	20	163.8 ± 2.6	162.6-165.0	1.00	0.12	0.63
Female	20	164.6 ± 3.6	162.9-166.3			
Culmen						
Male	20	26.0 ± 0.8	25.7-26.4	1.02	-0.18	0.45
Female	20	25.5 ± 1.3	25.0-26.1			
Bill depth						
Male	20	10.1 ± 0.4	9.9-10.3	1.09	-0.30	0.20
Female	20	9.3 ± 0.4	9.1-9.5			
Head plus bill						
Male	20	76.4 ± 0.9	76.0-76.8	1.01	0.36	0.12
Female	20	75.5 ± 1.7	74.7-76.3			
Body mass [b]						
Male	9	382.8 ± 20.3	367.2-398.4	0.99	0.20	0.61
Female	9	386.1 ± 19.1	371.4-400.8			

[a] Dimorphism index calculated as male/female ratio, where 1.0 = monomorphism.
[b] Body mass was used only for individuals not subject to experimental manipulation, see text for details.

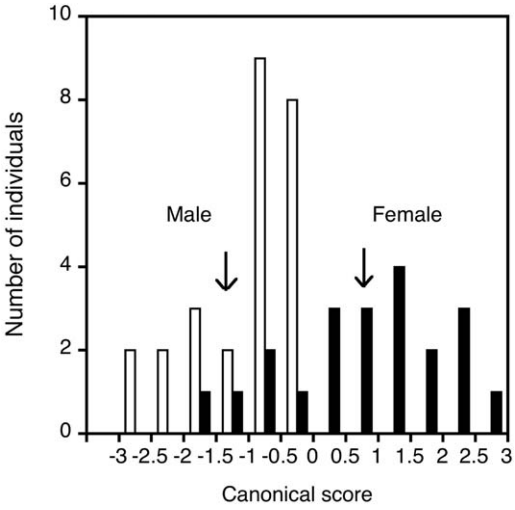

Figure 1. Frequency distribution of Black Guillemot canonical scores of the discriminant function. The mean canonical score for females is 1.34 and males is -1.08. The mean scores are depicted by an arrow. □ Males (N = 26); ■ Females (N = 21).

morphological traits. A lack of assortative mating in Black Guillemots based on size alone is perhaps not surprising given the structural similarity between males and females (the most dimorphic trait [bill depth] was only 8% larger in males). Nonetheless, assortative mating based on size has been reported for three other alcids: Razorbills (*Alca torda*), Atlantic Puffins (*Fratercula arctica*) (Wagner 1999) and Least Auklets (*Aethia pusilla*) (Jones and Montgomerie 1991). In contrast to Black Guillemots, Razorbills and Atlantic Puffins have ornamented bills; therefore, positive assortative mating for bill morphology in these species may reflect mate choice for ornaments (as previously proposed by Wagner 1999). If assortative mating occurs among Black Guillemots, it may be based on ornamental traits such as plumage (Jones and Montgomerie 1991) or foot color, rather than a structural trait such as tarsus length. Overall, SSD in bill depth in Black Guillemots is likely not the result of mate choice.

SSD in bill morphology has been hypothesized to reflect sex differences in foraging behavior (Lauro and Nol 1995; González-Solis 2004). However, it is not known whether sex differences in foraging exist in Black Guillemots. Such differences do not appear in Atlantic alcids (Moody and Hobson 2007 and references therein), although they do appear in Norway (Lorentsen and Anker-Nilssen 1999). We hypothesize that one mechanism underlying SSD in the bill depth of Black Guillemots may be territorial defence. Black Guillemots bite and bill-jab opponents when defending their nesting and roosting territories (Asbirk 1979), and a greater bill depth may confer an advantage when defending territories. Fighting and competing among males has been proposed as the mechanisms driving SSD in bill morphology (Stewart 1992; Babbit and Frederick 2007). However, further study in Black Guillemots on known-sex individuals is needed because both sexes may be involved in defending nesting territories, consistent with other alcids (Birkhead 1985).

For Black Guillemots, bill depth combined with wing length generated a discriminant function that accurately classified the sex of 91% of individuals. When applying the discriminant function to other populations of Black Guillemots, we recommend that investigators incorporate the use of this discriminant function and derive a cut-off score for the measurements of their specific population (following van Franeker and ter Braak 1993). Also, we recommend one observer perform all morphological measurements to eliminate inter-observer differences as the degree of dimorphism in Black Guillemots is small.

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