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ORIGINAL ARTICLE

Sexual size dimorphism and discriminant functions for predicting the sex of Atlantic Puffins (*Fratercula arctica*)

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Abstract Assortative mating is an important aspect of mate choice, especially in species where both sexes express ornamentation. Such ornaments could function as signals of individual quality and could result in individuals mating with partners of similar quality. We tested for assortative mating by measuring 63 pairs of Atlantic Puffins (*Fratercula arctica*) at two Canadian colonies (Gull Island, Witless Bay, Newfoundland and Labrador; and Machias Seal Island, New Brunswick), and constructed a function to predict the sex of puffins from Witless Bay. Male and female puffins have similar plumage, and both sexes have fleshy rosettes at the base of their bill, which are supposedly ornaments. We also examined changes in measurements over time in 5–30-year-old puffins recaptured at Machias Seal Island. Our discriminant function correctly predicted the sex of 88 % of puffins from Witless Bay. Overall, males were larger than females in all measurements, but within pairs, some females were larger in 4–27 % of individual measurements. We found no evidence of positive assortative mating or of assortative mating by rosette size, and rosette area did not increase with age. The importance of puffins' rosettes as indicators of quality requires further investigation.

Keywords Atlantic Canada · Atlantic Puffin · Discriminant function · Mate choice · Sexual size differences

Zusammenfassung

Geschlechtsbezogener Größendimorphismus und Diskriminanzfunktionen zur Geschlechtsbestimmung bei Papageitauchern (*Fratercula arctica*)

Assortative Paarung ist ein wichtiger Aspekt der Partnerwahl. Das gilt besonders für Arten, bei denen beide Geschlechter ornamentale Merkmale aufweisen. Solche Ornamente könnten als Signale individueller Qualität fungieren und dazu führen, dass Individuen sich mit Partnern ähnlicher Qualität verpaaren. Wir untersuchten die Frage assortativer Paarung, indem wir 63 Papageitaucherpaare (*Fratercula arctica*) aus zwei kanadischen Kolonien (Gull Island, Witless Bay, Neufundland und Labrador sowie Machias Seal Island, New Brunswick) vermaßen, und entwickelten eine Funktion zur Vorhersage des Geschlechts bei den Papageitauchern von Witless Bay. Bei Papageitauchern haben Männchen und Weibchen ähnliches Gefieder und beide Geschlechter tragen an der Schnabelbasis fleischige Rosetten, die vermutlich ornamentale Funktion besitzen. Außerdem betrachteten wir die zeitlichen Veränderungen der Maße bei 5–30 Jahre alten Papageitauchern, die auf Machias Seal Island wiedergefangen wurden. Unsere Diskriminanzfunktion konnte bei 88 % der Papageitaucher von Witless Bay das Geschlecht

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korrekt vorhersagen. Generell waren die Männchen in allen Maßen größer als die Weibchen, innerhalb der Paare waren allerdings manche Weibchen in 4–27 % der Einzelmaße größer. Es gab keine Belege für eindeutig assortative Paarung. Es gab keine Hinweise auf assortative Paarung anhand der Rosettengröße; die Rosettenfläche nahm mit dem Alter auch nicht zu. Die Bedeutung der Rosetten als Qualitätsmerkmale bei Papageitauchern bedarf weiterer Untersuchungen.

Introduction

Mate choice is just one component of sexual selection, but it has important evolutionary consequences (Andersson and Iwasa 1996). It can evolve by selection for a phenotypic characteristic that indicates quality (Møller and Jennions 2001), or a bias for a feature that first evolves under natural selection and is then favoured by mate choice (Endler and Basolo 1998; Ryan 1998). Ornamentation in animals has long been puzzling within the context of natural selection, because the survival benefit conferred by ornaments is not always clear (Jones et al. 2004). This problem was addressed by sexual selection theory, where preference for ornaments evolved due to the benefits of choosing the fittest mate (Darwin 1871; Fisher 1930). In species that are sexually dimorphic with respect to ornaments, the unornamented sex, generally female, can increase its fitness by using ornaments as honest indicators of quality and viability when choosing among multiple males (Møller and Jennions 2001; Pradhan and Van Schaik 2009). Though it is most common for one sex to be ornamented, with the unornamented sex doing the choosing, sexually monomorphic seabirds provide a way to study mate choice in species where both males and females are ornamented (Andersson 1994).

In cases of ornamentation in both sexes, there are often subtle differences in ornament and body size. Individuals may use these differences themselves in mate choice, which can result in non-random mating based on size (size assortative mating; Fairbairn 2007). These sexual size differences (SSD) can be the product of sexual or natural selection, and can be used to determine the sex of individuals without meticulous behavioural observations, dissection, or molecular genetic analysis (Jones 1993; Devlin et al. 2004; Gonzalez-Solis 2004).

In monomorphic species, the presence of ornaments may be due to direct mutual sexual selection by both males and females for that trait, or the presence of the trait in females could be due to genetic correlation (Darwin 1871; Lande 1980). Female ornamentation is often related to offspring quality, but overall, the pattern is equivocal (Nordeide et al. 2013). There is evidence of mutual sexual selection in Crested Auklets (*Aethia cristatella*), where both males and females have responded to experimentally accentuated ornaments in the opposite sex (Jones and Hunter 1993, 1999). Johnstone et al. (1996) proposed that if the male and the female similarly provide parental care, then both sexes are involved in mate choice. Mutual mate choice may lead to assortative mating if males and females are choosing mates based on the same selection criteria (Johnstone 1997). However, there are many studies of monomorphic ornamented species that have found no evidence of assortative mating with respect to ornamentation (Murphy 2008; Berzins et al. 2009; van Rooij and Griffith 2012).

The ability to use a discriminant function to quantify the probability of an individual being male or female is important for answering many ecological and conservation questions, but is often ignored in species with similar-appearing sexes (Forero et al. 2002; Peck and Congdon 2006; Welcker et al. 2009; Robinson et al. 2011). The size of some species may also change over time, independent of age-related changes (Sheridan and Bickford 2011; Barrett et al. 2012; Tomassini et al. 2014), making older discriminant functions obsolete or older measurements incompatible with newer functions, though the variance introduced by this directional change is likely inconsequential.

Male and female Atlantic Puffins (*Fratercula arctica*) are identical in appearance, but like several other auks, show SSD in many characters (Nelson 1981; Barrett et al. 1985; Jones 1993; Lowther et al. 2002; Grecian et al. 2003; Jakubas and Wojczulanis 2007; Berzins et al. 2009).

Our objectives were to (1) determine whether puffins mated assortatively, using measurements of birds from two colonies separated by more than 1100 km; (2) determine whether measurements changed with age; (3) derive a discriminant function to separate sexes based on field measurements for puffins in Witless Bay (such a function already exists for Machias Seal Island, but the latitudinal gradient in body size means it is not applicable in Witless Bay); and (4) compare the measurements and function to those from other sites.

Methods

We captured both adults of 26 pairs of breeding puffins on Gull Island, Witless Bay, Newfoundland and Labrador (47.26°N, 52.77°W), in 2012, and 37 pairs breeding on Machias Seal Island, New Brunswick (44.50°N, 67.10°W), in 2008–2011. As none of these birds was of known age, we analysed a second dataset of 211 known-age puffins marked as chicks on Machias Seal Island to determine the effect of age on body and ornament size.

Individuals were captured in active burrows during early incubation, and had at least two bill grooves and a completely bare brood patch, suggesting they were breeders

(Harris 1979). We measured flattened straightened right wing length (from the carpal joint to the tip of the longest primary (Witless Bay) or natural wing chord (Machias Seal Island) to the nearest 1 mm with a stopped ruler. Using dial callipers, we measured the following to the nearest 0.1 mm: total head + bill (distance from the tip of the bill to the back of the head in a straight line), culmen (Witless Bay: distance from the base of the feathers to the tip of the bill, including the cere; Machias Seal Island: distance from the distal edge of the cere to the tip of the bill), bill depth (greatest vertical distance), bill length (from the tip of the bill to the beginning of the gape, excluding the rosette; subset of 26 individuals), rosette length (longest axis), and rosette width (shortest axis). All measurements followed Harris (1979), except culmen, which followed "length of exposed culmen" in Baldwin et al. (1931). We used Eq. 6 from Friars and Diamond (2011; correcting a typographic error in the original) to provisionally classify puffins from Machias Seal Island as male or female:

$$D = (0.28 \times \text{bill depth}) + (0.36 \times \text{head} + \text{bill}) - 39.53 \tag{1}$$

where D is the discriminant function score.

Finally, we took a small blood sample from the brachial vein stored on #2 filter paper (Whatman's plc, London; Witless Bay) or feather sample (Machias Seal Island) for genetic sexing. Samples were kept in individual envelopes in a sealed container with desiccant or frozen at −4 °C. Blood samples were frozen upon return from the field.

Sex determination

DNA from Witless Bay puffins was extracted from filter paper using the DNeasy Blood and Tissue Kit (Qiagen Inc., Toronto, ON, CA), following the manufacturer's protocol. Sex was determined by amplifying the chromo-helicase-DNA 1 gene (CHD1), which is linked to both sex chromosomes, but when amplified with primers 2550F and 2718R (Fridolfsson and Ellegren 1999), different-sized bands are produced for each sex chromosome. Polymerase chain reactions (PCR) were carried out in a volume of 25 µL containing 1× Promega PCR master mix (Promega Corp., Madison, WI, USA), 2.5 µM of each primer, and approximately 25 ng of DNA. Thermal cycling was performed in a GeneAmp 9700 (Applied Biosystems Inc., Foster City, CA, USA) under the following conditions: 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 45 °C for 1 min, and 72 °C for 1.5 min, and a final extension at 72 °C for 5 min. PCR products were stained (EZ-Vision Three DNA dye: Amresco LLC, Solon, OH, USA) and visualized on 2 % agarose gel by electrophoresis.

Puffins from Machias Seal Island were first classified as male or female based on an existing discriminant function (Friars and Diamond 2011). For those individuals where the discriminant function gave an ambiguous classification (i.e., <75 % certainty; 23/74 individuals), we sexed the birds genetically. DNA was extracted using the method from Devlin et al. (2004). The proximal end of the calamus was removed from two breast feathers and placed in a solution of 10 µL extraction buffer (0.1 M NaCl, 0.05 M Tris–HCl, 0.01 M Na2EDTA, pH, 8.0), 1 µL 10 % Tween, and 1 µL Proteinase K (Devlin et al. 2004). Samples were incubated at 65 °C for 120 min (vortexed every 30 min), and Proteinase K was denatured at 95 °C for 10 min. Sex was determined as for puffins from Witless Bay (Fridolfsson and Ellegren 1999). For each sample, 1 µL of extracted DNA was added to a 9-µL solution (1 µL 10× ThermoPol buffer (New England Biolabs, Ipswich, Massachusetts, USA), 2 µM each DNTP, 0.5 µL 10 µM $MgSO_4$, 5.42 µL dH_2O, 0.5 µL 5 µM each "forward" and "reverse" primer, and 0.08 µL ThermoPol Taq). The DNA was amplified starting with a 180-s denaturation step at 95 °C, followed by 40 cycles (30 s at 95 °C, 30 s at 50 °C, and 45 s at 72 °C), with a final extension at 72 °C for 180 s. Amplified regions were visualized on agarose gel (30 ml TBE and 0.3 g agarose) using 0.5 µL SYBR Safe (Invitrogen, Carlsbad, California, USA).

For both techniques, individuals with two bands (CHD1 W and CHD1Z, approximately 450 and 650 bp in size, respectively) were scored as female, and those with one band (CHD1 W band only) as male.

Statistical methods

We first determined whether puffins exhibited SSD using a multivariate analysis of variance followed by trait-specific univariate analyses, and tested for assortative mating using Pearson's correlation corrected for the false discovery rate (Benjamini and Hochberg 1995; García 2004). We calculated a dimorphism index (DI) for each measurement within pairs and at the population level (using means) using:

$$(\text{Male}/\text{female}) - 1 \tag{2}$$

where positive values indicate that males are larger, negative values that females are larger, and zeroes that the sexes are identical in size (Fairbairn 2007). We did not consider mass as a character by which puffins mate assortatively, as it fluctuates over the breeding season.

Using the separate sample of known-age puffins comprising 211 captures of 190 birds captured throughout the breeding season and measured between 1997 and 2011 on Machias Seal Island, we determined whether their measurements changed with age, which could indicate assortative mating by age, indirectly resulting in assortative mating by size. Birds were measured by 37 observers over the 15 years. We used only data for birds ≥ 5 years old, their age of first breeding, and before which birds are growing relatively rapidly (Harris and Wanless 2011). We used a linear mixed model with the package lme4 (Bates et al. 2014) in R 3.1.2 (R Core Team 2014) to determine whether there was a relationship between bird age (range 5–30 years old) and mass, wing chord, culmen, bill depth, head + bill, and rosette size (the elliptical area defined by the rosette's length and width), treating bird ID as a random factor. Models were compared with a null (intercept-only) model using a likelihood ratio test. The sex of known-age birds was unknown. Differences within and among observers were likely very small relative to the value of the measurements, and the number of individuals (>15) were unlikely to introduce any directional change in measurements, but would increase variance, making our estimates of change conservative (Arnqvist and Mårtensson 1998).

We built a linear discriminant function for puffins breeding in Witless Bay using the function *lda* in the package MASS in R 3.1.2 (Ripley et al. 2012; R Core Team 2014) using backward selection and leave-one-out cross-validation, with wing length, culmen, bill depth, and head + bill as predictors of sex. We excluded rosette measurements because they are soft tissues, and their small size makes them more prone to measurement error (Arnqvist and Mårtensson 1998; Perktaş and Gosler 2010). This discriminant function allowed us to calculate the discriminant score, *D*, for varying probabilities of assignment to a particular sex (Fig. 1). We used Cohen's κ (Cohen 1960) to test whether assignment from the discriminant function was better than chance. The assumptions of both multivariate and discriminant function analyses were tested with Shapiro–Wilk's (1965) and Levene's (1960) tests. All statistical analyses were conducted in R 3.1.2 (R Core Team 2014), and results were considered significant at $p < 0.05$.

Results

Discriminant function for Witless Bay puffins

Data were normally distributed (Shapiro–Wilk test, all $W = 0.950$–0.984, all $p = 0.23$–0.71) and lacked heteroscedasticity (Levene's test, all $F_{1,50} = <0.01-1.31$, all $p = 0.26$–0.95), satisfying the statistical assumptions for our analyses.

Our full model (i.e., all variables retained by backward selection: wing length, culmen, bill depth, and head + bill) correctly classified 88 % of individuals (85 % of females, 92 % of males), and achieved an 87 % correct classification rate during cross-validation. This was significantly better than chance (Cohen's $\kappa = 0.77$, $p < 0.001$). The following function correctly classified 46/52 individuals (Fig. 1):

$$D = (0.85 \times \text{culmen}) + (0.98 \times \text{head} + \text{bill}) + (0.82 \times \text{bill depth}) + (0.28 \times \text{wing length}) - 196.3. \quad (3)$$

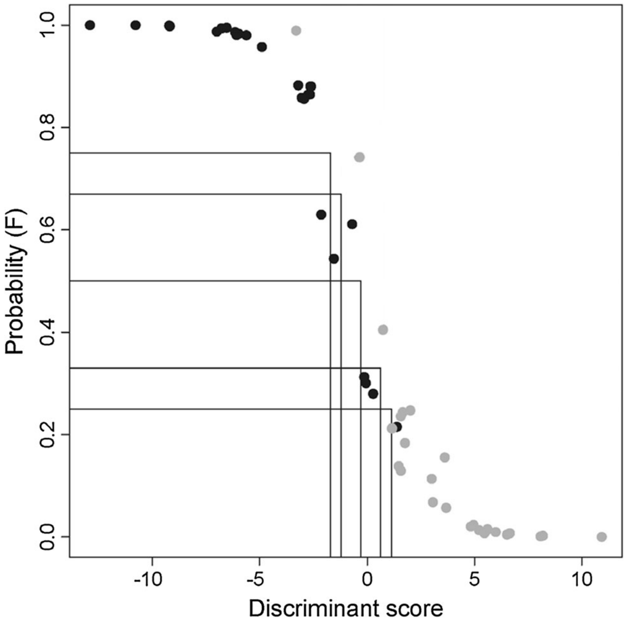

Fig. 1 Atlantic Puffins from Witless Bay, Newfoundland, can be separated by sex according to four measurements. Discriminant values (*D*) for the probability of classifying an individual as female (p_F) were −1.73 ($p_F = 0.75$), −1.22 ($p_F = 0.67$), −0.30 ($p_F = 0.50$), +0.61 ($p_F = 0.33$), and +1.12 ($p_F = 0.25$). Females = black, males = gray. Individuals where $D > -0.30$ were classified as male, while those with $D < -0.30$ were classified as female

Sexual size dimorphism

At the population level, male puffins from Witless Bay were larger than their female counterpart in every measurement (MANOVA, $F_{1,24} = 5.95$, $p = 0.001$; *t* tests, all $p < 0.01$), and the dimorphism index ranged from 0.03 to 0.34 (Table 1). Within pairs, males were 55 g heavier, with wing chords 4 mm longer, head + bill 2.7 mm larger, culmen 3.2 mm longer, and bills 2.6 mm deeper and 1.2 mm longer on average. Rosettes were 1.2 mm longer, 1.4 mm wider, and 17.3 mm^2 larger on average in males.

On Machias Seal Island, males were larger than females (*t* tests, all $p < 0.01$), and the degree of dimorphism ranged from 0.03 to 0.23 (Table 2). Within pairs, males were 30 g heavier, had wings 4 mm longer, head + bill 2.8 mm longer, and culmen 2.6 mm longer, with bills 2.1 mm deeper. Males also had rosettes 1.0 mm longer, 0.9 mm wider, and 13.1 mm^2 greater than females.

Table 1 Male Atlantic Puffins from Witless Bay, Newfoundland and Labrador, were significantly larger than their female partner in all measurements; $n = 52$ (26 males, 26 females)

Measurement	Female	Male	Male–female difference (range)	% Females larger	DI	t_{25}	p
Body mass (g)	425 ± 30	480 ± 25	−30 to +140	8	0.13	6.86	<0.001
Wing length (mm)	169 ± 4	173 ± 3	−3 to +12	23	0.02	3.90	<0.001
Culmen (mm)	47.0 ± 1.5	50.1 ± 1.5	−0.1 to +5.3	4	0.07	11.09	<0.001
Bill depth (mm)	34.7 ± 1.7	37.3 ± 1.9	−4.1 to +8.6	19	0.07	4.51	<0.001
Bill length (mm)[a]	29.7 ± 1.1	30.9 ± 1.4	−2.2 to +3.1	12	0.04	2.87	0.014
Head + bill (mm)	77.4 ± 1.9	80.1 ± 1.6	−3.4 to +6.4	12	0.03	5.90	<0.001
Rosette length (mm)	9.2 ± 0.8	10.5 ± 1.0	−0.1 to +2.6	4	0.15	6.61	<0.001
Rosette width (mm)	7.1 ± 0.6	8.3 ± 0.6	−0.7 to +2.9	15	0.17	8.26	<0.001
Rosette area (mm^2)	51.1 ± 7.6	68.3 ± 8.7	−2.6 to +37.6	8	0.34	9.00	<0.001

Male–female differences are differences within pairs relative to male measurements. Values are presented as mean ± SD

DI dimorphism index (see text)

[a] $n = 13$ pairs, $df = 12$

Table 2 Male Atlantic Puffins from Machias Seal Island, New Brunswick, were significantly larger than their female partner in all measurements; $n = 74$ (37 males, 37 females)

Measurement	Female	Male	Male–female difference (range)	% females larger	DI	t_{36}	P
Body mass (g)	425 ± 30	455 ± 30	−45 to +100	22	0.07	4.71	<0.001
Wing length (mm)	157 ± 5	161 ± 6	−12 to +17	27	0.03	3.45	0.001
Culmen (mm)	46.3 ± 1.7	48.9 ± 2.0	−2.6 to +9.5	16	0.06	5.84	<0.001
Bill depth (mm)	35.7 ± 1.6	37.9 ± 2.0	−1.5 to +8.3	19	0.06	5.39	<0.001
Head + bill (mm)	79.6 ± 1.6	82.4 ± 2.4	−2.3 to +8.9	11	0.04	6.97	<0.001
Rosette length (mm)	9.5 ± 1.0	10.5 ± 0.8	−2.3 to +3.9	16	0.10	5.83	<0.001
Rosette width (mm)	7.5 ± 0.7	8.4 ± 0.8	−1.3 to +2.8	16	0.12	4.89	<0.001
Rosette area (mm^2)	56.3 ± 9.9	69.4 ± 9.4	−23.8 to +32.4	14	0.23	6.51	<0.001

Values are presented as mean ± SD

DI dimorphism index (see text)

Assortative mating

While it appeared that puffins mated assortatively by culmen in Witless Bay ($r = +0.54$, $p = 0.005$), and possibly by head + bill on Machias Seal Island ($r = +0.32$, $p = 0.05$), these were not significant when accounting for the false discovery rate (Table 3).

Effect of age on puffin size

We found that puffins' mass increased with age (likelihood ratio test, $\chi^2 = 6.46$, $p = 0.011$, $\beta = 1.54 \pm 0.59$; Fig. 2), while all other measurements did not (wing chord: LRT $\chi^2 = 0.35$, $p = 0.55$; culmen: LRT $\chi^2 = 2.50$, $p = 0.11$; bill depth: LRT $\chi^2 = 1.75$, $p = 0.19$; head + bill: LRT $\chi^2 = 0.57$, $p = 0.45$; rosette length: LRT $\chi^2 = 0.65$, $p = 0.42$; rosette width: LRT $\chi^2 = 0.11$, $p = 0.74$; rosette area: LRT $\chi^2 = 0.49$, $p = 0.48$).

Discussion

Sexual size dimorphism and assortative mating

Male puffins were larger than their female partner in every measurement. The degree of SSD in puffins from Witless Bay was similar to that in puffins from Machias Seal Island and the Isle of May, Scotland (Harris and Wanless 2011). Relatively larger bills in males is a trait common in auks (Nelson 1981; Jones 1993; Grecian et al. 2003; Friars and Diamond 2011) and, more broadly, among the Charadriiformes (Burger 1980; Mawhinney and Diamond 1999; Devlin et al. 2004; De Marchi et al. 2012), though it is not universal throughout the order (Baker 1974; Hallgrimsson et al. 2008). In puffins, the males' bill is roughly 10 % larger in surface area, and this difference has been related to their role in nest defence (Creelman and Storey 1991; Harris and Wanless 2011),

Table 3 Atlantic Puffins showed no evidence of assortative mating after correcting for the false discovery rate (FDR)

Measurement	Witless Bay		Significant p (FDR)	Machias Seal Island		Significant p (FDR)
	Pearson's r	p		Pearson's r	p	
Body mass	−0.04	0.85	0.03	0.14	0.39	0.01
Wing length	0.11	0.59	0.03	0.08	0.63	0.02
Culmen	0.54	0.01	0.004	−0.02	0.90	0.02
Bill depth	−0.27	0.18	0.02	0.06	0.71	0.02
Bill length[a]	0.28	0.35	0.05	–	–	–
Head + bill	0.09	0.65	0.03	0.32	0.05	0.003
Rosette length	0.29	0.16	0.01	0.17	0.32	0.01
Rosette width	0.26	0.20	0.02	0.17	0.33	0.01
Rosette area	0.29	0.16	0.01	0.19	0.25	0.01

Correlations are significant when $p <$ significant p (FDR)

[a] $n = 13$ pairs

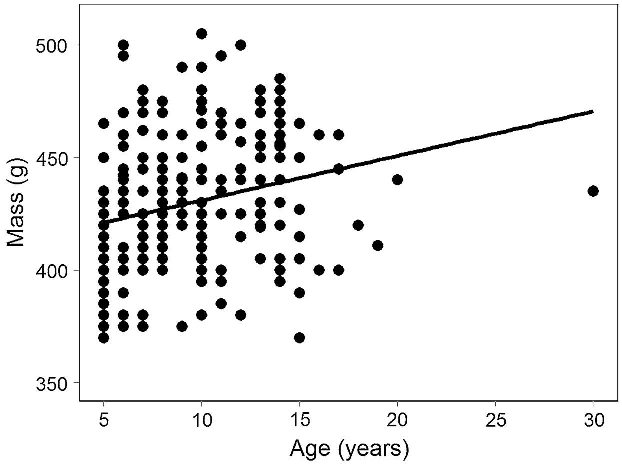

Fig. 2 The mass of breeding-age adult Atlantic Puffins from Machias Seal Island, New Brunswick, increased with age. Note that *dots* may represent more than one data point

but could also be a sexually selected character used in intra-specific aggression by males, as in Crested Auklets (Jones 1993; Székely et al. 2007). Puffins also use their bills to dig burrows, a task performed largely by males (Harris and Wanless 2011).

Puffins' fleshy rosette is also likely a sexually selected character (Douletrent et al. 2013), but we found no evidence for assortative mating based on its size or area (Tables 1, 2). When there is high parental investment from both males and females, and both sexes are ornamented, we expect to see mutual sexual selection for ornamental traits (Jones and Hunter 1993). However, in puffins, there does not appear to be assortative mating by the size ornaments such as the rosettes, suggesting that puffins do not use this character in mate choice; rosette colour, however, may be an important feature in choice of mate (Douletrent et al. 2013).

The lack of assortative mating by rosettes may mean that the ornaments are not used for mate selection by either sex. Murphy (2008) found no evidence of assortative mating in the monomorphic, elaborately ornamented Turquoise-browed Motmot (*Eumomota superciliosa*), and suggested that alternative mechanisms for the persistence of this trait amongst females, such as genetic correlation or natural selection, should be considered. Female ornamentation can be explained by genetic correlation, especially in cases where female ornamentation is not an indication of quality or reproductive success (Lande 1980; Cuervo et al. 1996; Wolf et al. 2006; Murphy and Pham 2012). Indeed, less than half of studies looking at the relationship between female ornamentation and reproductive success have found a positive correlation, suggesting that a genetic correlation rather than selection by males as an indication of female quality is responsible for maintaining these ornaments in females (Nordeide et al. 2013). Alternatives to sexual selection in monomorphic, ornamented species must be considered, including the role of ornaments as indicators of status when there is intra-specific competition for resources (Tarvin and Murphy 2012). Puffins may, however, mate assortatively by other ornamental features of the bill or ocular plates that we did not measure. The absence of a relationship between partners' rosette size may also reflect, in part, the larger measurement error associated with soft tissues and small measurements relative to precision (Yezerinac et al. 1992; Arnqvist and Mårtensson 1998; Perktaş and Gosler 2010).

Size changes with age

We found evidence of age-associated growth only of puffins' mass. Other seabirds mate assortatively by factors such as age; studies in Common Terns (*Sterna hirundo*) found that age could be selected for actively or passively due to the later arrival of younger terns at the colony or experienced birds selecting other experienced birds (Ludwig and Becker 2008). Palestis et al. (2012) found assortative mating by tail length in Roseate Terns (*Sterna dougallii*), but the correlation in size may have been an indirect result of age, and puffin bill size does generally increase with age during the first 6 years (Harris 2014).

It is possible that the increase of mass with age is the result of sampling bias, where heavier individuals are measured only later in life, rather than a within-individual effect of individuals increasing in mass with age. Statistical models to test this hypothesis (van de Pol and Verhulst 2006; van de Pol and Wright 2009) failed to converge.

Predicting puffin sex from measurements

Using a discriminant function, the sex of individuals can be determined if there is sufficient SSD, but such functions rarely account for spatial or temporal variation in measurements (Johnstone and Niven 1989; Devlin et al. 2004). Spatial or temporal variations in size reduce the applicability of discriminant functions (Moen 1991; Anker-Nilssen et al. 2003; Meiri and Dayan 2003). Puffins show a latitudinal gradient in size on both sides of the Atlantic (Moen 1991; Lowther et al. 2002; Harris and Wanless 2011), so the application of colony-specific discriminant functions is required.

Previous studies have also used linear measurements to determine the sex of puffins. Corkhill (1972) found that sexes at a colony in Wales could be separated by bill depth and length, as did Harris and Wanless (2011) in Scotland, noting that the separating function varied with breeding colony. In North America, sexes of puffins in New Brunswick could be separated using a combination of culmen, bill depth, and head + bill, with an accuracy of about 80 % (Friars and Diamond 2011). This discriminant function determined the sex of both birds in 23 pairs from Machias Seal Island in this study, but in 11 pairs, both individuals were small and were classified as female, and in two pairs, both individuals were classified as male. This model was expected to correctly classify only 78 % of males and 83 % of females (Friars and Diamond 2011). Using results from genetic sexing, the female was larger than the male in 4–10 (11–27 %) pairs on Machias Seal Island, and in 1–6 pairs (4–23 %) from Witless Bay, depending on the measurement.

We were able to achieve a higher rate of correct classification in our discriminant function for Witless Bay puffins (88 %) in part because we specifically measured both members of pairs when creating the function (Sandvik 2001; Fletcher and Hamer 2003; Nisbet et al. 2007). Our initial discriminant model for Witless Bay puffins with four variables (wing length, culmen, bill depth, head + bill) was supported by our backward selection procedure. These measurements do not vary within the breeding season (unlike mass), and are generally reproducible within and among observers (Perktaş and Gosler 2010). We should note, however, that as our function is based on 26 males and 26 females, a larger sample size may have a different correct classification rate.

Discriminant functions for other auks in particular, and Charadriiformes in general, use mainly bill and head measurements, and achieve >80 % correct classification (Nelson 1981; Jones 1993; Wagner 1999; Grecian et al. 2003; Berzins et al. 2009). Smaller auks (e.g., Dovekie, *Alle alle* and Least Auklet *Aethia pusilla*) do show SSD, but also have a higher degree of overlap in measurements between the sexes, making separation using a discriminant function challenging (Jakubas and Wojczulanis 2007; Bond et al. 2013).

Mate choice in puffins

If there is variation in quality among individuals as well as a high level of parental investment, assortative mating may arise due to mutual mate choice (Crespi 1989; Kokko and Johnstone 2002). There was no assortative mating by rosette size, which was surprising, as this is a presumed ornament that we expected to be used for sexual selection. Puffins, being monomorphic and ornamented, were expected to show assortative mating by the size of their ornaments (signals of quality), but there was no evidence of this using rosette size. Rosette colour, however, may be an important feature in mate choice (Douletrent et al. 2013). The use of these ornaments as indicators of quality, breeding success, and status should be studied in order to better understand their role in mate choice and sexual selection in both males and females.

In summary, we developed a function to predict the sex of breeding puffins from field measurements at their largest North American breeding colony, and found a lack of assortative mating based on several morphometrics. Bill rosettes, ornaments supposedly used in mate choice, seem not to be, and their possible role as indicators of quality should be further investigated.

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