No evidence for assortative mating in the Atlantic puffin

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Statements and Declarations

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Data availability - All data and R scripts for the analyses and production of figures are available as supplementary material. Raw samples (e.g., pictures) are available by request.

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Abstract

Assortative mating occurs when individuals with similar phenotypes mate together more often than by chance and can contribute to increases in homozygosity, linkage disequilibrium between loci, and premating isolation in a phenotypically divergent population. While this phenomenon has been well documented in many avian species, evidence is relatively scarce in seabirds. Most seabirds are long-lived, monogamous, and form multi-year pair bonds, so assortative mating is hypothesized to occur if individuals pair early in life and change predictably over time. Furthermore, most seabirds are sexually monomorphic, which may in part result from mutual mate choice on the same traits. In this study, we looked for evidence of assortative mating in Atlantic Puffins (*Fratercula arctica*) across 11 traits: current body condition, structural size (wing length), bill size (depth and culmen length), bill shape (three measures from geometric morphometric analyses), and bill colouration (four regions). Despite a large sample size, we detected only weak support for non-random pairing. Specifically, we found evidence for positive assortative mating on cere colour, but no other trait under investigation. We discuss these results in the context of poorly understood mate choice behaviours in Atlantic Puffins and argue that determining the individual age of these long-lived animals would help resolve many unanswered questions.

Keywords: Seabird, *Fratercula arctica*, visual ecology, mate choice, ornamentation, elaborate monomorphism

Introduction

Assortative mating is the non-random pairing of breeding individuals based on one or more phenotypic traits (Burley, 1983, Jiang et al., 2013). Pairing between individuals with similar trait values (i.e., positive assortative mating) can promote locus homozygosity and linkage disequilibrium between loci, as well as contribute to premating isolation (Coyne and Orr 2004; Janicke et al., 2019). In contrast, pairing between individuals with dissimilar trait values (i.e., negative assortative mating) can increase heterozygosity and limit inbreeding depression (Pusey and Wolf 1996). Correlations between phenotypic trait values such as size, colour, behaviour, and/or diet in mated individuals have been detected in a wide range of taxa including amphibians (Lu et al., 2009; Swierk and Langkilde, 2021), fish (Snowberg and Bolnick, 2008; Ward et al., 2004), and birds (Caro et al., 2021; Indykiewicz et al., 2017; Pryke and Griffith, 2007; Tamin and Doligez, 2022).

Assortative mating generally occurs when individuals with similar traits mate based on convenience or availability, or through explicit mate choice (Newton et al., 1981). For example, individuals with similar traits may arrive to the breeding grounds around the same time (Dittrich et al., 2018; Village, 1985; Wiebe, 2000), resulting in population segregation where individuals must choose mates that are similar to themselves (Jiang et al., 2013). Territorial defence or site fidelity can also lead to assortative mating if individuals with similar traits occupy or prefer the same space (e.g., Ferrer and Penteriani, 2003). Similarly, intrasexual competition for nest sites may also lead to assortative mating if high quality individuals access and defend the best breeding sites, thus attracting similarly high quality mates (Bitton et al., 2008; Harris and Siefferman, 2014). Positive assortative mating may persist in a population if monogamously mated individuals age together (Woodman et al., 2024), and their phenotypes change predictably with age. Traits known to change as individuals age in avian species include structural size (e.g., longer wing length with age *Turdus merula*, Piliczewski et al., 2018), bill size and shape (wider bill with age in *Fulica americana* Alisauskas, 1987; longer with age in *Fratercula arctica* and *Turdus merula* Petersen, 1976; Piliczewski et al., 2018), ornament size (longer/larger with age in *Hirundo rustica*, *Ficedula albicollis*, and *Passer domesticus* Adámková et al. 2022; Evans et al. 2011; Laucht and Dale, 2012), and plumage colouration (*Tachycineta bicolor* Bitton et al., 2008). Alternatively, when phenotypic traits honestly advertise quality and lead to reproductive or survival advantages in both sexes, assortative mating may occur because higher quality individuals preferentially choose to mate with one another (Wiley and Poston, 1996). Several experiments have provided strong evidence for mutual mate choice in birds based on various traits. Some of these traits are structural (e.g., tail length in bearded tit *Panurus biarmicus*, Romero-Pujante et al., 2002), but most studies have focused on elaborate, often colourful, ornaments (e.g., crest length, Jones and Hunter 1993; UV colour, Nolan et al., 2010). Therefore, mutual mate choice leading to assortative mating is especially likely to occur in mutually ornamented species. Furthermore, because of the mechanisms that lead to non-random pairing, positive assortative mating has been reported more often than negative assortative mating (Jiang et al., 2013; Partridge, 1983).

As elaborate monomorphic seabirds, Atlantic Puffins (*Fratercula arctica*) provide an excellent system for assessing the strength and direction of assortative mating based on ornamental traits. While sub-adults between age one and three display relatively drab features, adults display a bright red-orange bill, orange rosette, and orange feet during the breeding season. During the winter months, Atlantic Puffins shed their bill sheath and regrow it before the start of each breeding season, such that the colourful bill could reflect nonbreeding condition or serve another signalling role (Lowther et al., 2020). They are long-lived with high adult survival (0.909–0.965 in Sandvik et al. 2008, > 0.915 in five colonies over ~10 years in Harris et al. 2005), are socially and genetically monogamous, form multi-year pair bonds, and obligately share parental care to raise a single chick each year (Anker-Nilssen et al., 2008; Creelman and Storey, 1991; Lowther et al., 2020). Breeding attempts in this species are particularly energetically costly and can negatively impact body condition and survival (Erikstad et al., 2009), so mutual choice for a high-quality partner could be critical to ensure successful breeding (Henderson et al., 2013). However, little is known about pair formation and the criteria by which Atlantic Puffins choose their mates (Lowther et al., 2020).

Thus far, Bond et al. (2016) only found weak evidence for assortative mating in Atlantic puffins based on culmen length in a Newfoundland, Canada, population and head + bill length in a New Brunswick, Canada, population. However, these statistically significant correlations were found to be non-significant after applying Type I error correction for conducting multiple tests. It is unknown if Atlantic Puffins mate assortatively based on other untested morphological traits like bill shape. In many species, carotenoid-based colouration in plumage and fleshy ornaments provide information on aspects of individual quality such as foraging ability and overall health and condition (Caro et al., 2021; Kirschel et al., 2020) and are therefore used to select high quality mates (Hernández et al., 2021). In Atlantic Puffins, bill and rosette colouration has been found to correlate with body condition (Doutrelant et al. 2013, but see Kochvar et al. 2024), and chick growth rates (Kochvar and Bitton, 2025). However, no studies to date have searched for evidence of assortative mating in Atlantic Puffins based on the shape and colour of their ornaments.

In this study we investigated whether Atlantic Puffins breeding in Newfoundland and Labrador, Canada, mate assortatively based on body condition, structural traits, and bill characteristics. Because 1) individuals are long-lived and exhibit low divorce rate, and 2) they are mutually ornamented, we predicted that paired individuals would display positive assortative mating.

Methods

Study site and subjects

This study was conducted on Gull Island in the Witless Bay Ecological Reserve of Newfoundland and Labrador, Canada (47.26N, 52.77W) during the chick rearing phases (July and August) of 2019, 2020, 2021. The reserve supports over 300,000 breeding pairs of Atlantic Puffins, 120,000 of which breed on Gull Island (Wilhelm et al., 2015). Our full dataset consisted of 351 individuals including 75 confirmed mated adult pairs, as determined by a genetically sexed male and female occupying the same burrow containing a chick within the same breeding season. All 75 pairs were used to investigate assortative mating by morphometrics, and a subsample of 57 mated pairs were used to investigate assortative mating by bill colouration.

Field methods

Individuals were captured in randomly selected active burrows between the hours of 22:00-03:00. Upon capture, adults were placed inside a small breathable cloth bag and transferred to a banding station to be processed. We banded each puffin with a uniquely numbered Canadian Wildlife Service steel band, measured their weight (600 g Pesola spring scale to the nearest 5 g) and wing chord (flat wing ruler to the nearest mm), and collected a small blood sample (less than 75 µl) from the brachial vein. Puffins were then taken into a blind where ultraviolet (UV) and visual spectrum RAW images (20 megapixel) were taken of the left and right sides of the head with a UV converted Samsung NX1000 (following instructions from Troscianko, 2018) using two 2-inch Baader lens filters (UV: 300-400 nm; visual: 400-700 nm; Figure S1). The puffins were illuminated with a full-spectrum metal halide bulb (150 W; Iwasaki eyeColour MT70D E27 6500K) placed behind a light diffuser to avoid shadows, and all photos included optic grade white (99 % reflectance) and dark (10 % reflectance) standards, a ruler, and a small white board to record the date and band number of the individual. The bill was cleaned of debris with a toothbrush prior to photo capture and was held in place in a standardized position using a custom-built wooden stabilizer (Figure S1). Three pictures in the visible range and three pictures in the UV range were obtained using the camera bracketing function set at -0.5, 0, and 0.5.

Molecular sexing

DNA was isolated from blood samples collected in the field on #2 filter paper (Whatman™, Cytiva, Maidstone, UK) and stored at room temperature. A ~1 cm^2 section of the saturated filter paper was removed with sterilized scissors and placed into a 1.5 ml collection tube. A DNeasy Blood & Tissue kit (Qiagen Inc., Toronto, ON, CA) was used to extract DNA following standard protocols. All samples were stored in a freezer at -20 °C for later use.

A polymerase chain reaction (PCR) was run on an Eppendorf Mastercycler® ep gradient S for determining sex of the individuals based on the amplification of the chromo-helicase-DNA 1 (CHD1) gene found on the W and Z chromosomes of birds. This protocol is the standard used for sexing seabirds (Fridolfsson and Ellegren, 1999). For each sample, this included the use of 12.5 µl Thermo Fisher Promega PCR Master Mix, 2 µl each of primers 2550F and 2718R, and 2 µl of extracted DNA. Each batch also included a no-template control. The PCR was run on a program of initial denaturation at 95 °C for 5 minutes, then 35 cycles of denaturing, annealing, and extension at 94 °C for 30 seconds, 50 °C for 30 seconds, 72 °C for 60 seconds, followed by a final extension at 72 °C for 7 minutes and a cooling period of 4 °C for 10 minutes. Samples were stored in a research freezer at -20 °C if not used immediately.

The amplified samples were run on a RedSafe™ agarose gel (iNtRON Biotechnology, Inc., South Korea) along with 100 base pair reference ladders and a no template control on a Thermo Scientific™ EC 300 XL for 50 minutes at 130 amps. The gels were then imaged and stored digitally. Individuals associated with one band on the gel were assigned as males and individuals with two bands were assigned as females.

Bill size and shape through geometric morphometrics

Bill size measurements were obtained directly from the visual spectrum images of the left side of puffin bills using ImageJ (Rasband, 2021). After setting the scale using the ruler included in the image, we extracted the culmen length (distance from points 2 to 4; Figure 1) and bill depth (distance from points 2 to 6; Figure 1). To quantify the shape of the bill we used geometric morphometric methods (Zelditch et al. 2012) applied to our complete dataset of 150 females and 201 males. The inclusion of individuals other than the 150 of interest (75 mated pairs) improves the reliability of the metrics extracted by capturing variation that is more representative of the whole population. Basic operations (i.e., file management, positioning of landmarks and semi-landmarks) were conducted using the series of tps software (i.e., tpsUtil, tpsDig2, tpsRelw; Rohlf, 2015), and multivariate analyses on the landmarks were performed in

MorphoJ (Klingenberg, 2011). We scaled the images using the ruler in each photograph and placed landmarks and semi-landmarks at locations on the bill that best represented their geometric form and were easily recognisable and repeatable (Figure 1). Landmarks were positioned at: (1) the point where the forehead meets the uppermost part of the cere, (2) the uppermost edge of the cere, (3) the point where the upper mandibles' orange colouration dissipates into the lamella, (4) the tip of the nail, (5) the point where the mandibular tomium straightens laterally after its curvature from the nail, (6) the most proximal point of the lower mandible, (7) the commissure, (8) the lowermost part of the cere, (9) the closest point of the rosette to the cere, and (10) the outermost dorsal and (11) outermost posterior edge of the nare (Badikova and Dzerzhynsky, 2015; Lockley, 1962; Petersen, 1976). Semi-landmarks were positioned: (12-14) along the curve of the upper mandible, (15) at the most concave and (16) convex area of the lower mandible, at the middle of the (17) lower and (18) upper lamella, (19) the middle of the cere, and (20) where the lowest point of the lamella meets the cere (Badikova and Dzerzhynsky, 2015; Burnham et al., 2020).

Raw landmark coordinates were made comparable using tpsRelw software by removing the variation in digitizing orientation, location and scale, and superimposing them in a common coordinate system (Adams et al., 2004). Distances between landmarks were treated as multivariate data and processed with a Procrustes analysis in MorphoJ (Adams et al., 2004), which finds the transformations that best superimpose the coordinates of all individuals. This was followed by a principal component analysis (PCA) to assess the magnitude of shape variation among the landmarks. The resulting PCs described shape variation, with values near zero associated with the average shape of the bills, and high values, both negative and positive, associated with strong deviations from the average shape (see Results for details, Klingenberg, 2016; Slice, 2007).

Bill colour analysis

For each individual, a multispectral image of the left side of the bill was generated using the plugin micaToolbox version 2.2 in ImageJ (Troscianko and van den Berg, 2021; van den Berg et al. 2020). One visual spectrum and one UV RAW photo were chosen for alignment using the 'Photoscreening' tool, selecting the most illuminated sample without evidence of RGB channel oversaturation. Visual spectrum and UV images were aligned and merged with the ‘Affine align’ tool by selecting four landmarks along the bill of each image. To evaluate the success of the alignment, we created a false colour image with the ‘Make Presentation Image’ tool, using yellow for the normalised red channel and blue for the normalised uvB channel. False colour images with little to no blue colouration visible around the bill were deemed effectively aligned and suitable for analysis. From the aligned multispectral images, we generated cone catch images using the spectral sensitivity of a violet-sensitive (VS) avian visual system with the peak sensitivity of the four photoreceptors set at 410 nm (SWS1), 450 nm (SWS2), 505 nm (MWS), and 565 nm (LWS) (Endler and Mielke, 2005; Ödeen et al., 2010), and standard illuminant D65 as a typical ambient light spectrum. While no data on the photoreceptor sensitivities of Atlantic Puffins are available, closely related species (i.e., common murre *Uria aalge* and razorbills *Alca torda*), do not possess a UV sensitive photoreceptor (based on SWS1 sequencing, Ödeen et al. 2010).

The average quantum catch values for four regions of interest (ROIs) on the bill were extracted from the cone catch images, each of which consisted of a 30x30 pixel circle. The locations of the four ROIs included the tips of the upper and lower mandible, the semi-fleshy cere, and the fleshy rosette (Figure 1). The colours represented by the average quantum catch values from the ROIs were modelled in a tetrachromatic colourspace to obtain Cartesian coordinates (x,y,z) with the ‘colspace’ function in the R package pavo (Maia et al., 2013, 2019).

Study sample

Our sample size for assortative mating analyses was determined by identifying all the mated pairs of puffins sampled within the same year, resulting in an initial sample size of 84 pairs. Pairs that were sampled in multiple years were only used once in the analysis. In cases where one member of the pair changed in a subsequent year, both pairs were included in the analysis. Additional pairs were excluded from the analyses because one or both individuals were missing a blood sample (4 pairs) or missing photos (2 pairs), as well as in unusual cases where three adults were sampled from the same burrow (2 pairs) or two individuals of the same sex only were found (1 pair). After the removal of these individuals, the final sample size was 75 pairs for the morphological analysis and 57 pairs for colour analyses (available UV and VIS images). Most pair members were captured within a few days of one another (mean = 4.52 ± 5.51 SD), but a subset (19/75) were captured 10 or more days apart.

Statistical analysis

We conducted repeatability measures for bill shape, bill size, and wing length as recommended by Nakagawa and Schielzeth (2010), using a linear mixed model approach with the rptR package (Stoffel et al. 2017). Repeatability measures for the colour metrics have been reported elsewhere (Kochvar et al. 2024).

The condition of a bird is often calculated using a major axis regression of mass over size (Labocha and Hayes, 2012). However, the mass of Atlantic Puffins is different between the sexes (Lowther et al. 2020) and decreases over the course of the breeding season (unpublished data). Therefore, we calculated an index of condition using ordinary least squares regression residuals obtained from a linear model with mass as the dependent variable and sex, Julian date, and wing chord length as independent variables. We initially included all two-way interactions in a full model and performed stepwise removal of non-significant terms to achieve a final model. We assessed the assumptions of multiple regression (no multicollinearity, homoscedasticity, no outliers, linear relation) on the initial full model and final model.

For morphological traits, we assessed the presence or absence of assortative mating using Pearson correlations between the male and female trait values (i.e., direct measurements on the bill, principal components from the geometric morphometric analyses, and body condition). To assess assortative mating based on colouration, we first assessed the pairwise differences between the colours of mated individuals by calculating the Euclidean distance between their Cartesian coordinates in a tetrahedral colourspace. We calculated this separately for each ROI to assess the similarity in colour between mated individuals across different regions. We compared the mean Euclidean distance between mated individuals to a null distribution of expected mean values generated by randomly pairing males and females over 1000 iterations. We determined whether assortative mating occurred based on the position of the observed mean distance compared to the null distribution; positive assortative mating was defined as observed values smaller than the 25th smallest value in the null distribution (i.e., two-tailed test at p = 0.05), and negative assortative mating was defined as observed values greater than the 25th largest value in the null distribution. *P* values were then adjusted with the false discovery rate correction to reduce the probability of Type I error (Benjamini & Hochberg, 1995).

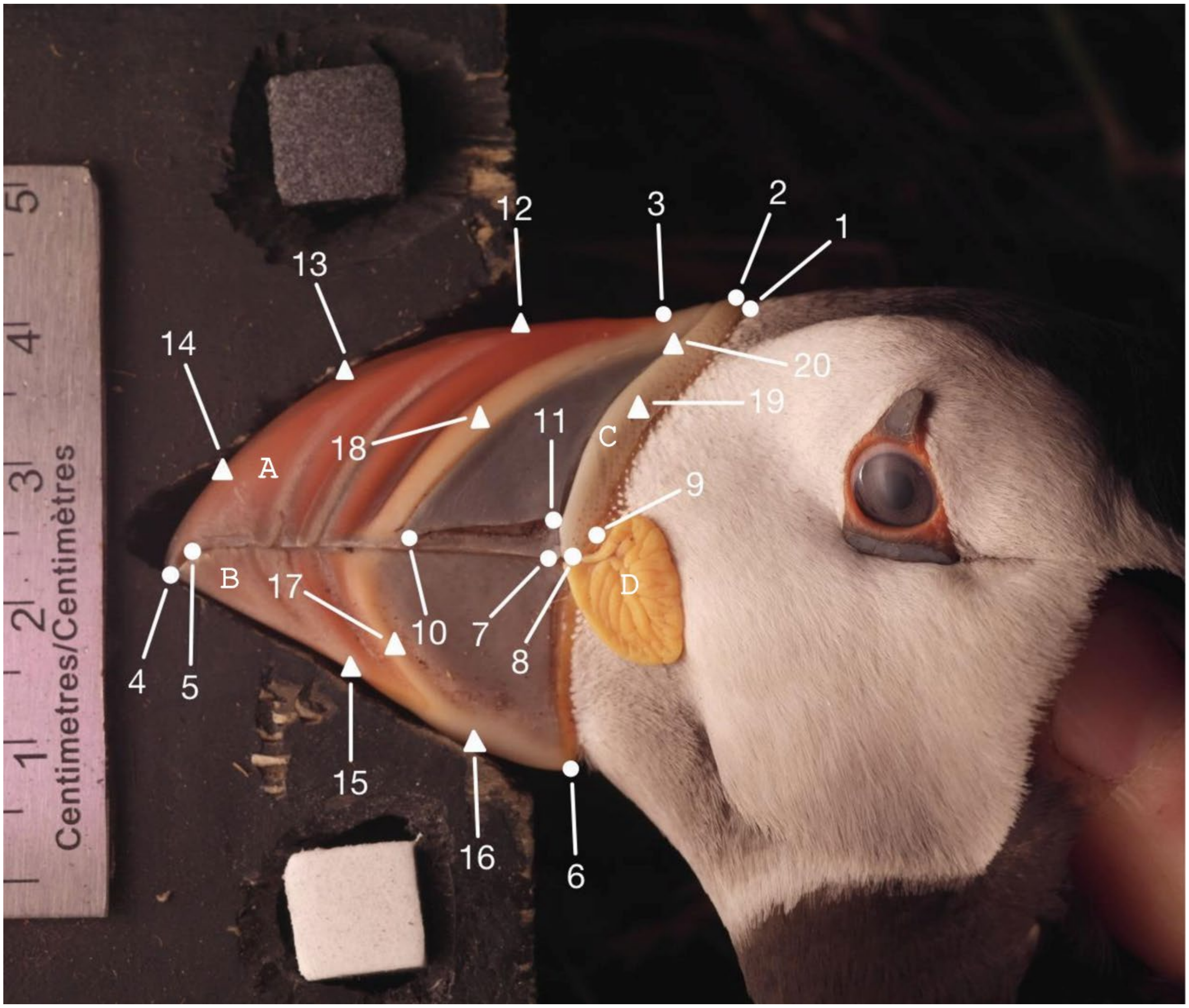

Figure 1. Exemplar puffin bill image with landmarks (solid circles) and semi-landmarks (solid triangles) for geometric morphometric analysis. Points 2 and 4 were used to calculate bill length and points 2 and 6 were used to calculate bill depth. Letters A through D represent regions of interest from which colour data were obtained: A) upper mandible, B) lower mandible, C) cere, and D) rosette.

Results

Body condition

The measurements for wing length used in the evaluation of body condition and assortative mating were highly repeatable ($R = 0.96$, 95 % CI = 0.92 – 0.98). All main effect variables (i.e., sex, wing length, Julian date), but none of the interactions, were retained in our final model of body condition (adjusted R^2 = 0.56; Table S1). Mass was significantly greater in males than females (parameter estimate: 39.94 ± 3.77 SE, $F_{1,145} = 112.11$, $p < 0.001$), in individuals with longer wing lengths (parameter estimate: 1.55 ± 0.42, $F_{1,145} = 13.41$, $p < 0.001$), and in individuals sampled earlier in the breeding season (parameter estimate: -0.50 ± 0.17, $F_{1,145} = 8.40$, $p = 0.004$). We retained the residuals of the full model as the index of the individuals' body condition.

Geometric morphometrics and bill size

The first seven principal components retrieved from the Procrustes analysis captured 83 % of the variation in bill morphologies. However, the first three, which captured over 50 % of the variation, were the most easily interpretable based on a visual inspection of the mesh planes (Figure S2, S3, and S4).

The first principal component (PC1 – *R* = 0.87, 95 % CI = 0.78 – 0.93); Figure S2) identified variance in the relationship between the depth and culmen length of the bill: individuals with large negative values had relatively long and shallow bills while individuals with large positive values had relatively short and deep bills. The second principal component (PC2 – *R* = 0.78, 95 % CI = 0.64 – 0.87); Figure S3) identified differences in the way the lamella converged on the cere: individuals with large negative values displayed a thick connection between the lamella and the cere whereas individuals with large positive values had a thin (if any) connection between the lamella and the cere. Finally, the third principal component (PC3 – *R* = 0.56, 95 % CI = 0.33 – 0.74), Figure S4) pointed to differences in the posterior curvature of the bill (where the bill meets the face): individuals with large negative values displayed a relatively straight posterior bill curvature, whereas individuals with large positive values had a more pronounced posterior bill curvature. The intraclass correlation coefficient for bill depth (*R* = 0.96, 95 % CI = 0.92 – 0.98) and culmen length (*R* = 0.95, 95 % CI = 0.90 – 0.97) were both very high.

Assortative mating by morphometrics

We did not find any evidence for assortative mating based on the morphological traits measured. Mated individuals did not pair according to body condition (n = 74, r = -0.065, 95% CI: -0.289 – 0.166, p = 0.58), body size (wing length: n = 74, r = -0.346, 95% CI: -0.255 – 0.202, p = 0.81), or bill size (bill depth: n = 75, r = -0.130, 95% CI: -0.346 – 0.100, p = 0.27; culmen: n = 75, r = -0.087, 95% CI: -0.308 – 0.143, p = 0.46). We also did not find evidence for assortative mating based on the three retained descriptors of bill shape (PC1: n = 75, r = 0.103, 95% CI: -0.127 – 0.322, p = 0.38; PC2: n = 75, r = 0.063, 95% CI: -0.167 – 0.285, p = 0.59; PC3: n = 75, r = -0.197, 95% CI: -0.406 – 0.031, p = 0.09).

Assortative mating by bill colour

The repeatability of colour metrics obtained from our images was generally high with most values above *R* = 0.60 (Kochvar et al. 2024). Because colours of the bill and rosette can change over time, and since some mated individuals were captured more than 10 days apart, we conducted two sets of analyses: one with individuals captured less than 10 days apart (n = 44 pairs, mean = 1.50 ± 2.37 SD days), and one with all individuals (n = 57 pairs). The results from the analyses were quantitatively similar and did not change our inferences. Therefore, we present only the results for all mated pairs. We found evidence for positive assortative mating by cere colour only. The mean distance between male and female cere colour was 0.046 (unitless), significantly below the mean of the null distribution (0.056 ± 0.003 SD, p = 0.012; Figure 2). For all other regions, there was no sign of positive or negative assortative mating (upper mandible: observed mean = 0.060; null distribution mean = 0.062, p = 0.67; lower mandible: observed mean = 0.039; null distribution mean = 0.042, p = 0.31; rosette: observed mean = 0.057; null distribution mean = 0.061, p = 0.31; Figure 2).

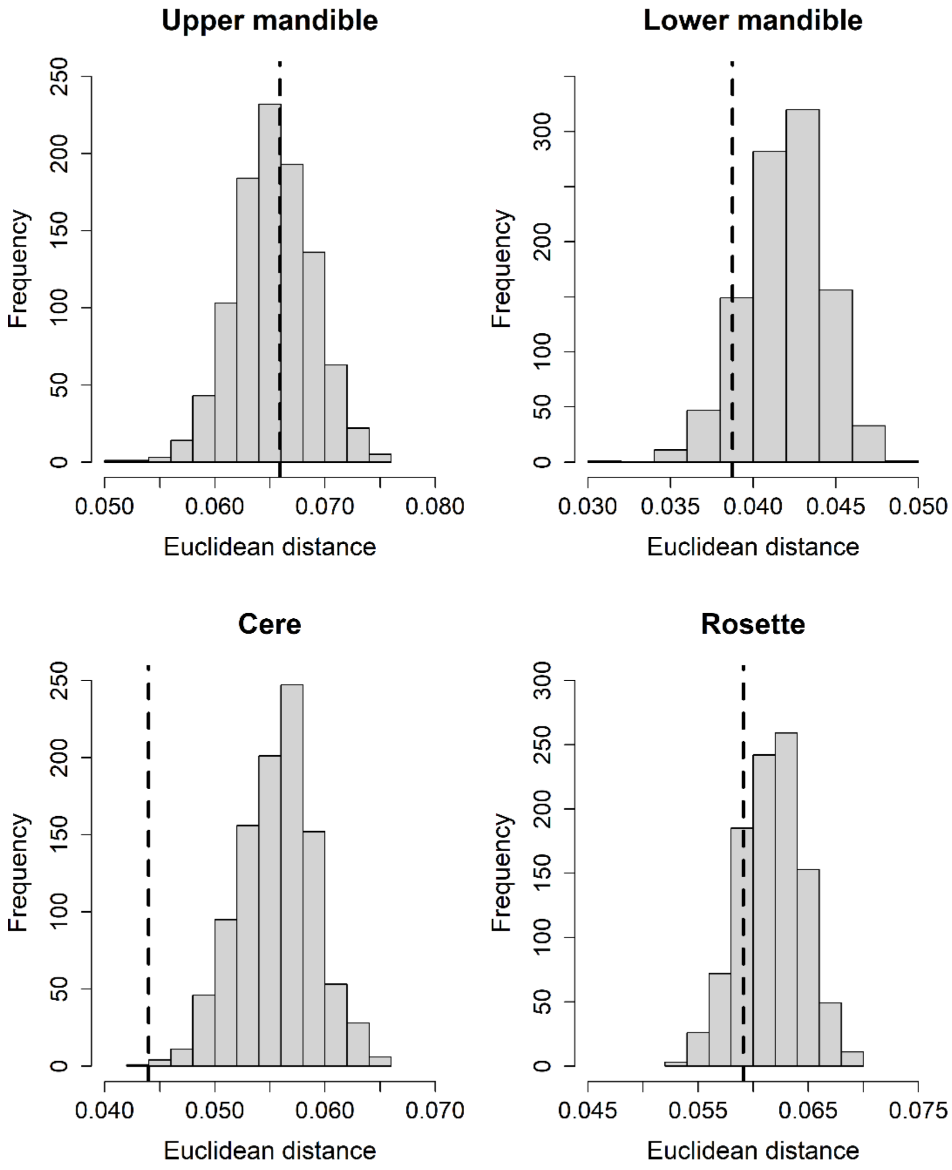

Figure 2. Pairs of Atlantic Puffins mated assortatively by cere colour, but not by any other bill colour trait. Each panel presents a null frequency distribution of Euclidean colour distances between randomly paired males and females (n = 1000 iterations) in a tetrahedral colourspace. Smaller distances indicate greater similarity between the colours. The dashed line marks the observed colour distance between mated individuals.

Discussion

Evidence of assortative mating has been found in a few Alcid species, including Lesser Auklets [*Aethia pusilla*; plumage colour (positive), tarsus length (positive), and facial plume size (negative); Jones and Montgomerie, 1991], Crested Auklets [*Aethia cristatella*; crest size (positive); Jones and Hunter, 1993], and Little Auks [*Alle alle*; wing length (positive), extent of white area on upper eyelid (positive), hormonal stress response (positive); Wojczulanis-Jakubas et al., 2018]. Despite a relatively large sample size we found only weak evidence for assortative mating. Of the 11 characteristics we explored, we found no relationships between male and female morphology (bill size and shape, wing length), body condition (corrected for covariates), or bill mandible and rosette colours. Only the colour of the fleshy cere was significantly correlated between mated individuals. Thus, our hypotheses predicting positive assortative mating are inconsistent with our findings. Our results are complimentary to those of Bond et al. (2016) and replicate their findings demonstrating a lack of assortative mating based on culmen length. We suggest that more detailed investigations of mating behaviours across the lifespan in this species is necessary to understand reproductive strategies and mating patterns.

Because of the low adult mortality rate and low reported divorce rate in this species, we hypothesized that Atlantic Puffins pairing for the first time would do so mostly with other young individuals. Furthermore, because puffins form life-long pair bonds, we further hypothesized that these mated pairs would age together. Detecting assortative mating under these conditions would further require that traits predictably change over time. One or all premises of this assortative mating mechanism could be incorrect. Adult puffins disperse to multiple locations during the winter, and individuals that breed together do not necessarily overwinter at the same sites (Guilford et al., 2011, Burnham et al., 2021, Baran et al., 2022, Charrier et al., 2024). While the precise mechanism of how previously mated individuals find each other at the breeding grounds is unclear, some have hypothesized that they reunite on the water close to the colony (Haris and Wanless 2011). When, where, and how individuals pairing for the first time initially encounter and court is undocumented (Lowther et al., 2020). Individuals that have lost their mate through mortality or divorce could favour a breeding location, potentially at or near their previous burrow, rather than actively seek a mate that displays specific traits. Fewer than 0.3 % of tracked breeding adults have been found to renest further than 100 m away from a previously used burrow (Harris and Wanless, 2011). Furthermore, an annual mortality rate of ~ 3 % combined with a divorce rate of ~ 7 % would lead to 10 % reshuffling of relationships every year, which could be sufficient to eliminate evidence of assortative mating even with the initial pairing of young individuals. Whether or not morphological traits change predictably with age is also uncertain. While the size and shape of the bill of Atlantic Puffins has been found to change in an expected way over the first six years of life (Petersen 1976), this period does not include most adults of breeding age considering their long lifespan (oldest North American puffin aged at 33 years; USGS, 2023). Subsequent research using repeated measures from birds of known age did not find evidence of age-related change continuing later in life (Bond et al. 2016). If morphological traits do not change predictably in older adults, assortative mating on these traits would not necessarily be maintained after initial age-based pairing.

Because male and female Atlantic puffins are ornamented, we predicted assortative mating on traits that could be associated with mate choice. While Atlantic puffin bill colouration is dynamic within the breeding season (Kochvar et al., 2024), evidence that bill colour is correlated with condition is mixed (no relationship, Kochvar et al., 2024; positive relationship stronger in female than males, Doutrelant et al., 2013). Moreover, there is evidence that female colouration, but not male colouration, is positively related to offspring growth rate constants (mass and 10th primary; Kochvar and Bitton, 2025). For assortative mating to be achieved through mutual mate choice, the same traits need to signal some measure of quality in both males and females (Johnstone, 1997), and most pairings at the population level should occur relatively synchronously. Of the 11 traits we evaluated, only cere colouration was more similar in mated pairs than expected by chance. In females only, cere hue has been associated with offspring mass at fledging (Kochvar and Bitton, 2025) but was not associated with body condition in either males or females (Kochvar et al., 2024). The opportunity for mutual mate choice on this trait seems limited, but bare part ornament colouration such as the cere and rosette are dynamic and honest signals of current condition in many species (Iverson and Karubian 2017). Since our measurements were obtained from adults caring for nestlings, measurements earlier in the breeding season when pairing typically occurs may provide stronger evidence for mutual mate choice.

Detection of assortative mating can be further hampered by spatial, temporal, and behavioural mechanisms. Studies have demonstrated that traits under selection can differ among populations (Whittingham et al., 2015), and that natural and sexual selection can lead to a mosaic of phenotypic trait values across a species range (Gosden and Svensson, 2008). Recent work on Atlantic Puffins has also shown that within population trait values can quickly change (Major et al. 2024). Even if we had found evidence of assortative mating, caution would have been needed in making broad inferences. The strength and direction of assortative mating can also be temporally variable: a study in blue tits (*Cyaniste caeruleus*) reported large interannual variation in assortative mating, with both positive and negative assortment (Fargevieille et al., 2017). Our study included data from three different years, which could have masked interannual variation, but sample size limitations precluded us from analysing each year separately. In addition, because we obtained measurements of temporally variable traits later in the breeding season, it would be difficult to evaluate if the correlation between males and females is evidence of assortative mating or mutual adjustment. If paired individuals compensate for one another during the breeding season, then condition dependent traits may converge even if they were different at the time of mating (i.e., increased investment when paired to a partner with low trait value; Harris and Uller, 2009; Limbourg et al., 2013).Basic studies of mating behaviours when Atlantic Puffins first arrive at the breeding grounds are needed to better understand the factors that influence individual pairing.

Several of the premises underlying our hypotheses could be resolved with an accurate way to age Atlantic Puffins. For instance, we could assess if paired individuals are generally of similar age and whether divorced individuals choose a mate of similar age. Combined with our multispectral bill images, it would also be possible to determine if the traits we measured (e.g., bill shape, size, and colour) predictably change after the age of six. Ultimately, this information would give us insight into the breeding ecology and mating behaviours of Atlantic Puffins and potentially clarify why there is no evidence of assortative mating in this species.

References

Adámková M, Tomášek O, Albrecht T (2022) An unexpected age-related pattern in feather growth contributes to age-dependent ornament expression in a passerine bird. J Ornithol 163:987-996. https://doi.org/10.1007/s10336-022-01990-5

Adams DC, Rohlf FJ, Slice, DE (2004) Geometric morphometrics: ten years of progress following the 'revolution'. Ital J Zool 71:5-16. doi.org/10.1080/11250000409356545

Anker-Nilssen T, Kleven O, Aarvak T, Lifjeld JT (2008) No evidence of extra-pair paternity in the Atlantic Puffin *Fratercula arctica*. Ibis 150:619–622. https://doi.org/10.1111/j.1474-919X.2008.00810.x

Alisauskas RT (1987) Morphometric correlates of age and breeding status in American Coots. The Auk 104:640-646. https://doi.org/10.1093/auk/104.4.640

Badikova AA, Dzerzhynsky FY (2015) Functional morphology and adaptive features of the jaw apparatus in puffins (Fraterculini, Alcidae, and Charadriiformes). Biol Bulletin 42:622-632.

Baran MA, Kress SW, Shannon P, Lyons DE, Major HL, Diamond AW (2022) Overwinter movement of Atlantic puffins (*Fratercula arctica*) breeding in the Gulf of Maine: inter-and intra-colony effects. Waterbirds 45:1-16. https://doi.org/10.1675/063.045.0103

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological) 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bitton P-P, Dawson RD, Ochs CL (2008) Plumage characteristics, reproductive investment and assortative mating in Tree Swallows *Tachycineta bicolor*. Behav Ecol Sociobiol 62:1543–1550. https://doi.org/10.1007/s00265-008-0583-7

Bond AL, Standen RA, Diamond AW, Hobson KA (2016) Sexual size dimorphism and discriminant functions for predicting the sex of Atlantic Puffins (*Fratercula arctica*). J Ornithol 157:875–883. https://doi.org/10.1007/s10336-016-1332-8

Burley N (1983) The meaning of assortative mating. Ethol Sociobio 4:191–203.

https://doi.org/10.1111/j.1096-0031.2011.00374.x

Burnham KK, Burnham JL, Johnson JA (2020) Morphological measurements of Atlantic Puffin (*Fratercula arctica naumanni*) in High-Arctic Greenland. Polar Res 39. https://doi.org/10.33265/polar.v39.5242

Burnham KK, Burnham JL, Johnson JA, Huffman A (2021) Migratory movements of Atlantic puffins *Fratercula arctica naumanni* from high Arctic Greenland. PLoS ONE 16:e0252055. https://doi.org/10.1371/journal.pone.0252055

Caro S, Pierre L, Bergès M, Bakker R, Doutrelant C, Bonadonna F (2021) Mutual mate preferences and assortative mating in relation to a carotenoid-based color trait in Blue Tits. Behav Ecol 32: 1171-1182. https://doi.org/10.1093/beheco/arab080

Charrier J, Anker-Nilssen T, Fort J, Jessopp M, Strøm H, Espinasse B (2024) Intracolony variability in winter feeding and migration strategies of Atlantic puffins and black-legged kittiwakes. Marine Biol 171:79. https://doi.org/10.1007/s00227-023-04384-0

Coyne J, Orr H (2004) Speciation. Sinauer, Sunderland, MA.

Creelman E, Storey AE (1991) Sex differences in reproductive behavior of Atlantic Puffins. Condor 93:390–398. https://doi.org/10.2307/1368955

Dittrich C, Rodríguez A, Segev O, Drakulić S, Feldhaar H, Vences M, Rödel M-O (2018) Temporal migration patterns and mating tactics influence size-assortative mating in Rana temporaria. Behav Ecol 29:418–428. https://doi.org/10.1093/beheco/arx188

Doutrelant C, Grégoire A, Gomez D, Staszewski V, Arnoux E, Tveraa T, Faivre B, Boulinier T (2013) Colouration in Atlantic Puffins and Black-legged Kittiwakes: Monochromatism and links to body condition in both sexes. J Avian Biol 44:451-460. https://doi.org/10.1111/j.1600-048X.2013.00098.x

Endler JA, Miekle PW (2005) Comparing entire colour patterns as birds see them. Biol J Linnean Soc 86:405–431. https://doi.org/10.1111/j.1095-8312.2005.00540.x

Erikstad KE, Sandvik H, Fauchald P, Tveraa T (2009) Short-and long-term consequences of reproductive decisions: an experimental study in the puffin. Ecology 90:3197-3208. https://doi.org/10.1890/08-1778.1

Evans SR, Gustafsson L, Sheldon BC (2011) Divergent patterns of age-dependence in ornamental and reproductive traits in the collared flycatcher. Evolution 65:1623-1636. https://doi.org/10.1111/j.1558-5646.2011.01253.x

Fargevieille A, Grégoire A, Charmantier A, del Rey Granado M, Doutrelant C (2017) Assortative mating by colored ornaments in blue tits: space and time matter. Ecol Evol, 7:2069-2078.

Ferrer M, Penteriani V (2003) A process of pair formation leading to assortative mating: passive age-assortative mating by habitat heterogeneity. Anim Behav 66:137-143. https://doi.org/10.1006/anbe.2003.2158

Fridolfsson A-K, Ellegren H (1999) A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol 30:116-121. https://doi.org/10.2307/3677252

Gosden TP, Svensson EI (2008) Spatial and temporal dynamics in a sexual selection mosaic. Evolution 62:845-856. https://doi.org/10.1111/j.1558-5646.2008.00323.x

Guilford T, Freeman R, Boyle D, Dean B, Kirk H, Phillips R, Perrins C (2011) A dispersive migration in the Atlantic puffin and its implications for migratory navigation. PLoS One 6:e21336. https://doi.org/10.1371/journal.pone.0021336

Harris MP, Anker-Nilssen T, McCleery RH, Erikstad KE, Shaw DN, Grosbois V (2005) Effect of wintering area and climate on the survival of adult Atlantic puffins *Fratercula arctica* in the eastern Atlantic. Marine Ecol Prog Series 297:283-296. doi:10.3354/meps297283

Harris MR, Siefferman L (2014) Interspecific competition influences fitness benefits of assortative ating for territorial aggression in Eastern Bluebirds (*Sialia sialis*). PLoS ONE 9:e88668. https://doi.org/10.1371/journal.pone.0088668

Harris WE, Uller T (2009) Reproductive investment when mate quality varies: Differential allocation versus reproductive compensation. Phil Trans Roy Soc B 364:1039–1048.

Harris MP, Wanless S (2011). The puffin. A&C Black, London.

Henderson LJ, Heidinger BJ, Evans NP, Arnold KE (2013) Ultraviolet crown coloration in female blue tits predicts reproductive success and baseline corticosterone. Behav Ecol 241299-1305. https://doi.org/10.1093/beheco/art066

Hernández A, Martínez-Gómez M, Beamonte-Barrientos R, Montoya B (2021) Colourful traits in female birds relate to individual condition, reproductive performance and male-mate preferences: a meta-analytic approach. Biology Letters 17:20210283. https://doi.org/10.1098/rsbl.2021.0283

Indykiewicz P, Podlaszczuk P, Surmacki A, Kudelska K, Kosicki J, Kamiński M, Minias, P (2017) Scale-of-choice effect in the assortative mating by multiple ornamental and non-ornamental characters in the black-headed gull. Behav Ecol Sociobiol 71:1-14. https://doi.org/10.1007/s00265-017-2411-4

Iverson EN, Karubian J (2017) The role of bare parts in avian signaling. Ornithol Advances, 134:587-611. https://doi.org/10.1642/AUK-16-136.1

Janicke T, Marie-Orleach L, Aubier TG, Perrier C, Morrow EH (2019) Assortative mating in animals and its role for speciation. Am Nat 194:865-875. https://doi.org/10.1086/705825

Jiang Y, Bolnick DI, Kirkpatrick M (2013) Assortative mating in animals. Am Nat 181 E125-E138. https://doi.org/10.1086/670160

Johnstone RA (1997) The tactics of mutual mate choice and competitive search. Behav Ecol Sociobiol 40:51-59. https://doi.org/10.1007/s002650050315

Jones IL, Hunter FM (1993) Mutual sexual selection in a monogamous seabird. Nature 362:238–239. https://doi.org/10.1038/362238a0

Jones IL, Montgomerie R (1991) Mating and remating of Least Auklets (*Aethia pusilla*) relative to ornamental traits. Behav Ecol 2:249–257. https://doi.org/10.1093/beheco/2.3.249

Kirschel A, Nwankwo E, Pierce D, Moysi M, Ogolowa B, Monadjem A, and Brelsford A (2020) CYP2J19 mediates carotenoid colour introgression across a natural avian hybrid zone. Mol Ecol 29:4970-4984. https://doi.org/10.22541/au.158880215.54508683/v2

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol

Res 11:353-357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

Klingenberg CP (2016) Size, shape, and form: Concepts of allometry in geometric morphometrics. Develop Genes Evol 226:113–137. https://doi.org/10.1007/s00427-016-0539-2

Kochvar K, Bitton, P-P (2025) Hatch timing and maternal bill colouration are associated with chick growth in a mutually ornamented seabird. Authorea Preprint under review DOI:10.22541/au.173707203.31611712/v1

Kochvar KH, Wilson AC, Bitton P-P (2024) Bill color is dynamic across the breeding season but not condition-dependent in Atlantic puffins. Ethology 130:e13417. https://doi.org/10.1111/eth.13417

Labocha MK, Hayes JP (2012) Morphometric indices of body condition in birds: a review. J Ornithol 153:1-22. https://doi.org/10.1007/s10336-011-0706-1

Laucht S, Dale J (2012) Correlations of condition, testosterone, and age with multiple ornaments in male house sparrows: patterns and implications. Condor 114:865-873. https://doi.org/10.1525/cond.2012.110194

Limbourg T, Mateman AC, Lessells CM (2013) Opposite differential allocation by males and females of the same species. Biol Lett 9:20120835. https://doi.org/10.1098/rsbl.2012.0835

Lowther PE, Diamond AW, Kress SW, Robertson GJ, Russell K, Nettleship DN, Kirwan GM, Christie D, Sharpe CJ, Garcia E, Boesman PFD (2020) Atlantic Puffin (*Fratercula arctica*), version 1.0. Birds of the World. https://doi.org/10.2173/bow.atlpuf.01

Lu X, Ma X, Li Y, Fan L (2009) Breeding behavior and mating system in relation to body size in *Rana chensinensis*, a temperate frog endemic to northern China. J Ethol 27:391-400. https://doi.org/10.1007/s10164-008-0132-x

Maia R, Eliason CM, Bitton P-P, Doucet SM, Shawkey MD (2013) pavo: An R package for the analysis, visualization and organization of spectral data. Methods Ecol Evol 4:906–913. https://doi.org/10.1111/2041-210X.12069

Maia R, Gruson H, Endler JA, White TE (2019) pavo 2: new tools for the spectral and spatial analysis of colour in R. Methods Ecol Evol 10:1097-1107. https://doi.org/10.1111/2041-210X.13174

Major HL, Rivers JE, Carvey QB, Diamond AW (2024) The incredible shrinking puffin: Decreasing size and increasing proportional bill size of Atlantic puffins nesting at Machias Seal Island. Plos one, 19:e0295946.

Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev, 85:935-956.

Newton I, Marquiss M, Moss D (1981) Age and breeding in sparrowhawks. J Anim Ecol 50:839-853. https://doi.org/10.2307/4141

Nolan PM, Stephen Dobson F, Nicolaus M, Karels TJ, McGraw KJ, Jouventin P (2010) Mutual mate choice for colorful traits in king penguins. Ethology 116:635-644. https://doi.org/10.1111/j.1439-0310.2010.01775.x

Ödeen A, Håstad O, Alström P (2010) Evolution of ultraviolet vision in shorebirds (Charadriiformes). Biol Letters 6:370–374. https://doi.org/10.1098/rsbl.2009.0877

Partridge L (1983) Mate choice. In: Bateson P (ed) Mate Choice. Cambridge University Press, Cambridge, pp 227–256

Petersen A (1976) Size variables in puffins *Fratercula arctica* from Iceland, and bill features as criteria of age. Ornis Scand 7:185-192. https://doi.org/10.2307/3676188

Piliczewski P, Jankowiak Ł, and Wysocki D (2018) Age-dependent changes in biometrics indicate senescence in the European Blackbird *Turdus merula*. Bird Study, 65219-224. https://doi.org/10.1080/00063657.2018.1451821

Pryke SR, Griffith SC (2007) The relative role of male vs. female mate choice in maintaining assortative pairing among discrete colour morphs. J Evol Biol 20:1512-1521. https://doi.org/10.1111/j.1420-9101.2007.01332.x

Pusey A, Wolf M (1996) Inbreeding avoidance in animals. Trends Ecol Evol 11:201–206. DOI: 10.1016/0169-5347(96)10028-8

Rasband W (2021) ImageJ. National Institutes of Health, USA. http://imagej.nih.gov/ij

Rohlf FJ (2015) The tps series of software. Hystrix 26:9-12. DOI: 10.4404/hystrix-26.1-11264

Romero-Pujante M, Hoi H, Blomqvist D, Valera F (2002) Tail length and mutual mate choice in bearded tits (*Panurus biarmicus*). Ethology 108:885-895. https://doi.org/10.1046/j.1439-0310.2002.00821.x

Sandvik H, Erikstad KE, Fauchald P, Tveraa T (2008) High survival of immatures in a long-lived seabird: insights from a long-term study of the Atlantic puffin (*Fratercula arctica*). Auk 125:723-730. https://doi.org/10.1525/auk.2008.07059

Slice DE (2007) Geometric Morphometrics. Annual Rev Anthropol, 36:261–281. https://doi.org/10.1146/annurev.anthro.34.081804.120613

Snowberg LK, Bolnick DI (2008) Assortative mating by diet in a phenotypically unimodal but ecologically variable population of stickleback. Am Nat 172:733–739. https://doi.org/10.1086/591692

Stoffel MA, Nakagawa S, Schielzeth H (2017) rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8:1639-1644.

Swierk L, Langkilde T (2021) Size-assortative mating in explosive breeders: A case study of adaptive male mate choice in anurans. Behaviour 158:1–20. https://doi.org/10.1163/1568539X-bja10098

Tamin T, Doligez B (2022) Assortative mating for between-patch dispersal status in a wild bird population: Exploring the role of direct and indirect underlying mechanisms. Journal of Evolutionary Biology, 35(4), 561-574. https://doi.org/10.1111/jeb.13925

Troscianko J, van den Berg C (2021) MicaToolbox (2.2). Empirical Imaging.

van den Berg CP, Troscianko J, Endler JA, Marshall NJ, Cheney KL (2020) Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. Methods Ecol Evol 11:316-332. https://doi.org/10.1111/2041-210X.13328

Village A (1985) Spring arrival times and assortative mating of kestrels in south Scotland. J Anim Ecol 54:857-868. https://doi.org/10.2307/4383

Ward AJW, Hart PJB, Krause J (2004) The effects of habitat- and diet-based cues on association preferences in three-spined sticklebacks. Behav Ecol 15:925–929.

https://doi.org/10.1093/beheco/arh097

Whittingham LA, Freeman-Gallant CR, Taff CC, Dunn PO (2015) Different ornaments signal male health and MHC variation in two populations of a warbler. Molec Ecol, 24:1584-1595.

Wiebe KL (2000) Assortative mating by color in a population of hybrid Northern Flickers. Auk 117:525-529. https://doi.org/10.1093/auk/117.2.525

Wiley RH, Poston J (1996) Perspective: Indirect mate choice, competition for mates, and coevolution of the sexes. Evolution 50:1371–1381. https://doi.org/10.2307/2410875

Wilhelm SI, Mailhiot J, Arany J, Chardine JW, Robertson GJ, Ryan PC (2015) Update and trends of three important seabird populations in the Western North Atlantic using a geographic information system approach. Marine Ornithol 43:211–222. http://doi.org/10.5038/2074-1235.43.2.1133

Wojczulanis-Jakubas K, Drobniak SM, Jakubas D, Kulpińska-Chamera M, Chastel O (2018) Assortative mating patterns of multiple phenotypic traits in a long-lived seabird. Ibis 160:464-469. https://doi.org/10.1111/ibi.12568

Woodman JP, Gokcekus S, Beck KB, Green JP, Nussey DH, Firth JA (2024) The ecology of ageing in wild societies: linking age structure and social behaviour. Phil Trans B, 379:20220464. https://doi.org/10.1098/rstb.2022.0464

Zelditch M, Swiderski D, Sheets HD (2012) Geometric morphometrics for biologists: a primer. Academic Press, Cambridge, Massachusetts