Short communication

Assortative mating patterns of multiple phenotypic traits in a long-lived seabird

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Choosing the right mate is crucial for successful breeding, particularly in monogamous species with long and extensive bi-parental care, and when the breeding pair is presumed to last many seasons. We investigated the degree of assortative mating in the Little Auk *Alle alle*, a long-lived seabird with long-term pair bonds and bi-parental care for fixed (morphological) and labile (physiological, behavioural) traits. Using randomization tests, we suggest assortative mating with respect to wing length, extent of the white area on the upper eyelid and hormonal stress response (the difference between stress-induced and baseline corticosterone levels). We discuss how the assortative mating patterns that we found in the Little Auk may be adaptive.

Keywords: labile traits, mate choice, non-random mating.

Choosing the right mate is key to successful breeding (Bateson 1983). A mating partner has to be fertile and possess certain traits that will be advantageous to the offspring through either heredity or parental performance, or both (Bateson 1983, Gibson & Langen 1996). In socially and genetically monogamous breeding systems with bi-parental care and long-term pair bonds, choosing the right partner may have a decisive effect on lifetime breeding success. In such breeding systems one therefore may expect strong selective pressure for mutual sexual selection (Jones & Hunter 1993).

The first step in determining how selection operates on mate choice is to identify traits that could be considered quality indicators by the mating partners. Typically, a significant correlation between the values of a homologous phenotypic trait across pair members is considered indicative of active mate choice (assortative mating) in regard to such a trait (Jiang *et al.* 2013). In this study, we analysed mating patterns in the Little Auk *Alle alle* with regard to a number of biologically divergent traits. This species is a small pelagic seabird, exhibiting all the characteristics of that ecological group: it is long-lived (at least 15 years, own unpubl. data) with long-term pair bonds (at least some years, if not the whole life, K. Wojczulanis-Jakubas & D. Jakubas unpubl. data), socially and genetically monogamous with long and extensive bi-parental care (Stempniewicz 2001, Wojczulanis-Jakubas *et al.* 2009). All these features make the Little Auk a suitable species for examining the question of mate choice.

First, we considered the wing lengths and head–bill lengths of the partners that are routine proxies for body size and are positively correlated with each other (Wojczulanis-Jakubas *et al.* 2011). Significant positive correlations of body size parameters between mated individuals have been commonly reported across numerous divergent phylogenetic lineages (Arnqvist *et al.* 1996, Jiang *et al.* 2013). Although the evolutionary underpinnings of the assortativity of such a mating are not fully understood, its widespread occurrence strongly suggests its importance.

Secondly, we investigated some characteristics of the Little Auk's plumage, as a proxy of an individual's quality (e.g. Jones & Montgomerie 1992, MacDougall & Montgomerie 2003, Masello & Quillfeldt 2003). Little Auks have a conspicuous pattern of black and white feathers distributed across their bodies (Jakubas & Wojczulanis-Jakubas 2012), including white patches on the upper eyelids and outer rectrices (present year-round). Males and females appear to expose these patches deliberately in greeting displays (Evans 1981, K. Wojczulanis-Jakubas & D Jakubas pers. obs.), which suggests that the white patches may be of importance in mate choice.

Thirdly, we examined hormonal and behavioural proxies of the stress response. As the manner in which individuals handle stressful situations while raising their offspring can affect their breeding success (Cockrem 2007, Angelier & Chastel 2009), the stress response

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seems to be a pertinent candidate trait in the context of mate choice. We also examined aggressive behaviour towards other individuals, as aggressive interactions can be important in establishing territories and/or the social status of individuals (Piper 1997). However, unlike the morphological and plumage traits that do not vary over a season, the hormonal and behavioural traits may vary with environmental conditions (labile traits) and have been rarely considered in the context of non-random mating in animals, as reviewed in Jiang *et al.* (2013).

METHODS

We carried out the study in Hornsund, SW Spitsbergen (77°00′N, 15°33′E). Given the high genetic homogeneity of the global population of the Little Auk (Wojczulanis-Jakubas *et al.* 2014a) and the fact that we performed the study in a single colony, we assumed homogeneous distribution of the traits within the study population (i.e. pooling of mating pairs from heterogeneous samples might potentially lead to 'false–positive' results, Fernández-Meirama *et al.* 2017).

To analyse assortative mating for wing and head–bill lengths we used data collected from several projects, performed across the whole breeding season over 7 years. We marked the birds with a metal ring of unique number to monitor their identity. We measured the length of the flattened left wing (with a ruler accurate to 1 mm), and the head-bill length (with a calliper accurate to 0.1 mm; D.J. measured all the birds). We obtained a complete set of measurements for a total of 236 pairs, 49 of which were measured more than once.

To measure the size of the white patches on the outermost rectrices and on the upper eyelid, we photographed and took video shots, respectively, with the bird held in the same position and distance above a scaled background. From the videos we extracted the best single frame showing the eye of the bird closed (i.e. entire white area on the upper eyelid fully exposed). We considered 55 pairs in this dataset. We collected these data during the pre-laying period, when the tail feathers were freshly grown and not yet abraded by rocks during the various activities of the birds in the colony.

We examined the hormonal stress response of pair members during the incubation period in 2014. For this purpose we removed the birds by hand from the nest, applying the standard capture-and-restraint protocol (Wingfield *et al.* 1998, Romero & Reed 2005) as validated and described in detail for the Little Auk in Wojczulanis-Jakubas *et al.* (2013). By taking blood samples from each bird within 3 min of the bird first being grasped and 30 min thereafter, we established baseline and stress-induced concentrations of corticosterone, respectively. In the subsequent analysis, we used the baseline concentrations and the differences between the stress-induced and baseline levels (hereafter hormonal stress response) as dependent variables. We sampled 20 pairs.

We collected data on behavioural stress response and aggressive behaviour in 2011 as part of a synergistic project of which some results have been published in Wojczulanis-Jakubas *et al.* (2013), with details of the fieldwork described there. In short, we modified the immediate vicinity of the nests of the target pairs by placing small, foreign objects (a bamboo stick cross with six plastic strips on the crossbar, and a sheet of blue plastic) on the rock close to the nest chamber entrance. We then recorded the time of occurrence of the first bird and its reaction from the moment of the birds' appearance in the colony and compared control (without foreign objects) and experimental conditions. The difference between the two values was used as a proxy for the behavioural stress response. We observed the aggressive behaviour of pair members of 18 pairs for seven consecutive days during the pre-laying period. We recorded the total number of aggressive interactions for an individual standardized by the time it spent in the colony.

As sexual dimorphism in the Little Auk is negligible (Jakubas & Wojczulanis 2007), we sexed all the birds molecularly based on blood samples collected for that purpose. The partners of birds handled during the mating period were established on the basis of behavioural observations following Wojczulanis-Jakubas *et al.* (2009, 2014b). The partners of birds handled during the incubation and chick-rearing periods were established on the basis of nest identity.

Baseline and stress-induced levels of total corticosterone were assayed at the Centre d'Etudes Biologiques de Chizé (CEBC) by radioimmunoassay following procedures described in detail in Wojczulanis-Jakubas *et al.* (2013). Minimal detectable corticosterone levels were 0.3 ng. The intra-assay variation for total corticosterone level was within the range 5–12%. Molecular sexing was performed based on variation in the CHD gene, using primers F2550 and R2718, and P2 and P8 (Griffiths *et al.* 1998).

The white areas on the upper eyelid and outer rectrices areas were measured relative to a reference (millimetre grid or 1-mm accurate in the background) using ImageJ (Ferreira & Rasband 2012), with a standard thresholding procedure.

We handled the birds and performed all experiments following international standards, with permission of the Norwegian Animal Research Authority and the Governor of Svalbard. We did not record any deviation in the behaviour of birds nor their survival in any of the procedures.

To analyse the data, we generated null hypothesis distributions for each within-pair correlation of traits that we measured using a randomization procedure, as

many of the traits were not normally distributed. In each trait we randomly 'paired' available males and females 1000 times, and for each such pairing we calculated the Pearson correlation coefficient to obtain its distribution under the null hypothesis of no assortative mating. We calculated the type I error as the proportion of randomizations that generated a correlation equal to or more extreme (in absolute terms, i.e. values equal or higher for positive correlations, equal or lower for negative correlations) than the correlation obtained from original male–female pairings. As our biometric data could be affected by measurement error and pseudoreplication, we evaluated these issues as detailed in Supporting information Appendix S1 concluding that both were minimal. We performed the analysis in R 3.3.1 (R Core Team 2016).

RESULTS

We found significant positive relationships between pair members with respect to three traits: wing length ($r = 0.11$, $n = 296$, $P = 0.03$), eyelid white area ($r = 0.32$, $n = 50$, $P = 0.01$) and hormonal stress response ($r = 0.49$, $n = 16$, $P = 0.02$). We found no evidence of assortative mating with regard to the other traits (head–bill length: $r = 0.004$, $n = 296$, $P = 0.46$; tail white area: $r = 0.10$, $n = 53$, $P = 0.19$; baseline corticosterone concentration: $r = 0.35$, $n = 16$, $P = 0.10$; behavioural stress response: $r = 0.03$, $n = 13$, $P = 0.43$; aggressive interactions: $r = -0.18$, $n = 18$, $P = 0.25$; Fig. 1).

DISCUSSION

On examining eight phenotypic traits in the Little Auk pair members, we found significant positive correlations between the partners in three of them: wing length, white plumage area on the upper eyelids and hormonal stress response. While examining the pattern of wing length across pair members, we assumed that the trait was related to overall body size. If that had been the case, we should have found the same pattern in the head–bill length, as these two traits are correlated (Wojczulanis-Jakubas *et al.* 2011). However, head–bill length was not correlated between the partners. Hence, assortativity in wing length requires an alternative explanation to assortativity in overall body size: this could be related to the migration pattern. Although speculative, a link between wing length and migration distance has been demonstrated in several other avian species (Leisler & Winkler 1990, O'Hara *et al.* 2006). If that were also the case in the Little Auk, their assortative mating with respect to wing length might be a reflection of their migration patterns. As migration distance can be heritable (Müller *et al.* 2015), assortative mating with regard to such a trait could prevent the production of offspring that would have a

non-adaptive mixture of migration distance, i.e. an unclear migration area (Bearhop *et al.* 2005).

We also found a significant correlation between the mates in one plumage trait: the extent of the white patches on the upper eyelids. Achromatic plumage has rarely been considered in the context of assortative mating but existing studies indicate that white plumage parts seem to be influenced by developmental and/or genetic factors (Mennill *et al.* 2003), and the size of the white patches may be associated with the quality of individuals: those with a more extensive white area are of better quality. Consequently, therefore, given the assortative mating pattern involving the white patch on the upper eyelid in the Little Auk, we can hypothesize that the revealed pattern reflects mate choice informed by individual quality.

We found a significant and positive correlation between pair members in the hormonal stress response, which suggests assortative bonding with regard to the Little Auk partners' styles in coping with stressful situations. The similarity of the stress response in the mates may be adaptive. If conditions in foraging areas are unfavourable, both parents need to increase their efforts to ensure that a given breeding attempt is successful (Jakubas *et al.* 2016). As increased parental efforts in such stressful situations are mediated by changes in corticosterone levels (Harding *et al.* 2009, 2011, Welcker *et al.* 2009), a similar stress response in both members of a pair may be a mechanism that increases the chance to breed successfully.

This interpretation of the correlations between the partners with regard to corticosterone levels should be treated with caution, however, as the hormone levels are a labile trait (i.e. can change over the time). As it is measured in pair members during the breeding season, the positive relationships may be due to the pair experiencing similar environmental conditions (e.g. Ouyang *et al.* 2013, 2014) and/or the similar age of the two birds in the pair (Barbraud & Barbraud 1999, Weiß *et al.* 2010, Hirschenhauser 2012). A recent study by Class *et al.* (2017) has shown that this issue could be addressed by measuring labile traits such as the hormonal stress response in multiple breeding seasons. Such data are difficult to obtain and are not available for the Little Auk at the moment. However, the similarity of hormonal stress response of the partners is an interesting finding. The study of Ouyang *et al.* (2014), which is also correlational and performed over a restricted time-period, suggests the possibility that hormonal mechanisms may be also partially under sexual selection. Both studies clearly show that there is a great need for future studies focusing on the endocrine similarity of the partners, preferentially being performed over a wider time-scale and/or using an experimental approach.

We did not find significant correlations between pair members in head–bill length, white tail tips, baseline
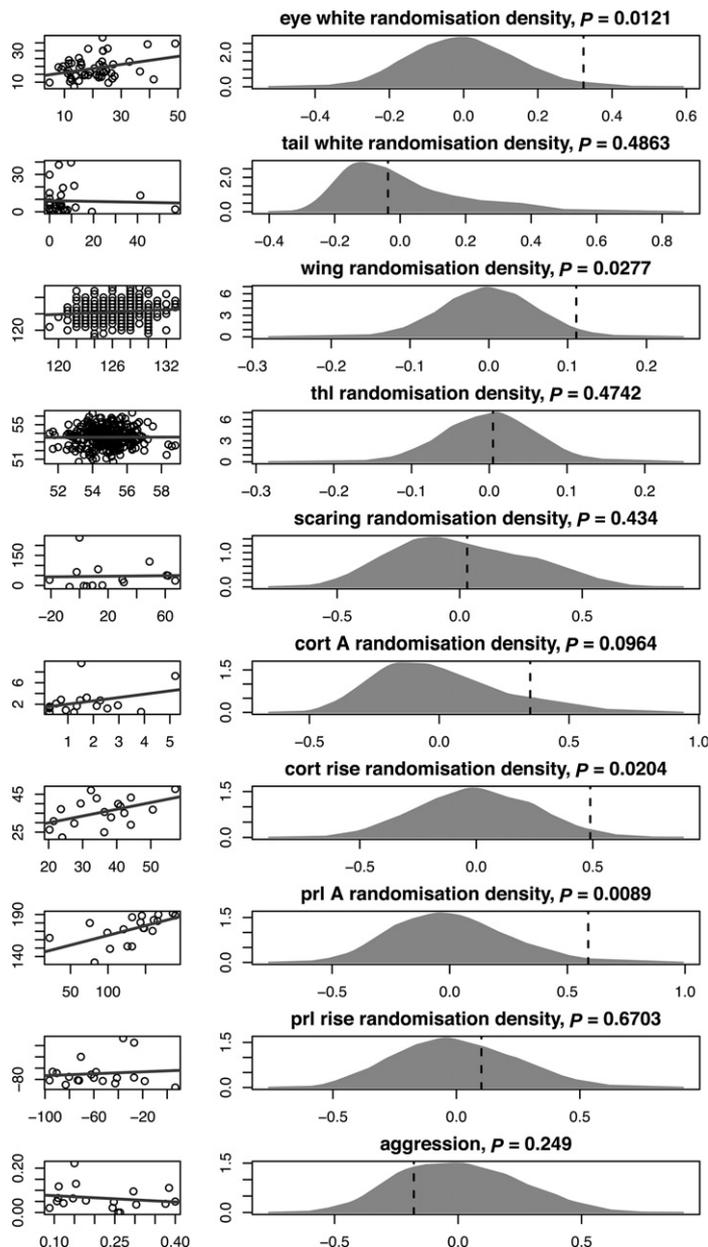
Figure 1. Male–female pairwise scatterplots of all traits (left-hand panel) with reduced major axis regression lines (solid lines), and null-hypothesis distributions of correlations (kernel density estimators, right-hand panel) with overlaid original data correlations (dashed lines).

corticosterone concentration, behavioural stress response and number of aggressive interactions. This may be due to some traits being labile (baseline hormone concentration and behavioural parameters), measured with an error (behavioural stress response and aggressive interactions) and/or relatively small sample size (baseline hormone concentration). We also cannot exclude the possibility that some of these traits (e.g. tail white patches, head–bill length) are not under mutual mate choice.

Our study suggests the assortativity of multiple phenotypic traits in the Little Auk, a species that, because of its life-history traits, should particularly benefit from finely tuned mate choice. The range of significant correlation coefficients values found here (0.11–0.49) contains the average value of 0.28 reported in similar

contexts for other animal populations, and suggests that these traits indeed may be under sexual selection (Jiang *et al.* 2013). However, as our study is purely correlational, further research examining the influence of similarity of the partners in a given trait on breeding success (e.g. Gonzales-Solis 2004) is necessary to identify the processes responsible for the observed patterns.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Data analysis details