MHC-IIB disassortative mate choice in a genetically monogamous seabird

Abstract

Among species reproducing sexually, mating strategies represent a major component of individual fitness. The Major Histocompatibility Complex (MHC) is an extremely diverse set of genes responsible for immunological recognition and defence against pathogens. Although MHC dissimilarity between mates has been proposed to drive mate choice through increased offspring pathogen resistance, evidence is mixed. In addition, exploration of the role of MHC in other mating strategies, such as divorce, is rare. We investigated whether MHC-IIB dissimilarity is associated with mate choice and divorce probability in the genetically monogamous black-legged kittiwake (*Rissa tridactyla*). We found that first-time male breeders, as well as divorced males, were paired with more MHC-IIB dissimilar females than expected under random mating. We did not find evidence for mate choice based on MHC-IIB dissimilarity when considering females. In addition, in the studied population, divorce probability was very low compared to other populations and did not significantly vary with the MHC-IIB dissimilarity of the pair. Our results pave the way to a better understanding of the complex role of MHC dissimilarity in mating decisions of species displaying mutual choice and biparental care.

Keywords: Black-legged kittiwake, *Rissa tridactyla*, Divorce, Reproduction, Major Histocompatibility Complex, Disassortative mate choice

Introduction

Mating strategies are the set of behavioural tactics used by individuals to attract, select, change or retain mates to promote individual fitness. Understanding their driving factors has long been a major topic in evolutionary biology [1;2]. For instance, mate choice has repeatedly been shown to rely on traits that honestly reflect the quantity or quality of resources, the care or the genes that the mate can provide [3;4;5]. In addition, in several species, individuals preferentially choose mates which are more genetically or behaviourally compatible to avoid inbreeding or to enhance behavioural coordination or task complementation between mates [6;7;8;9;10]. However, individuals may face energetic, time or spatial constraints in choosing mates [11]. Under such circumstances, individuals are forced to choose from a limited pool of potential mates and thus may accept suboptimal partners [12]. A possible response to this limitation in iteroparous species is divorce, defined as one breeding individual changing partner for a new mate while the original mate is still alive [13]. Theoretical studies have suggested that individuals should divorce when pairing with a higher-quality or more compatible mate improves their reproductive success [14]. Accordingly, divorce is common after a reproductive failure [15;16;17], and usually leads to increased reproductive success [18;19]. Like mate choice, divorce may be associated with the quality of the ornaments of the mate, or the behavioural or genetic compatibility of the pair (e.g. [20;21]).

Concerning the genetic component of the pool of heritable factors that can affect mating strategies, the major histocompatibility complex (MHC, called HLA in humans) has been the focus of much research [22;23;24]. The MHC, one of the most polymorphic sets of genes in vertebrates, encodes cell-surface proteins that are essential for immunological recognition and defence against pathogens. Because each MHC molecule can bind a limited set of antigens [25], higher MHC diversity increases the number of antigens recognized,

48 thereby providing resistance to a wider range of parasites [26;27]. Accordingly, several
49 studies have found that higher MHC diversity increases health, reproductive success and
50 survival [28;29]. Because the MHC allelic composition of the offspring results from the
51 combination of those of the parents, multiple studies have found evidence for mate choice
52 maximising MHC dissimilarity between mates (e.g. [30;31;32;33]). However, high MHC
53 diversity can also increase the risk of autoimmune diseases and reduce the T cell receptors
54 diversity [34;35]. In a few species, intermediate MHC diversity has thus been shown to be
55 optimum for fitness, and to be an important factor in mate choice (e.g. [36;37;38;39]).
56 Although there is evidence that the MHC composition or diversity of the parents also
57 influences other reproductive strategies (e.g. cryptic female choice [40]; extra-pair copulation
58 [41]), to our knowledge, a single study on Seychelle warblers (*Acrocephalus sechellensis*)
59 explored the association between parental MHC similarity and divorce rate and found no
60 evidence [42].
61 Here we tested whether mate choice and divorce depend on dissimilarity at MHC
62 class-II B (hereafter MHC-IIB dissimilarity) in the black-legged kittiwake (*Rissa tridactyla*),
63 a long-lived genetically monogamous seabird with no extra-pair copulation [43]. In black-
64 legged kittiwakes, divorce is fairly common (i.e. 15 % to 50 % of the pairs divorce each
65 year), and more frequent among first-time breeders and after reproductive failure [44;45;46]
66 [10]. Coulson [44] suggested therefore that some kinds of "incompatibility" between mates
67 result in unsuccessful breeding, thereby providing costs that select for divorce. In black-
68 legged kittiwakes, individuals can recognize MHC-IIB dissimilarity using body odours [47],
69 and MHC-IIB similarity between mates results in reduced offspring MHC-IIB diversity [48],
70 which incurs costs for female chicks such as slower growth, poor tick clearance efficiency
71 and lower survival [49]. We therefore hypothesised that, in this species, mating decisions
72 based on MHC-IIB dissimilarity between mates may have evolved to maximise the MHC-IIB

diversity of the offspring. However, the association between MHC diversity and fitness has never been explored in adults in this species, so one cannot exclude that intermediate MHC diversity is optimum when considering lifetime fitness. For all our analyses, we thus also explored the intermediate optimum hypothesis that predicts that individuals should mate with partners with intermediate MHC dissimilarity [36].

First, we tested whether individuals chose a mate according to MHC dissimilarity (Test 1). In several seabirds, including the black-legged kittiwake, young or inexperienced individuals arrive later to the breeding ground than older or more experienced breeders [50;51;52;53], which may increase the temporal constraints and limit the pool of potential mates. Inexperienced breeders may also be less efficient in sampling mates and less able to make mating discrimination or to anticipate alternative choices [54]. For instance, in grey mouse lemurs (*Microcebus murinus*), young females in their first mating season do not express mate choice, while experienced females prefer MHC-IIB dissimilar males [55]. The mate choice of first-time breeders may thus be more constrained than that of experienced breeders. We therefore tested the existence of MHC-IIB dependent mate choice in first-time breeders (Test 1.1) and experienced breeders experiencing a divorce (Test 1.2). Second, if divorcing allows individuals paired with a suboptimal mate to optimise the MHC-IIB diversity of their future offspring, we expected the rate of divorce to depend on the MHC-IIB dissimilarity between mates (Test 2). Third, we expected that, after a divorce, the new pair should be more MHC-IIB compatible (maximised or intermediate dissimilarity) than the original one (Test 3). In black-legged kittiwakes, although both mates participate fairly equally in incubation and chick rearing, sexes slightly differ in energetic investment in reproduction [56;57]. We thus tested these predictions separately in males and females to explore potential sex differences in mating decisions.

Materials and methods

Study area and population

This study was conducted between 1996 and 2023 in a colony of black-legged kittiwakes nesting in an abandoned U.S. Air Force radar tower on Middleton Island (59°26'N, 146°20'W), Gulf of Alaska, located approximately 100 km south of the mainland. Individuals were tagged with a unique combination of coloured and metal bands for long-term monitoring [58]. About 400 nest sites were monitored on the tower each year. All the nests were checked daily to record the identity of the present individual(s). This study was approved by a USGS Institutional Animal Care and Use Committee, with permits from the United States Fish and Wildlife Service and the Alaska Department of Fish and Game. For each individual of the study, sex was determined, in order of priority, by: 1) molecular assessment (n = 840 individuals [59]); 2) behavioural observations (n = 438 individuals [60]); 3) comparison of the relative size of both members of a pair, the largest bird being considered male (n = 6 individuals); 4) when only one individual of a pair was sexed, we attributed the opposite sex to its mate (n = 214 individuals).

Like many populations monitored in the long term, our population has been subjected to several treatments during the study period (e.g. supplemental feeding, brood manipulation, feather clipping, use of anti-copulatory devices). These treatments can affect reproductive success [58;61] and therefore pair stability. To limit any bias (e.g. artificial increase of reproductive success, and thus decreased divorce probability due to supplemental feeding), we excluded pairs that were subjected to experiments from the analysis on the probability of divorce (Test 2).

We defined a pair as two individuals of opposite sex involved in a reproductive event, i.e. attending the same nest and laying at least one egg during a given reproductive season. Individuals usually remain and breed in the same colony during their whole life [44]. We thus

123 defined the first mate choice as the pair formed during the first observed breeding attempt.
124 Each individual was involved in on average 4.75 ± 3.74 (SE) reproductive events (range = [1;
125 21]; Supporting information 1) over the course of the study. To study divorces, we focused
126 on individuals that were observed with an identified mate during at least one year and that re-
127 mated with a new mate the next year, the original mate being re-observed at least once during
128 the following years [10].
129 We performed all analyses using the R software [62]. All datasets and codes used in
130 the analyses are available from the Zenodo Repository
131 (https://doi.org/10.5281/zenodo.14509255).
132
133 *Molecular analyses*
134 We focused on the MHC class II which exhibits stronger selection than the MHC class I in
135 non-passerine birds such as the black-legged kittiwake [63]. Dissimilarity at MHC-IIB was
136 assessed following the methods described in Pineaux et al. [49;47]. Briefly, DNA was
137 extracted from blood with the blood and tissue DNA extraction kit (Qiagen), and a 258-pb
138 fragment of exon 2 of MHC-IIB was amplified using specific primers (forward: 5′-
139 GCACGAGCAGGGTATTTCCA, and reverse: 5′-GTTCTGCCACACACTCACC).
140 Amplicons were then sequenced with an Illumina MiSeq platform, using the 2 x 300-bp
141 protocol (Fasteris SA, Switzerland). Amplicon sequences were analysed with ampliSaS, a
142 three-step pipeline that consists of read demultiplexing, unique sequence clustering and
143 erroneous sequence filtering (substitution errors: 1 %; indel errors: 0.001 %; minimum
144 frequency with respect to dominant: 33 %; minimum amplicon sequence frequency: 2.5 %
145 [64]). We calculated the MHC-IIB functional distance between individuals based on amino
146 acid variations in the peptide-binding region (PBR) inferred from Leclaire et al. [65].

We described the chemical binding properties of each amino acid in the PBR with Sandberg's five physicochemical descriptors (z-descriptors [66]) and computed a Sandberg's distance matrix between sequences using the *DistCalc* function of the *MHCTools* package [67]. Then, we calculated an UPGMA dendrogram of sequences (*hclust* function), which represents clusters of functionally similar MHC-IIB sequences (hereafter, "supertypes"). The MHC-IIB functional distance between a male and a female was estimated using the tree distance (*treedist* function in the *vegan* package [68]), which is found by combining sequences in a common dendrogram and calculating how much of the sum of the branch length is shared and how much is unique. In addition, we calculated the difference in MHC-IIB supertype sharing between mates derived from Strandh et al. [69]:

$$100 \times [1 - (Nb_{shared\ supertypes} / Nb_{unique\ supertypes})],$$

where $Nb_{shared\ supertypes}$ corresponds to the number of supertypes carried by both the female and the male and $Nb_{unique\ supertypes}$ corresponds to the total number of unique supertypes possessed by the pair (where common supertypes count as 1). This index thus varies between 0 for individuals sharing all their supertypes and 100 for individuals sharing no supertype. The MHC-IIB functional distance was highly correlated with the difference in MHC-IIB supertype sharing (Spearman correlation test: $\rho = 0.79$, $P < 0.0001$, $n = 959$ pairs; Supporting information 2).

Mate choice for MHC-IIB dissimilarity may not only be governed by evolutionary processes that specifically target these genes, but may also be affected by mechanisms operating at the level of the entire genome (e.g. inbreeding avoidance [70]). To test whether the associations between MHC-IIB dissimilarity and mate choice may be driven by genome-wide relatedness, we used RAD sequencing (n = 255 individuals) to estimate genome-wide relatedness between individuals (see detailed methods in Supporting information 3).

172 *Statistical analyses*

173 To test the association between mate choice and MHC-IIB dissimilarity (Test 1), we
174 used bootstrapping to generate random pairs, where we let each individual choose randomly
175 10000 times between all available opposite-sex mates of the same year, without replacement
176 (i.e. one mate can be selected only once during a given year [71;72]). To test for the
177 maximisation hypothesis, we calculated p-values as the proportion of iterations in which the
178 random MHC-IIB dissimilarity mean was greater than the observed MHC-IIB dissimilarity
179 mean, plus the proportion of iterations in which the random MHC-IIB dissimilarity mean was
180 smaller than the symmetrical (relative to the mean of random MHC-IIB dissimilarity means)
181 of the observed MHC-IIB dissimilarity mean (two-tailed test, following the method described
182 in [55]). To test for the intermediate optimum hypothesis, we used a similar procedure, but
183 instead of comparing the observed and random means of MHC-IIB dissimilarity, we
184 compared the observed and random variance in MHC-IIB dissimilarity (following the method
185 described in [72]). In the case of a mate choice for intermediate MHC-IIB dissimilarity, we
186 expect the observed and random means of MHC-IIB dissimilarity between partners to be
187 similar. However, because individuals are expected to prefer a mate that allows them to reach
188 the intermediate MHC-IIB dissimilarity optimum, we expect the observed variance in MHC-
189 IIB dissimilarity to be smaller than the random variance. We tested MHC-IIB based mate
190 choice for first-time breeders (n = 534 females and n = 554 males; Test 1.1) and for breeders
191 experiencing a divorce (n = 113 divorces in females and n = 83 divorces in males; Test 1.2)
192 separately.

193 To test whether divorce probability varied with the MHC-IIB dissimilarity of the pair
194 during the year n (Test 2), we defined a binary response variable as 1 = divorce between the
195 year n and n+1, and 0 = no divorce between the year n and n+1. We tested females (n = 1244)
196 and males (n = 1232) separately. We used Generalised Linear Mixed Models (GLMM) with a

197 binomial distribution of error and a logit link (*glmer* function of the *lme4* package [73]). To
198 test for the intermediate optimum hypothesis, we included the quadratic term of the MHC-IIB
199 dissimilarity [74]. If this variable did not have a significant effect (i.e. $P > 0.05$), we removed
200 the quadratic term and then tested for the maximisation hypothesis with the linear term of the
201 MHC-IIB dissimilarity. The reproductive outcome is also known to influence the probability
202 of divorce in black-legged kittiwakes [46;10]. We thus defined the reproductive outcome as a
203 binary variable recording the presence (i.e. "success") or absence (i.e. "failure") of at least
204 one chick fledged at the end of the reproductive season and included this variable as an
205 additive effect. In the models, we set the *nAGQ* argument (number of points per axis for
206 evaluating the adaptive Gauss-Hermite approximation to the log-likelihood) of the *glmer*
207 function to 0 to help for convergence, when necessary. We tested the effect of individual
208 identity and year as random intercepts with the *ranef* function of the *lme4* package[73], and
209 removed these variables when they had no effect. We removed pairs that were subjected to
210 experiments from this analysis. As we used GLMM with binomial data, we provided the log
211 odd (and its standard error), which is equivalent to the coefficient of the slope in a linear
212 model (i.e. estimate in the model summary). It corresponds to the probability of an event in
213 the log scale, which is the transformed scale to satisfy the assumptions of a linear model for
214 GLMM with binomial data. For convenience, we also provided the odd ratio, which
215 corresponds to the exponential of the log odd. An odd ratio of 1 means no effect, whereas an
216 odd ratio > 1 (< 1, resp.) corresponds to an increase (decrease, resp.) of the response variable
217 when the explanatory variable (here, reproductive outcome or MHC-IIB dissimilarity)
218 increases by 1 unit. We calculated the confidence intervals from the model on the fixed
219 effects using the *predictInterval* function of the *merTools* package (n iterations = 10000,
220 [75]).

To test whether, after divorce, the new mate was more MHC-IIB dissimilar than the original mate (i.e. maximisation hypothesis), we used Linear Mixed Models (LMM; Test 3). The response variable was the MHC-IIB dissimilarity gain, i.e. the difference between the MHC-IIB dissimilarity to the original mate and the MHC-IIB dissimilarity to the new mate. Using the gain in a linear modelling framework allowed us to include individual and year as random effects. The MHC-IIB composition of the individuals available in the population might vary with year, thereby potentially affecting the ability to find MHC-IIB dissimilar mates. The use of random effects allowed us to account for this potential source of variability. We tested the effect of individual identity and year as random intercepts with the *ranef* function of the *lme4* package [73], and removed these variables when they had no effect. We tested the intermediate optimum hypothesis by comparing the variance of the MHC-IIB dissimilarity between the pool of original pairs and the pool of new pairs with a Fisher exact test (or its non-parametric equivalent, the Levene test, when necessary). In the case of mate choice following the intermediate hypothesis, we expect the variance of the MHC-IIB dissimilarity in the new pair to be lower than the variance of the MHC-IIB dissimilarity in the original pair.

Power analyses

We conducted test-specific power analyses to assess the robustness of our results. When studying the MHC-IIB dissimilarity with the new mate compared to a random mate (Test 1.1 and 1.2), we evaluated the likelihood of error as detailed in Hoover and Nevitt [71] and Hoover et al.[76]. For example, for males involved in divorces, we resampled between 10 and 83 males (the actual number of individuals in this dataset) and conducted the same analysis as described previously (n_{boots} = 500 instead of 10000 for reasons of computational limitations). We then assigned a “1” to a significant result and a “0” to a non-significant result for every

246 sample size tested, and used a GLM with a binomial distribution of error and a logit link
247 (*glmer* function of the *lme4* package [73]) to describe the relationship between sample size
248 and likelihood of error. We also provided the effect size measured as the difference between
249 the observed MHC-IIB dissimilarity mean (resp. variance) and the random MHC-IIB
250 dissimilarity mean (resp. variance).

251 When assessing the probability of divorce according to MHC-IIB dissimilarity (Test
252 2), our dataset was large enough (> 1000 individuals), but largely unbalanced between
253 faithful and divorcing pairs (approx. 20 times less divorced than faithful pairs in both females
254 and males). We thus randomly resampled the faithful pairs to obtain the same sample size as
255 for divorced pairs and adjusted the same models (n_{boots} = 1000) as described previously on the
256 balanced dataset using GLMs. We then estimated the probability to obtain a significant effect
257 for each explanatory variable (i.e. quadratic and linear terms of MHC-IIB dissimilarity, and
258 reproductive outcome).

259 When assessing the gain in MHC-IIB dissimilarity between the original and the new
260 pair after a divorce (Test 3), we calculated the effect sizes for our analysis using Cohen's d
261 statistic [77].

262

263 **Results**

264 *Mate choice in first-time breeders (Test 1.1)*

265 Most of the first-time breeders mated with first-time breeders (62 % when considering males
266 and 65 % when considering females). For their first breeding attempt, males tended to mate
267 with females which had a significantly greater difference in supertype sharing than expected
268 under random mating (P = 0.05, effect size = 1.61; Table S4.1; Figure 1b). The probability to
269 find a significant result with the available sample size (n = 554 mate choice events) was 0.40
270 (Table S4.1; Figure S4.1b). This effect was however not significant when considering the

271 functional distance between mates (P = 0.30, effect size = 0.07; Probability to find a
272 significant result = 0.03; Figure S4.2c and S4.2d). Females and their first mates did not show
273 significantly greater difference in supertype sharing or greater functional distance than
274 expected under random mating (functional distance: P = 0.89, effect size = -0.01, Figure
275 S4.2a; difference in supertype sharing: P = 0.72, effect size = 0.31, Figure 1a; Table S4.1).
276 Neither females (functional distance: P = 0.98; difference in supertype sharing: P = 0.88) nor
277 males (functional distance: P = 0.75; difference in supertype sharing: P = 0.49) mated with
278 significantly more intermediately dissimilar mates than expected under random mating (Table
279 S4.1; Figure S4.3 and S4.4).
280
281 *Probability of divorce (Test 2)*
282 In both sexes, the probability of divorce was significantly lower after a reproductive success
283 than after a reproductive failure (all P ≤ 0.01; Probability to find a significant result with a
284 balanced dataset > 0.80; Table S5.1; Figure S5.1). However, the probability of divorce did
285 not significantly vary with the MHC-IIB dissimilarity of the pair (Figure S5.2), when
286 considering either females (linear term: functional distance: P = 0.58 and difference in
287 supertype sharing: P = 0.74; quadratic term: functional distance: P = 0.18 and difference in
288 supertype sharing: P = 0.46; Table S5.1) or males (linear term: functional distance: P = 0.68
289 and difference in supertype sharing: P = 0.54; quadratic term: functional distance: P = 0.64
290 and difference in supertype sharing: P = 0.81; Table S5.1). The probability to find a
291 significant effect of MHC-IIB dissimilarity on the probability of divorce was consistently <
292 0.20 with a balanced dataset (Table S5.1).
293
294 *Mate choice in divorced individuals (Tests 3 and 1.2)*

Divorced individuals re-mated mainly with experienced breeders (63 % when considering males and 71 % when considering females). The difference in MHC-IIB dissimilarity between the new pair and the original pair was not different from 0, either in females or males (Test 3 – maximisation hypothesis; all P > 0.10; Table S6.1). The effect sizes revealed by Cohen's d statistics were very low (all Cohen's d < 0.20; Table S6.1; Figure S6.1).

However, after a divorce, males were paired with females which had a significantly greater difference in supertype sharing to them than expected under random mating (Test 1.2: P = 0.04, effect size = 4.52; Probability to find a significant result = 0.78; Table S7.1; Figure 2b and S7.1b). Females were not re-mated with males which had a significantly greater difference in supertype sharing than expected under random mating (Test 1.2: P = 0.06, effect size = 3.58; Probability to find a significant result = 0.23; Table S7.1; Figure 2a and S7.1a). These effects were not significant when considering functional distance (males: P = 0.09, effect size = 0.31 and females: P = 0.62, effect size = 0.08; Table S7.1; Figure S7.2).

When considering either males or females, after a divorce, the new pair was not more intermediately dissimilar than the original pair (Test 3 – intermediate hypothesis: all P > 0.10; Table S6.1) or than a random pair (Test 1.2 – intermediate hypothesis: all P > 0.10; Table S7.1).

MHC-IIB dissimilarity and genome-wide relatedness

We found that genome-wide relatedness was correlated neither with functional MHC-IIB similarity (Mantel test: r = 0.04, P = 0.09; Figure S3.1), nor with difference in MHC-IIB supertype sharing (Mantel test: r = 0.03, P = 0.10; Figure S3.2).

Discussion

319 In this study, we tested whether mating decisions (i.e. mate choice and divorce) of black-
320 legged kittiwakes vary with the MHC-IIB dissimilarity between mates. We found that first-
321 time male breeders (Test 1.1) as well as divorced males (and to a lower extent, divorced
322 females; Test 1.2) were paired with mates more MHC-IIB dissimilar to them than expected
323 under random mating. However, we did not detect an association between the MHC-IIB
324 dissimilarity of the pair and the probability of divorce.

325 First-time male breeders, as well as divorced males, were more MHC-IIB dissimilar
326 to their female partners than expected under random mating. These results may suggest active
327 mate choice for MHC-IIB dissimilarity. Like several other species (e.g. [78;79;26;80]),
328 including birds [81;82], black-legged kittiwakes are able to recognize MHC-IIB dissimilarity
329 using body odours [47]. In this species, the recognition of MHC-IIB dissimilarity might have
330 evolved as a mechanism to select MHC-IIB dissimilar mates to produce offspring with high
331 MHC-IIB diversity [48], and thus with higher early-life performance [49]. Similarly, in the
332 domestic cat (*Felis catus*), mating with MHC-IIB dissimilar mates increases offspring
333 survival and thus probably parental fitness [83].

334 In contrast to results when considering males, we did not detect MHC-IIB
335 disassortative mate choice when considering first-time female breeders. In addition, we
336 detected only a tendency for MHC-IIB disassortative mate choice when considering divorced
337 females (P = 0.06, with 23 % likelihood of obtaining a significant result). Our analysis
338 design, however, does not clearly allow us to determine whether the MHC-IIB disassortative
339 mate choice detected when considering males is driven by males' choice or females' choice.
340 For instance, a MHC-IIB disassortative mate choice detected in divorced males may suggest
341 that divorced males preferentially choose MHC-IIB dissimilar females or that females
342 preferentially select MHC-IIB dissimilar males when they have to choose amongst divorced
343 males. Similarly, a lack of MHC-IIB disassortative mate choice detected when considering

344 first-time female breeders may be due to first-time female breeders not choosing male
345 partners based on MHC-IIB dissimilarity or to males not choosing MHC-IIB dissimilar
346 female partners when they have to choose amongst first-time female breeders.

347 The contrasting results between males and females might however illustrate sex-
348 specific mating strategies. For instance, in Leach's storm-petrels (*Oceanodroma leucorhoa*),
349 while males show MHC-IIB disassortative mate choice, females' choice does not differ from
350 random mating [76]. In long-lived monomorphic species in which males and females equally
351 share parental care, such as the black-legged kittiwake [86;87;88], mate choice has been
352 suggested to be mutual [12]. However, the trade-off between the benefits obtained through
353 MHC disassortative mate choice and those obtained through mate choice based on the
354 intrinsic quality of the partner, such as its ability to provide good genes [89;90], good
355 parental care [91] or good-quality territory [92], can vary between individuals [93;89],
356 including between sexes. It may thus result in males and females having different preference
357 criteria for mate choice [94;95]. Sex-differences in the relative influence of MHC
358 dissimilarity vs. other traits on mating preference might be due to sex-specific variability in
359 either of these traits or to sex-specific variations in the direct benefits of these other traits to
360 the chooser. For instance, in mice, MHC dissimilarity is a more important criteria for mate
361 choice than scent-marking rate, only when variability in MHC dissimilarity between mates is
362 large [93]. In a cichlid fish (*Pelvicachromis taeniatus*), body size is a more important criteria
363 for mate choice than genetic compatibility for males than for females, which may be due to
364 the crucial role of female body size on fecundity [8].

365 When using Monte Carlo simulations to test mate choice, Hoover and Nevitt [71]
366 showed that a sample size of 500 pairs may be necessary to confidently reject the null
367 hypothesis when the effect size is small (see also [74]), small effect sizes being common
368 when studying MHC-based mate choice [74]. In the present study, the sample size of first-

369 time breeding males was ca. 500 individuals, and we found a P = 0.05 in males with 60 %
370 likelihood to find a non-significant result. This suggests at best a low effect size for mate
371 choice based on MHC-IIB dissimilarity in first-time breeders. In contrast, the sample size of
372 divorced males was largely smaller (i.e. only 83 males), but we found a significant MHC-IIB
373 disassortative mate choice (P = 0.04) with a much larger effect size in divorced individuals
374 than in first-time breeders (compare effects sizes in Tables S4.1 and S7.1). In several species,
375 the level of mate selectivity is influenced by age [96;55;97;98]. In kittiwakes, higher
376 selectivity in divorced individuals compared to first-time breeders may be related to their
377 earlier arrival on the nesting site [50], which may increase both decision time and the pool of
378 potential partners and thereby mating decision accuracy [99]. However, like for the sex-
379 specific variations detected in the mate choice analysis, we cannot determine whether the
380 higher selectivity for MHC-IIB dissimilar partners detected when considering divorcees than
381 when considering first-time breeders is due to divorced individuals being more selective in
382 choosing MHC-IIB dissimilar males or individuals being more selective in choosing MHC-
383 IIB dissimilar mates when they have to choose amongst divorced partners. For instance, in
384 bluethroats (*Luscinia svecica*), females seem to select males based on MHC-related traits
385 only when paired with young males [39]. In black-legged kittiwakes, in the context of a
386 balance between selecting mates for MHC-IIB dissimilarity vs. other individual quality traits,
387 the lower inter-individual variability in individual quality in experienced breeders than in
388 first-time breeders [100], may explain the higher selectivity for MHC-IIB dissimilarity when
389 the pool of potential partners are divorcees. Clearly, while our study provides invaluable
390 insight into the MHC-IIB disassortative mating strategy of black-legged kittiwakes, further
391 studies are needed to determine which sex and age-groups are more selective or selected
392 based on MHC-IIB dissimilarity, and why.

393 In accordance with previous studies in black-legged kittiwakes [45;101;46;10], we
394 found that the probability of divorce was greater in pairs that failed to fledge a chick than in
395 pairs that reproduced successfully. However, it does not significantly vary with the MHC-IIB
396 dissimilarity between mates. Also, after a divorce, individuals do not mate with partners more
397 MHC-IIB dissimilar to them than their original partners. Together, these results suggest that
398 in black-legged kittiwakes, MHC-IIB dissimilarity between the male and the female of the
399 pair is not a driving factor of divorce. In the present study, the proportion of divorce was
400 much lower than the proportions observed in other populations of black-legged kittiwakes
401 (annual mean ± SD = 4.4 ± 2.6 % in our study *vs.* 25 % in [44]; between 13 % and 47 % in
402 [45]; 19.1 % on average in [46]). On Middleton island, numerous black-legged kittiwakes
403 nest on other man-made structures than the tower [58]. These sites were not monitored,
404 except for occasional demographic surveys for certain years. It may therefore have limited
405 our ability to observe birds that changed nesting sites between breeding season, and thus to
406 detect all divorces, leading to a low sample size that might have affected our results and have
407 limited our ability to detect a significant effect.
408 We did not find any support for the intermediate optimum hypothesis in mating
409 decisions in black-legged kittiwakes. This result is consistent with Pineaux et al. [49], where
410 no benefits of intermediate MHC-IIB diversity were detected in black-legged kittiwake
411 chicks. In this species, the benefits of a larger MHC-IIB repertoire associated with increased
412 resistance to wider range of pathogens may outcompete the costs associated with increased
413 risks of autoimmune diseases and reduced T cell receptors diversity [35;34].
414 A previous study on the same population of black-legged kittiwakes showed that pairs
415 have a higher genetic dissimilarity (as measured by microsatellites) than expected under
416 random mating, and that the resulting increased heterozygosity of their chicks leads to higher
417 fitness [102]. Besides increasing the resistance to pathogens of the offspring, MHC-IIB

418 disassortative mate choice may limit inbreeding [79;103]. In the present study, we detected
419 MHC-based mate choice when MHC-IIB dissimilarity was measured based on difference in
420 supertype sharing, but not when based on functional distance. Similarly, in blue petrels
421 (*Halobaena caerulea*), yellow-rumped flycatchers (*Ficedula zanthopygia*) and little auks
422 (*Alle alle*), evidence for MHC-based mating strategies are stronger when considering
423 supertype or allele sharing than when considering functional distances [69;84;85] (but see
424 [55] for the opposite pattern). Understanding whether the difference detected between the two
425 indices illustrate that MHC-based mate choice is related to inbreeding avoidance in this
426 species require a larger number of individuals with genome-wide data.

427 In conclusion, we found evidence that black-legged kittiwakes choose partners based
428 on MHC-IIB dissimilarity. This finding supports the role of the benefits associated with high
429 MHC-IIB dissimilarity in the evolution of mating decisions in this population. It is consistent
430 with a previous study showing that, in this species, sex-allocation strategy varies with MHC-
431 IIB dissimilarity between parents [48]. In addition, our study suggests that mate choice for
432 MHC-IIB dissimilar partners varies with sex and age or experience of the chooser or the
433 selected mate. When the relative benefits of MHC disassortative mate choice vary with sex
434 and mate choice is mutual, a sexual conflict over mating strategies can arise and may select
435 for a rich repertoire of sex-specific behavioural adaptations [104;8]. Our study paves the road
436 for further studies of sex-specific mate choice in species with bi-parental care, a common trait
437 in birds [105].

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Figure legends

Figure 1: Distribution of 10000 means of MHC-IIB dissimilarity (as measured by the difference in MHC-IIB supertype sharing) between first-time breeders and their mates, calculated from bootstrap randomisations of potential pairs (see Materials and Methods section for details), when considering a) females, and b) males. The red vertical line is the observed mean of MHC-IIB dissimilarity. The black vertical line is the mean of the simulated distribution, and the black dashed lines are the 2.5 and 97.5 % quantiles of the simulated distribution showing the 95 % confidence intervals. See Figure S4.1 for a representation of the likelihood of a type II error according to the sample size.

Figure 2: Distribution of 10000 means of MHC-IIB dissimilarity (as measured by the difference in MHC-IIB supertype sharing) between divorced breeders and their new mates, calculated from bootstrap randomisations of potential pairs (see Materials and Methods section for details), when considering: a) females, and b) males. The red vertical line is the observed mean of the MHC-IIB dissimilarity. The black vertical line is the mean of the simulated distribution, and the black dashed lines are the 2.5 and 97.5 % quantiles of the simulated distribution showing the 95 % confidence intervals. See Figure S7.1 for a representation of the likelihood of a type II error according to the sample size.

775 **Figures**

776

777 Figure 1

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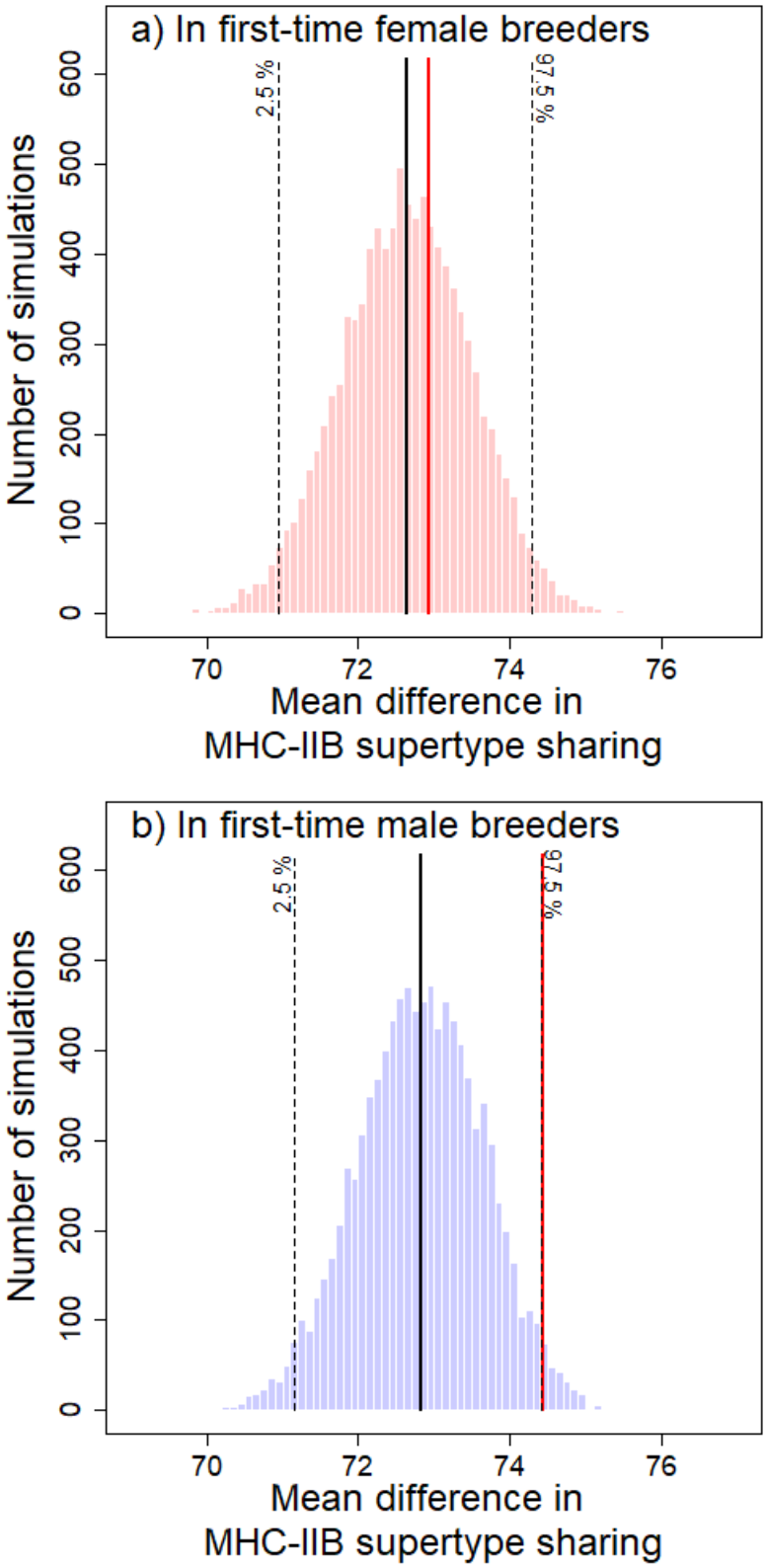
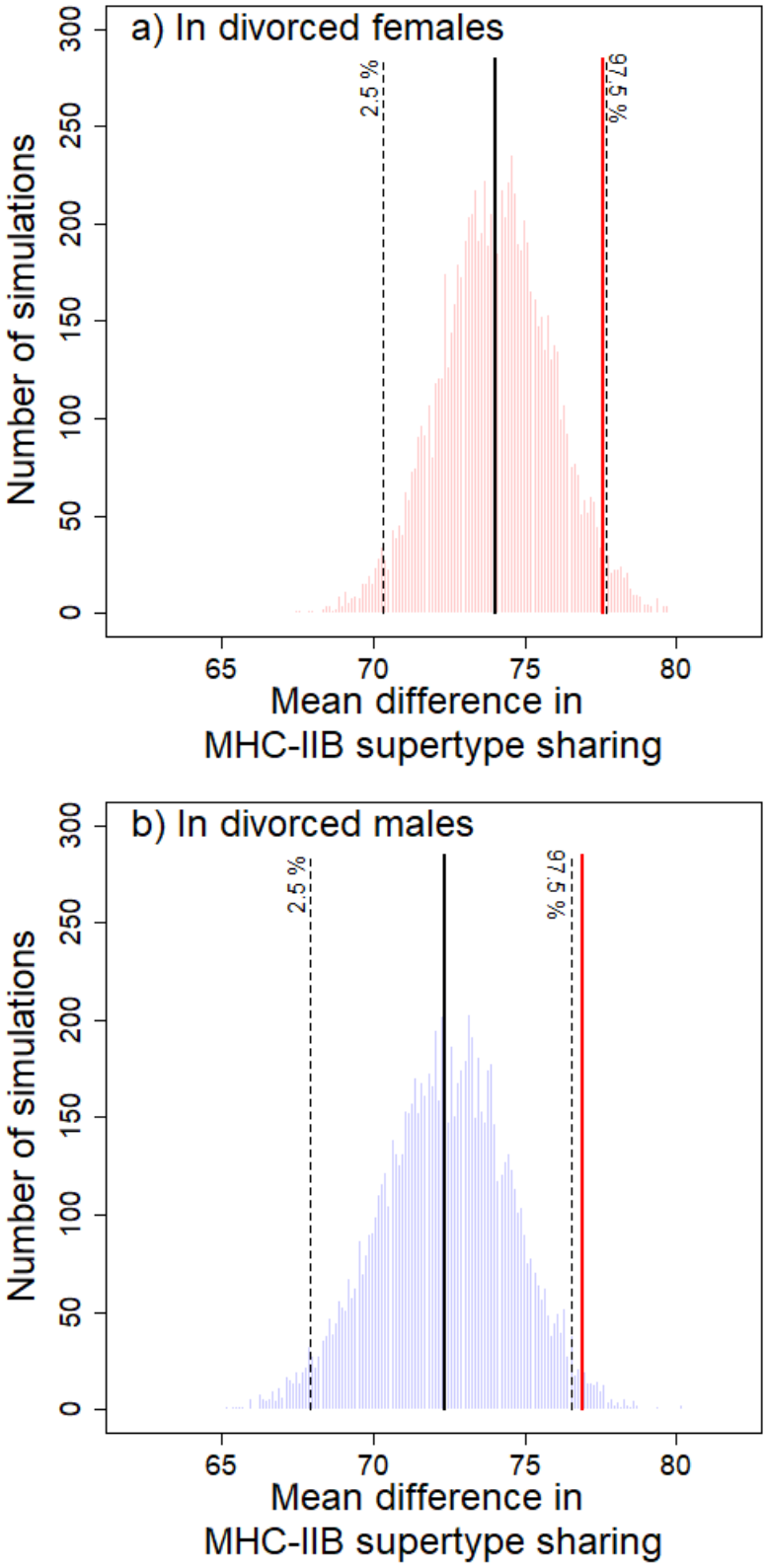


779 Figure 2

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