MHC-IIB disassortative mate choice in a genetically monogamous seabird

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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, explorations of the role of MHC in other mating strategies, such as divorce, are 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 females more MHC-IIB dissimilar 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

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,

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

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

91 First, we tested whether individuals chose their mates according to MHC dissimilarity
92 (Test 1). In several seabirds, including the black-legged kittiwake, young or inexperienced
93 individuals arrive later to the breeding ground than older or more experienced breeders
94 [50;51;52;53], which may increase the temporal constraints and limit the pool of potential
95 mates. Inexperienced breeders may also be less efficient in sampling mates and less able to
96 make mating discrimination or to anticipate alternative choices [54]. For instance, in grey
97 mouse lemurs (*Microcebus murinus*), young females in their first mating season do not
98 express mate choice, while experienced females prefer MHC-IIB dissimilar males [55]. The
99 mate choice of first-time breeders may thus be more constrained than that of experienced
100 breeders. We therefore tested the existence of MHC-IIB dependent mate choice in first-time
101 breeders (Test 1.1) and experienced breeders after a divorce (Test 1.2). Second, if divorcing
102 allows individuals paired with suboptimal mates to optimise the MHC-IIB diversity of their
103 future offspring, we expected the rate of divorce to depend on the MHC-IIB dissimilarity
104 between mates (Test 2). Third, we expected that, after a divorce, the new pair should be more
105 MHC-IIB compatible (maximised or intermediate dissimilarity) than the original pair (Test
106 3). In black-legged kittiwakes, although both mates participate fairly equally in incubation
107 and chick rearing, sexes slightly differ in energetic investment in reproduction [56;57]. We
108 thus tested these predictions separately in males and females to explore potential sex
109 differences in mating decisions.
110

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 probability of divorce 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

136 defined the first mate choice as the pair formed during the first observed breeding attempt.
137 Each individual was involved in on average 4.75 ± 3.74 (SE) reproductive events (range = [1;
138 21]; Supporting information 1) over the course of the study. To study divorce, we focused on
139 individuals that were observed with an identified mate during at least one year and that re-
140 mated with a new mate the next year, the original mate being re-observed at least once during
141 the following years [10].
142 We performed all analyses using the R software [62]. All datasets and codes used in
143 the analyses are available from the Zenodo Repository
144 (https://doi.org/10.5281/zenodo.14509255, [63]).
145
146 *Molecular analyses*
147 We focused on the MHC class II which exhibits stronger selection than the MHC class I in
148 non-passerine birds such as the black-legged kittiwake [64]. Dissimilarity at MHC-IIB was
149 assessed following the methods described in Pineaux et al. [47;49]. Briefly, DNA was
150 extracted from blood with the blood and tissue DNA extraction kit (Qiagen), and a 258-pb
151 fragment of exon 2 of MHC-IIB was amplified using specific primers (forward: 5′-
152 GCACGAGCAGGGTATTTCCA, and reverse: 5′-GTTCTGCCACACACTCACC).
153 Amplicons were then sequenced with an Illumina MiSeq platform, using the 2 x 300-bp
154 protocol (Fasteris SA, Switzerland). Amplicon sequences were analysed with ampliSaS, a
155 three-step pipeline that consists of read demultiplexing, unique sequence clustering and
156 erroneous sequence filtering (substitution errors: 1 %; indel errors: 0.001 %; minimum
157 frequency with respect to dominant: 33 %; minimum amplicon sequence frequency: 2.5 %
158 [65]). We calculated the MHC-IIB functional distance between individuals based on amino
159 acid variations in the peptide-binding region (PBR) inferred from Leclaire et al. [66].

We described the chemical binding properties of each amino acid in the PBR with Sandberg's five physicochemical descriptors (z-descriptors [67]) and computed a Sandberg's distance matrix between sequences using the *DistCalc* function of the *MHCTools* package [68]. 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 of the *vegan* package [69]), 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. [70]:

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

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 [71]). 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).

185 *Statistical analyses*

186 To test the association between mate choice and MHC-IIB dissimilarity (Test 1), we
187 used bootstrapping to generate random pairs, where we let each individual choose randomly
188 10000 times between all available opposite-sex mates of the same year, without replacement
189 (i.e. one mate can be selected only once during a given year [72;73]). To test for the
190 maximisation hypothesis, we calculated p-values as the proportion of iterations in which the
191 random MHC-IIB dissimilarity mean was greater than the observed MHC-IIB dissimilarity
192 mean, plus the proportion of iterations in which the random MHC-IIB dissimilarity mean was
193 smaller than the symmetrical (relative to the mean of random MHC-IIB dissimilarity means)
194 of the observed MHC-IIB dissimilarity mean (two-tailed test, following the method described
195 in [55]). To test for the intermediate optimum hypothesis, we used a similar procedure, but
196 instead of comparing the observed and random means of MHC-IIB dissimilarity, we
197 compared the observed and random variance in MHC-IIB dissimilarity (following the method
198 described in [73]). In the case of a mate choice for intermediate MHC-IIB dissimilarity, we
199 expected the observed and random means of MHC-IIB dissimilarity between partners to be
200 similar. However, because individuals are expected to prefer a mate that allows them to reach
201 the intermediate MHC-IIB dissimilarity optimum, we expected the observed variance in
202 MHC-IIB dissimilarity to be smaller than the random variance. We tested MHC-IIB based
203 mate choice for first-time breeders (n = 534 females and n = 554 males; Test 1.1) and for
204 experienced breeders after a divorce (n = 113 divorces in females and n = 83 divorces in
205 males; Test 1.2) separately.

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

210 binomial distribution of error and a logit link (*glmer* function of the *lme4* package [74]). To
211 test for the intermediate optimum hypothesis, we included the quadratic term of the MHC-IIB
212 dissimilarity [75]. If this variable did not have a significant effect (i.e. $P > 0.05$), we removed
213 the quadratic term and then tested for the maximisation hypothesis with the linear term of the
214 MHC-IIB dissimilarity. The reproductive outcome is also known to influence the probability
215 of divorce in black-legged kittiwakes [10;46]. We thus defined the reproductive outcome as a
216 binary variable recording the presence (i.e. "success") or absence (i.e. "failure") of at least
217 one chick fledged at the end of the reproductive season and included this variable as an
218 additive effect. In the models, we set the *nAGQ* argument (number of points per axis for
219 evaluating the adaptive Gauss-Hermite approximation to the log-likelihood) of the *glmer*
220 function to 0 to help for convergence, when necessary. We tested the effect of individual
221 identity and year as random intercepts with the *ranef* function of the *lme4* package [74], and
222 removed these variables when they had no effect. We removed pairs that were subjected to
223 experiments from this analysis. As we used GLMM with binomial data, we provided the log
224 odd (and its standard error), which is equivalent to the coefficient of the slope in a linear
225 model (i.e. estimate in the model summary). It corresponds to the probability of an event in
226 the log scale, which is the transformed scale to satisfy the assumptions of a linear model for
227 GLMM with binomial data. For convenience, we also provided the odd ratio, which
228 corresponds to the exponential of the log odd. An odd ratio of 1 means no effect, whereas an
229 odd ratio > 1 (< 1, resp.) corresponds to an increase (decrease, resp.) of the response variable
230 when the explanatory variable (here, reproductive outcome or MHC-IIB dissimilarity)
231 increases by 1 unit. We calculated the confidence intervals from the model on the fixed
232 effects using the *predictInterval* function of the *merTools* package (n iterations = 10000,
233 [76]).

To test whether, after divorce, the new mates were more MHC-IIB dissimilar than the original mates (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 mates and the MHC-IIB dissimilarity to the new mates. 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 [74], 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 expected the variance of the MHC-IIB dissimilarity in the new pairs to be lower than the variance of the MHC-IIB dissimilarity in the original pairs.

Power analyses

We conducted test-specific power analyses to assess the robustness of our results. When studying the MHC-IIB dissimilarity with the new mates compared to random mates (Test 1.1 and 1.2), we evaluated the likelihood of error as detailed in Hoover and Nevitt [72] and Hoover et al. [77]. 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

259 result for every sample size tested, and used a GLM with a binomial distribution of error and
260 a logit link (*glmer* function of the *lme4* package [74]) to describe the relationship between
261 sample size and likelihood of error. We also provided the effect size measured as the
262 difference between the observed MHC-IIB dissimilarity mean (resp. variance) and the
263 random MHC-IIB dissimilarity mean (resp. variance).

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

272 When assessing the gain in MHC-IIB dissimilarity between the original and the new
273 pairs after a divorce (Test 3), we calculated the effect sizes for our analysis using Cohen's d
274 statistic [78].

275

276 Results

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

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

284 functional distance between mates (P = 0.30, effect size = 0.07; Probability to find a
285 significant result = 0.03; Figure S4.2c and S4.2d). Females and their first mates did not show
286 significantly greater difference in supertype sharing or greater functional distance than
287 expected under random mating (functional distance: P = 0.89, effect size = -0.01, Figure
288 S4.2a; difference in supertype sharing: P = 0.72, effect size = 0.31, Figure 1a; Table S4.1).
289 Neither females (functional distance: P = 0.98; difference in supertype sharing: P = 0.88) nor
290 males (functional distance: P = 0.75; difference in supertype sharing: P = 0.49) mated with
291 significantly more intermediately dissimilar mates than expected under random mating (Table
292 S4.1; Figure S4.3 and S4.4).
293
294 *Probability of divorce (Test 2)*
295 In both sexes, the probability of divorce was significantly lower after a reproductive success
296 than after a reproductive failure (all P ≤ 0.01; Probability to find a significant result with a
297 balanced dataset > 0.80; Table S5.1; Figure S5.1). However, the probability of divorce did
298 not significantly vary with the MHC-IIB dissimilarity of the pair (Figure S5.2), when
299 considering either females (linear term: functional distance: P = 0.58 and difference in
300 supertype sharing: P = 0.74; quadratic term: functional distance: P = 0.18 and difference in
301 supertype sharing: P = 0.46; Table S5.1) or males (linear term: functional distance: P = 0.68
302 and difference in supertype sharing: P = 0.54; quadratic term: functional distance: P = 0.64
303 and difference in supertype sharing: P = 0.81; Table S5.1). The probability to find a
304 significant effect of MHC-IIB dissimilarity on the probability of divorce was consistently <
305 0.20 with a balanced dataset (Table S5.1).
306
307 *Mate choice in divorced individuals (Tests 3 and 1.2)*

308 Divorced individuals re-mated mainly with experienced breeders (63 % when considering
309 males and 71 % when considering females). The difference in MHC-IIB dissimilarity
310 between the new pair and the original pair was not different from 0, either in females or
311 males (Test 3 – maximisation hypothesis; all P > 0.10; Table S6.1). The effect sizes revealed
312 by Cohen's d statistics were very low (all Cohen's d < 0.20; Table S6.1; Figure S6.1).
313 However, after a divorce, males were paired with females which had a significantly
314 greater difference in supertype sharing to them than expected under random mating (Test 1.2:
315 P = 0.04, effect size = 4.52; Probability to find a significant result = 0.78; Figure 2b and
316 S7.1b; Table S7.1). Females were not re-mated with males which had a significantly greater
317 difference in supertype sharing than expected under random mating (Test 1.2: P = 0.06, effect
318 size = 3.58; Probability to find a significant result = 0.23; Figure 2a and S7.1a; Table S7.1).
319 These effects were not significant when considering functional distance (males: P = 0.09,
320 effect size = 0.31 and females: P = 0.62, effect size = 0.08; Table S7.1; Figure S7.2).
321 When considering either males or females, after a divorce, the new pairs were not
322 more intermediately dissimilar than the original pairs (Test 3 – intermediate hypothesis: all P
323 > 0.10; Table S6.1) or than random pairs (Test 1.2 – intermediate hypothesis: all P > 0.10;
324 Table S7.1).
325
326 *MHC-IIB dissimilarity and genome-wide relatedness*
327 We found that genome-wide relatedness was correlated neither with functional MHC-IIB
328 similarity (Mantel test: r = 0.04, P = 0.09; Figure S3.1), nor with difference in MHC-IIB
329 supertype sharing (Mantel test: r = 0.03, P = 0.10; Figure S3.2).
330
331 **Discussion**

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

338 First-time male breeders, as well as divorced males, were more MHC-IIB dissimilar
339 to their female partners than expected under random mating. These results may suggest active
340 mate choice for MHC-IIB dissimilarity. Like several other species (e.g. [26;79;80;81]),
341 including birds [82;83], black-legged kittiwakes are able to recognize MHC-IIB dissimilarity
342 using body odours [47]. In this species, the recognition of MHC-IIB dissimilarity might have
343 evolved as a mechanism to select MHC-IIB dissimilar mates to produce offspring with high
344 MHC-IIB diversity [48], and thus with higher early-life performance [49]. Similarly, in the
345 domestic cat (*Felis catus*), mating with MHC-IIB dissimilar mates increases offspring
346 survival and thus probably parental fitness [84].

347 In contrast to results when considering males, we did not detect MHC-IIB
348 disassortative mate choice when considering first-time female breeders. In addition, we
349 detected only a tendency for MHC-IIB disassortative mate choice when considering divorced
350 females ($P = 0.06$, with 23 % likelihood of obtaining a significant result). Our analysis
351 design, however, does not clearly allow us to determine whether the MHC-IIB disassortative
352 mate choice detected when considering males is driven by males' choice or females' choice.
353 For instance, a MHC-IIB disassortative mate choice detected in divorced males may suggest
354 that divorced males preferentially choose MHC-IIB dissimilar females or that females
355 preferentially select MHC-IIB dissimilar males when they have to choose amongst divorced
356 males. Similarly, a lack of MHC-IIB disassortative mate choice detected when considering

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

360 The contrasting results between males and females might however illustrate sex-
361 specific mating strategies. For instance, in Leach's storm-petrels (*Oceanodroma leucorhoa*),
362 while males show MHC-IIB disassortative mate choice, females' choice does not differ from
363 random mating [77]. In long-lived monomorphic species in which males and females equally
364 share parental care, such as the black-legged kittiwake [85;86;87], mate choice has been
365 suggested to be mutual [12]. However, the trade-off between the benefits obtained through
366 MHC disassortative mate choice and those obtained through mate choice based on the
367 intrinsic quality of the partner, such as its ability to provide good genes [88;89], good
368 parental care [90] or good-quality territory [91], can vary between individuals [88;92],
369 including between sexes. It may thus result in males and females having different preference
370 criteria for mate choice [93;94]. Sex-differences in the relative influence of MHC
371 dissimilarity vs. other traits on mating preference might be due to sex-specific variability in
372 either of these traits or to sex-specific variations in the direct benefits of these other traits to
373 the chooser. For instance, in mice, MHC dissimilarity is a more important criteria for mate
374 choice than scent-marking rate, only when variability in MHC dissimilarity between mates is
375 large [92]. In a cichlid fish (*Pelvicachromis taeniatus*), body size is a more important criteria
376 for mate choice than genetic compatibility for males than for females, which may be due to
377 the crucial role of female body size on fecundity [8].

378 When using Monte Carlo simulations to test mate choice, Hoover and Nevitt [72]
379 showed that a sample size of 500 pairs may be necessary to confidently reject the null
380 hypothesis when the effect size is small (see also [75]), small effect sizes being common
381 when studying MHC-based mate choice [75]. In the present study, the sample size of first-

382 time breeding males was ca. 500 individuals, and we found a P = 0.05 in males with 60 %
383 likelihood to find a non-significant result. This suggests at best a low effect size for mate
384 choice based on MHC-IIB dissimilarity in first-time breeders. In contrast, the sample size of
385 divorced males was largely smaller (i.e. only 83 males), but we found a significant MHC-IIB
386 disassortative mate choice (P = 0.04) with a much larger effect size in divorced individuals
387 than in first-time breeders (compare effects sizes in Tables S4.1 and S7.1). In several species,
388 the level of mate selectivity is influenced by age [55;95;96;97]. In black-legged kittiwakes,
389 higher selectivity in divorced individuals compared to first-time breeders may be related to
390 their earlier arrival on the nesting site [50], which may increase both decision time and the
391 pool of potential partners and thereby mating decision accuracy [98]. However, like for the
392 sex-specific variations detected in the mate choice analysis, we cannot determine whether the
393 higher selectivity for MHC-IIB dissimilar partners detected when considering divorcees than
394 when considering first-time breeders is due to divorced individuals being more selective in
395 choosing MHC-IIB dissimilar males or individuals being more selective in choosing MHC-
396 IIB dissimilar mates when they have to choose amongst divorced partners. For instance, in
397 bluethroats (*Luscinia svecica*), females seem to select males based on MHC-related traits
398 only when paired with young males [39]. In black-legged kittiwakes, in the context of a
399 balance between selecting mates for MHC-IIB dissimilarity vs. other individual quality traits,
400 the lower inter-individual variability in individual quality in experienced breeders than in
401 first-time breeders [99], may explain the higher selectivity for MHC-IIB dissimilarity when
402 the pool of potential partners are divorcees. Clearly, while our study provides invaluable
403 insight into the MHC-IIB disassortative mating strategy of black-legged kittiwakes, further
404 studies are needed to determine which sex and age-groups are more selective or selected
405 based on MHC-IIB dissimilarity, and why.

406 In accordance with previous studies in black-legged kittiwakes [10;45;46;100], we
407 found that the probability of divorce was greater in pairs that failed to fledge a chick than in
408 pairs that reproduced successfully. However, it does not significantly vary with the MHC-IIB
409 dissimilarity between mates. Also, after a divorce, individuals do not mate with partners more
410 MHC-IIB dissimilar to them than their original partners. Together, these results suggest that
411 in black-legged kittiwakes, MHC-IIB dissimilarity between the male and the female of the
412 pair is not a driving factor of divorce. In the present study, the proportion of divorce was
413 much lower than the proportions observed in other populations of black-legged kittiwakes
414 (annual mean ± SD = 4.4 ± 2.6 % in our study *vs.* 25 % in [44]; between 13 % and 47 % in
415 [45]; 19.1 % on average in [46]). On Middleton island, numerous black-legged kittiwakes
416 nest on other man-made structures than the tower [58]. These sites were not monitored,
417 except for occasional demographic surveys for certain years. It may therefore have limited
418 our ability to observe birds that changed nesting sites between breeding seasons, and thus to
419 detect all divorces, leading to a low sample size that might have affected our results and have
420 limited our ability to detect a significant effect.
421 We did not find any support for the intermediate optimum hypothesis in mating
422 decisions in black-legged kittiwakes. This result is consistent with Pineaux et al. [49], where
423 no benefits of intermediate MHC-IIB diversity were detected in black-legged kittiwake
424 chicks. In this species, the benefits of a larger MHC-IIB repertoire associated with increased
425 resistance to wider range of pathogens may outcompete the costs associated with increased
426 risks of autoimmune diseases and reduced T cell receptors diversity [34;35].
427 A previous study on the same population of black-legged kittiwakes showed that pairs
428 have a higher genetic dissimilarity (as measured by microsatellites) than expected under
429 random mating, and that the resulting increased heterozygosity of their chicks leads to higher
430 fitness [101]. Besides increasing the resistance to pathogens of the offspring, MHC-IIB

431 disassortative mate choice may limit inbreeding [80;102]. In the present study, we detected
432 MHC-based mate choice when MHC-IIB dissimilarity was measured based on difference in
433 supertype sharing, but not when based on functional distance. Similarly, in blue petrels
434 (*Halobaena caerulea*), yellow-rumped flycatchers (*Ficedula zanthopygia*) and little auks
435 (*Alle alle*), evidence for MHC-based mating strategies are stronger when considering
436 supertype or allele sharing than when considering functional distances [70;103;104] (but see
437 [55] for the opposite pattern). Understanding whether the difference detected between the two
438 indices illustrates that MHC-based mate choice is related to inbreeding avoidance in this
439 species require a larger number of individuals with genome-wide data.

440 In conclusion, we found evidence that black-legged kittiwakes choose partners based
441 on MHC-IIB dissimilarity. This finding supports the role of the benefits associated with high
442 MHC-IIB dissimilarity in the evolution of mating decisions in this population. It is consistent
443 with a previous study showing that, in this species, sex-allocation strategy varies with MHC-
444 IIB dissimilarity between parents [48]. In addition, our study suggests that mate choice for
445 MHC-IIB dissimilar partners varies with sex and age or experience of the chooser or the
446 selected mate. When the relative benefits of MHC disassortative mate choice vary with sex
447 and mate choice is mutual, a sexual conflict over mating strategies can arise and may select
448 for a rich repertoire of sex-specific behavioural adaptations [8;105]. Our study paves the road
449 for further studies of sex-specific mate choice in species with bi-parental care, a common trait
450 in birds [106].

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Figure captions

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.

789 **Figures**

790 Figure 1

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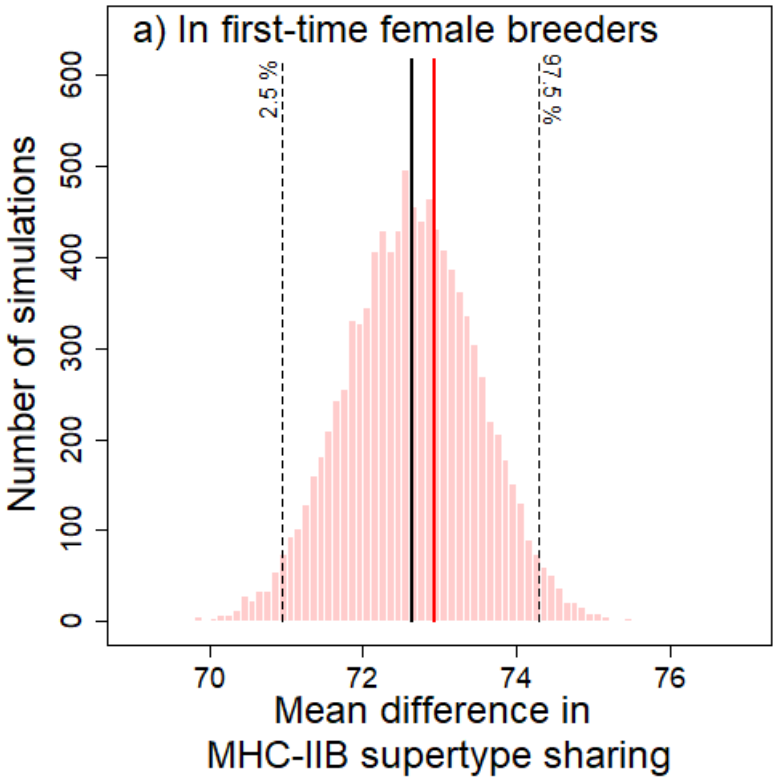

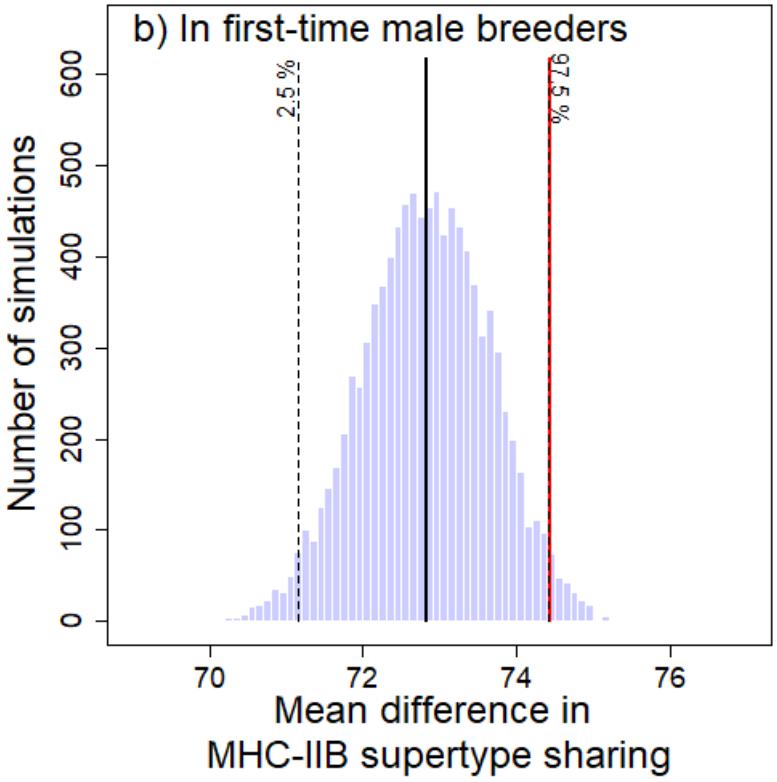

792 Figure 2

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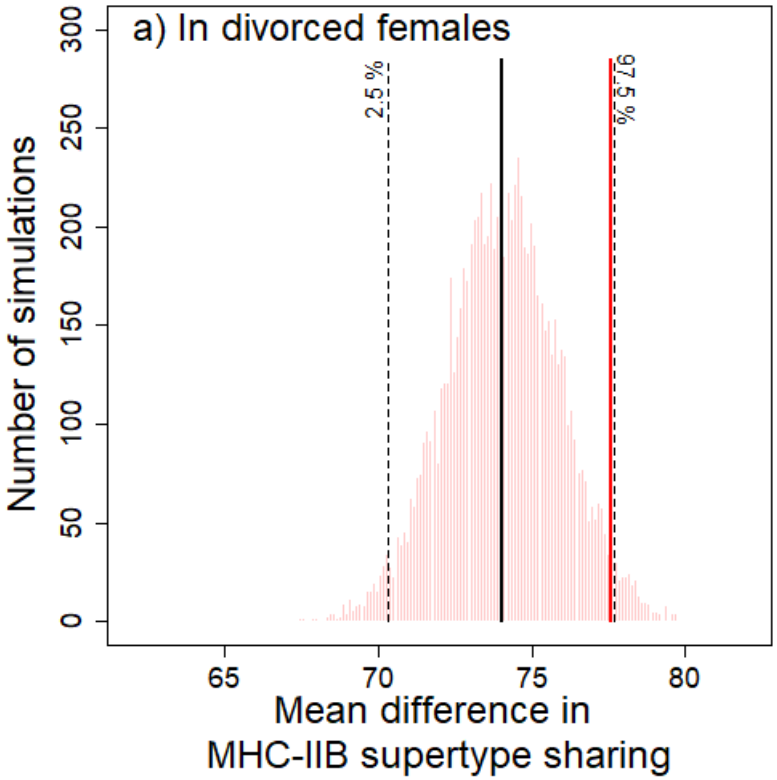

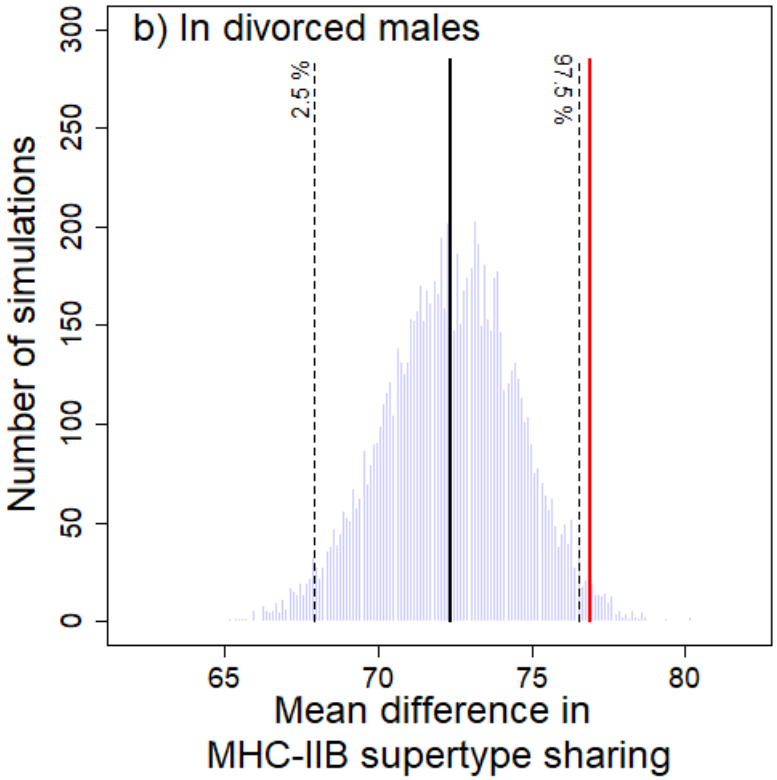

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Conflict of interest

The authors declare that they have no conflict of interest.

Data accessibility

Data available from the Zenodo Repository: https://doi.org/10.5281/zenodo.14509255.

Authors' contributions

Conceptualisation: LT, MP, PB, SL; Methodology: LT, MP, PB, SL; Data Curation: LT, PB, SL; Formal Analyses: LT, SL; Investigation: LT; Writing: Original Draft: LT, SL; Writing: Review & Editing: LT, MP, ED, PB, SL; Visualisation: LT, SL; Data acquisition: MP, LR; Project administration: ED, SW, SH, SL; Funding acquisition: ED, SH, SL; Supervision: PB, SL; Resources: SL.

Ethics

Research activities on Middleton Island, AK, were permitted by the United State Geological Survey (Banding Permit 23910) and the McGill Animal Care Committee (animal use protocol 2016–7814 and precursors).