Does MHC-IIB dissimilarity between partners drive mate choice and divorce in a genetically monogamous seabird?

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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

The experiment was conducted under the approval of the U.S. Geological Survey Alaska Science Center, the University of Alaska Institutional Animal Care and Use Committees, and the Institut Paul-Emile Victor Ethical Committee, in accordance with United States laws and under permits from the U.S. Fish and Wildlife Service and the State of Alaska.

Conflict of interest

The authors declare that they have no conflict of interest.

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Data availability statement

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

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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, support for other MHC based mating strategies, such as divorce, is rare. We investigated whether MHC-IIB dissimilarity (MHC-IIB functional distance and difference in MHC-IIB supertype sharing) 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 a female with a greater difference in MHC-IIB supertype sharing to them than expected under random mating. We did not find evidence for mate choice based MHC-IIB dissimilarity when considering females. In addition, divorce probability did not significantly vary with the MHC-IIB dissimilarity of the pair, and after a divorce, the newly formed pair was not more MHC-dissimilar than the original 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

24 ## Introduction

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

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

thereby providing resistance to a wider range of parasites [26;27]. Accordingly, several studies have found that higher MHC diversity increases health, reproductive success and survival [28;29]. Because the MHC allelic composition of the offspring results from the combination of those of the parents, multiple studies have found evidence for mate choice maximising MHC dissimilarity between mates (e.g. [30;31;32;33]). However, high MHC diversity can also increase the risk of autoimmune diseases and reduce the T cell receptors diversity [34;35]. In a few species, intermediate MHC diversity has thus been shown to be optimum for fitness, and to be an important factor in mate choice (e.g. [36;37;38;39]). Although there is evidence that the MHC composition or diversity of the parents also influences other reproductive strategies (e.g. cryptic female choice, [40]; extra-pair copulation, [41]), to our knowledge, a single study on Seychelle warblers (*Acrocephalus sechellensis*) explored the association between parental MHC similarity and divorce rate and found no evidence [42].

Here we tested whether mate choice and divorce depend on dissimilarity at MHC class-II B (hereafter MHC-IIB dissimilarity) in the black-legged kittiwake (*Rissa tridactyla*), a long-lived genetically monogamous seabird with no extra-pair copulation [43]. In black-legged kittiwakes, divorce is fairly common (i.e. 15 % to 50 % of the pairs divorce each year), and more frequent among first-time breeders and after reproductive failure [44;45;46] [10]. Coulson [44] suggested therefore that some kinds of "incompatibility" between mates result in unsuccessful breeding, thereby providing costs that select for divorce. In black-legged kittiwakes, individuals can recognize MHC-IIB dissimilarity using body odours [47], and MHC-IIB similarity between mates results in reduced offspring MHC-IIB diversity [48], which incurs costs for female chicks such as slower growth, poor tick clearance efficiency and lower survival [49]. We therefore hypothesised that, in this species, mating decisions 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). 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 defined the first mate choice as the pair formed during the first observed breeding attempt. Each individual was involved in on average 4.75 ± 3.74 (SE) reproductive events (range = [1;

21]; Supporting information 1) over the course of the study. To study divorces, we focused on individuals that were observed with an identified mate during at least one year and that re-mated with a new mate the next year, the original mate being re-observed at least once during the following years [10].

We performed all analyses using the R software [62]. All datasets and code used in the analyses are available from the Zenodo Repository (https://doi.org/10.5281/zenodo.14509255).

Molecular analyses

We focused on the MHC class II which exhibits stronger selection than the MHC class I in non-passerine birds such as the black-legged kittiwake [63]. Dissimilarity at MHC-IIB was assessed following the methods described in Pineaux et al. [49;47]. Briefly, DNA was extracted from blood with the blood and tissue DNA extraction kit (Qiagen), and a 258-pb fragment of exon 2 of MHC-IIB was amplified using specific primers (forward: 5′-GCACGAGCAGGGTATTTCCA, and reverse: 5′-GTTCTGCCACACACTCACC). Amplicons were then sequenced with an Illumina MiSeq platform, using the 2 x 300-bp protocol (Fasteris SA, Switzerland). Amplicon sequences were analysed with ampliSaS, a three-step pipeline that consists of read demultiplexing, unique sequence clustering and erroneous sequence filtering (substitution errors: 1 %; indel errors: 0.001 %; minimum frequency with respect to dominant: 33 %; minimum amplicon sequence frequency: 2.5 %; [64]). We calculated the MHC-IIB functional distance between individuals based on amino 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

149 [67]. Then, we calculated an UPGMA dendrogram of sequences (*hclust* function), which
150 represents clusters of functionally similar MHC-IIB sequences (hereafter, "supertypes"). The
151 MHC-IIB functional distance between a male and a female was estimated using the tree
152 distance (*treedist* function in the *vegan* package [68]), which is found by combining
153 sequences in a common dendrogram and calculating how much of the sum of the branch
154 length is shared and how much is unique. In addition, we calculated the difference in MHC-
155 IIB supertype sharing between mates similarly to Strandh et al. [69]:

156 $$100 \times [1 - Nb_{supertypes\ pair} / (Nb_{supertypes\ female} + Nb_{supertypes\ male})],$$

157 where $Nb_{supertypes\ female}$ corresponds to the number of supertypes possessed by the female,
158 $Nb_{supertypes\ male}$ corresponds to the number of supertypes possessed by the male, and $Nb_{supertypes}$
159 $_{pair}$ corresponds to the total number of supertypes possessed by the female and the male
160 together (where shared supertypes count as 1). This index thus varies between 0 for
161 individuals sharing no supertype and 100 for individuals sharing all their supertypes. The
162 MHC-IIB functional distance was highly correlated with the difference in MHC-IIB
163 supertype sharing (Spearman correlation test: $\rho = 0.79$, $P < 0.0001$, n = 959 pairs; Supporting
164 information 2).

165 Mate choice for MHC-IIB dissimilarity may not only be governed by evolutionary
166 processes that specifically target these genes, but may also be affected by mechanisms
167 operating at the level of the entire genome (e.g. inbreeding avoidance). To test whether the
168 associations between MHC-IIB dissimilarity and mate choice may be driven by genome-wide
169 relatedness, we used RAD sequencing (n = 254 individuals) to estimate genome-wide
170 relatedness between individuals. We found that genome-wide relatedness was correlated
171 neither with functional MHC-IIB similarity (Mantel test: r = 0.03, P = 0.09), nor with MHC-
172 IIB supertype sharing (Mantel test: r = 0.03, P = 0.17; Figure S3.1).
173

174 *Statistical analyses*

175 To test the association between mate choice and MHC-IIB dissimilarity (Test 1), we
176 used bootstrapping to generate random pairs, where we let each individual choose randomly
177 10000 times between all available opposite-sex mates of the same year, without replacement
178 (i.e. one mate can be selected only once during a given year; [70;71]). To test for the
179 maximisation hypothesis, we calculated p-values as the proportion of iterations in which the
180 random MHC-IIB dissimilarity mean was greater than the observed MHC-IIB dissimilarity
181 mean, plus the proportion of iterations in which the random MHC-IIB dissimilarity mean was
182 smaller than the symmetrical (relative to the mean of random MHC-IIB dissimilarity means)
183 of the observed MHC-IIB dissimilarity mean (two-tailed test, following the method described
184 in [55]). To test for the intermediate optimum hypothesis, we used a similar procedure, but
185 instead of comparing the observed and random means of MHC-IIB dissimilarity, we
186 compared the observed and random variance in MHC-IIB dissimilarity (following the method
187 described in [71]). In the case of a mate choice for intermediate MHC-IIB dissimilarity, we
188 expect the observed and random means of MHC-IIB dissimilarity between partners to be
189 similar. However, because individuals are expected to prefer a mate that allows them to reach
190 the intermediate MHC-IIB dissimilarity optimum, we expect the observed variance in MHC-
191 IIB dissimilarity to be smaller than the random variance. We tested MHC-IIB based mate
192 choice for first-time breeders (n = 534 females and n = 554 males; Test 1.1) and for breeders
193 experiencing a divorce (n = 113 divorces in females and n = 83 divorces in males; Test 1.2)
194 separately.

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

199 binomial distribution of error and a logit link (*glmer* function of the *lme4* package; [72]). To
200 test for the intermediate optimum hypothesis, we included the quadratic term of the MHC-IIB
201 dissimilarity [73]. If this variable did not have a significant effect (i.e. $P > 0.05$), we removed
202 the quadratic term and then tested for the maximisation hypothesis with the linear term of the
203 MHC-IIB dissimilarity. The reproductive outcome is also known to influence the probability
204 of divorce in black-legged kittiwakes [46];10]. We thus defined the reproductive outcome as
205 a binary variable recording the presence (i.e. "success") or absence (i.e. "failure") of at least
206 one chick fledged at the end of the reproductive season and included this variable as an
207 additive effect. In the models, we set the *nAGQ* argument (number of points per axis for
208 evaluating the adaptive Gauss-Hermite approximation to the log-likelihood; [74]) of the
209 *glmer* function to 0 to help for convergence, when necessary. We tested the effect of
210 individual identity and year as random intercepts with the *ranef* function of the *lme4* package
211 [72], and removed these variables when they had no effect. We removed pairs that were
212 subjected to experiments from this analysis. As we used GLMM with binomial data, we
213 provided the log odd (and its standard error), which is equivalent to the coefficient of the
214 slope in a linear model (i.e. estimate in the model summary). It corresponds to the probability
215 of an event in the log scale, which is the transformed scale to satisfy the assumptions of a
216 linear model for GLMM with binomial data. For convenience, we also provided the odd ratio,
217 which corresponds to the exponential of the log odd. An odd ratio of 1 means no effect,
218 whereas an odd ratio > 1 (< 1, resp.) corresponds to an increase (decrease, resp.) of the
219 response variable when the explanatory variable (here, reproductive outcome or MHC-IIB
220 dissimilarity) increases by 1 unit. We calculated the confidence intervals from the model on
221 the fixed effects using the *predictInterval* function of the *merTools* package (n iterations =
222 10000; [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 [72], 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 et al. [70;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 sample

248 size tested, and used a GLM with a binomial distribution of error and a logit link (*glmer*
249 function of the *lme4* package; [72]) to describe the relationship between sample size and
250 likelihood of error.

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 a female which had a significantly greater difference in supertype sharing than expected
268 under random mating (P = 0.05; Table S4.1; Figure 1c). The probability to find a significant
269 result with the available sample size (n = 554 mate choice events) was 0.40 (Table S4.1;
270 Figure 1d). This effect was however not significant when considering the functional distance
271 between mates (P = 0.30; Probability to find a significant result = 0.03; Figure S4.1c and
272 S4.1d). Females did not mate with a male which had a significantly greater difference in

supertype sharing or greater functional distance to them than expected under random mating (functional distance: P = 0.89; difference in supertype sharing: P = 0.72; Table S4.1; Figure 1a and Figure S4.1a). Neither females (functional distance: P = 0.98; difference in supertype sharing: P = 0.88) nor males (functional distance: P = 0.75; difference in supertype sharing: P = 0.49) mated with a significantly more intermediately dissimilar mates than expected under random mating (Table S4.1; Figure S4.2 and S4.3).

Probability of divorce (Test 2)

In both sexes, the probability of divorce was significantly lower after a reproductive success than after a reproductive failure (all $P \leq 0.01$; Probability to find a significant result with a balanced dataset > 0.80; Table S5.1). However, the probability of divorce did not significantly vary with the MHC-IIB dissimilarity of the pair (Figure S5.2), when considering either females (linear term: functional distance: P = 0.58 and difference in supertype sharing: P = 0.74; quadratic term: functional distance: P = 0.18 and difference in supertype sharing: P = 0.46; Table S5.1) or males (linear term: functional distance: P = 0.68 and difference in supertype sharing: P = 0.54; quadratic term: functional distance: P = 0.64 and difference in supertype sharing: P = 0.81; Table S5.1). The probability to find a significant effect of MHC-IIB dissimilarity on the probability of divorce was consistently < 0.20 with a balanced dataset (Table S5.1).

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

297 males (Test 3; all P > 0.10; Table S6.1). The effect sizes revealed by Cohen's d statistics were
298 very low (all Cohen's d < 0.20; Table S6.1).
299 However, after a divorce, males were paired with a female which had a significantly
300 greater difference in supertype sharing to them than expected under random mating (Test 1.2:
301 P = 0.04; Probability to find a significant result = 0.78; Table S7.1; Figure 2c & 2d). Females
302 did not re-mate with a male which had a significantly greater difference in supertype sharing
303 than expected under random mating (Test 1.2: P = 0.06; Probability to find a significant result
304 = 0.23; Table S7.1; Figure 2a). These effects were not significant when considering
305 functional distance (males: P = 0.09 and females: P = 0.62; Table S7.1; Figure S7.1).
306 When considering either males or females, after a divorce, the new pair was not more
307 intermediately dissimilar than the original pair (Test 3: all P > 0.10; Table S6.1) or than a
308 random pair (Test 1.2: all P > 0.10; Table S7.1).
309

310 **Discussion**

311 In this study, we tested whether mating decisions (i.e. mate choice and divorce) of black-
312 legged kittiwakes vary with the MHC-IIB dissimilarity between mates. We found that first-
313 time male breeders (Test 1.1) as well as divorced males (and to a lower extent, divorced
314 females; Test 1.2) were paired with a mate more MHC-IIB dissimilar to them than expected
315 under random mating. However, we did not detect an association between the MHC-IIB
316 dissimilarity of the pair and the probability of divorce.
317 First-time male breeders, as well as divorced males, were more MHC-IIB dissimilar
318 to their female partner than expected under random mating. These results may suggest active
319 mate choice for MHC-IIB dissimilarity. Like several other species (e.g. [78;79;26;80]),
320 including birds [81;82], black-legged kittiwakes are able to recognize MHC-IIB dissimilarity
321 using body odours [47]. In this species, the recognition of MHC-IIB dissimilarity might have

322 evolved as a mechanism to select MHC-IIB dissimilar mates to produce offspring with high
323 MHC-IIB diversity [48], and thus with higher early-life performance [49]. Similarly, in the
324 domestic cat (*Felis catus*), mating with MHC-IIB dissimilar mates increases offspring
325 survival and thus probably parental fitness [83]. We detected MHC-based mate choice when
326 MHC-IIB dissimilarity was measured based on difference in supertype sharing, but not when
327 based on functional distance. The causes of these discrepancies are unknown but are
328 consistent with the literature. For instance, in blue petrels (*Halobaena caerulea*), yellow-
329 rumped flycatchers (*Ficedula zanthopygia*) and little auks (*Alle alle*), evidence for MHC-
330 based mating strategies are stronger when considering supertype or allele sharing than when
331 considering functional distances ([69;84;85]; but see [55] for the opposite pattern).
332 In contrast to results when considering males, we did not detect MHC-IIB
333 disassortative mate choice when considering first-time female breeders. In addition, we
334 detected only a tendency for MHC-IIB disassortative mate choice when considering divorced
335 females (P = 0.06, with 23 % likelihood of obtaining a significant result). Our analysis
336 design, however, does not clearly allow us to determine whether the MHC-IIB disassortative
337 mate choice detected when considering males is driven by males' choice or females' choice.
338 For instance, a MHC-IIB disassortative mate choice detected in divorced males may suggest
339 that divorced males preferentially choose MHC-IIB dissimilar females or that females
340 preferentially select MHC-IIB dissimilar males when they have to choose amongst divorced
341 males. Similarly, a lack of MHC-IIB disassortative mate choice detected when considering
342 first-time female breeders may be due to first-time female breeders not choosing a male
343 partner based on MHC-IIB dissimilarity or to males not choosing a MHC-IIB dissimilar
344 females when they have to choose amongst first-time female breeders.
345 The contrasting results between males and females might however illustrate sex-
346 specific mating strategies. Similarly, in Leach's storm-petrels (*Oceanodroma leucorhoa*),

347 while males show MHC-IIB disassortative mate choice, females' choice does not differ from
348 random mating [76]. In long-lived monomorphic species in which males and females equally
349 share parental care, such as the black-legged kittiwake [86;87;88], mate choice has been
350 suggested to be mutual [12]. However, the trade-off between the benefits obtained through
351 MHC disassortative mate choice and those obtained through mate choice based on the
352 intrinsic quality of the partner, such as its ability to provide good genes [89;90], good
353 parental care [91] or good-quality territory [92], can vary between individuals [93;89],
354 including between sexes. It may thus result in males and females having different preference
355 criteria for mate choice [94;95]. Sex-differences in the relative influence of MHC
356 dissimilarity vs. other traits on mating preference might be due to sex-specific variability in
357 either of these traits or to sex-specific variations in the direct benefits of these other traits to
358 the chooser. For instance, in mice, MHC dissimilarity is a more important criteria for mate
359 choice than scent-marking rate, only when variability in MHC dissimilarity between mates is
360 large [93]. In a cichlid fish (*Pelvicachromis taeniatus*), body size is a more important criteria
361 for mate choice than genetic compatibility for males than for females, which may be due to
362 the crucial role of female body size on fecundity [8].
363 When using Monte Carlo simulations to test mate choice, Hoover et al. [70] showed
364 that a sample size of 500 pairs may be necessary to confidently reject the null hypothesis
365 when the effect size is small (see also [73]), small effect sizes being common when studying
366 MHC-based mate choice [73]. In the present study, the sample size of first-time breeding
367 males was ca. 500 individuals, and we found a P = 0.05 in males, with 60 % likelihood to
368 find a non-significant result. This suggests at best a low effect size for mate choice based on
369 MHC-IIB dissimilarity in first-time male breeders. In contrast, the sample size of divorced
370 males was largely smaller (i.e. only 83 males), but we found a significant MHC-IIB
371 disassortative mate choice (P = 0.04) with 22 % likelihood to find a non-significant result,

372 suggesting a much larger effect size in divorced males than in first-time male breeders. In
373 several species, the level of mate selectivity is influenced by age [96;55;97;98]. In kittiwakes,
374 higher selectivity in divorced individuals compared to first-time breeders may be related to
375 their earlier arrival on the nesting site [50], which may increase both decision time and the
376 pool of potential partners and thereby mating decision accuracy [99]. However, like for the
377 sex-specific variations detected in the mate choice analysis, we cannot determine whether the
378 higher selectivity for MHC-IIB dissimilar partners detected when considering divorcees than
379 when considering first-time breeders is due to divorced individuals being more selective in
380 choosing MHC-IIB dissimilar males or individuals being more selective in choosing MHC-
381 IIB dissimilar mates when they have to choose amongst divorced partners. For instance, in
382 bluethroats (*Luscinia svecica*), females seem to select males based on MHC-related traits
383 only when paired with young males [39]. In black-legged kittiwakes, in the context of a
384 balance between selecting mates for MHC-IIB dissimilarity vs. other individual quality traits,
385 the lower inter-individual variability in individual quality in experienced breeders than in
386 first-time breeders [100], may explain the higher selectivity for MHC-IIB dissimilarity when
387 the pool of potential partners are divorcees. Clearly, while our study provides invaluable
388 insight into the MHC-IIB disassortative mating strategy of black-legged kittiwakes, further
389 studies are needed to determine which sex and age-groups are more selective or selected
390 based on MHC-IIB dissimilarity, and why.

391 In accordance with previous studies in black-legged kittiwakes [45;101;46;10], we
392 found that the probability of divorce was greater in pairs that failed to fledge a chick than in
393 pairs that reproduced successfully. However, it does not significantly vary with the MHC-IIB
394 dissimilarity between mates. Also, after a divorce, individuals do not mate with a partner
395 more MHC-IIB dissimilar to them than their original partner. Together, these results suggest
396 that in black-legged kittiwakes, MHC-IIB dissimilarity between the male and the female is

397 not a driving factor of divorce. In the present study, the proportion of divorce was much
398 lower than the proportions observed in other populations of kittiwakes (annual mean ± SD =
399 4.4 ± 2.6 % in our study *vs.* 25 % in [44]; between 13 % and 47 % in [45]; 19.1 % on average
400 in [46]). On Middleton island, numerous black-legged kittiwakes nest on other man-made
401 structures than the tower [58]. These sites were not monitored, except for occasional
402 demographic surveys for certain years. It may therefore have limited our ability to observe
403 birds that changed nesting sites between breeding season, and thus to detect all divorces,
404 leading to a low sample size that might have affected our results.

405 We did not find any support for the intermediate optimum hypothesis in mating
406 decisions in the black-legged kittiwake. This result is consistent with Pineaux et al. [49],
407 where no benefits of intermediate MHC-IIB diversity were detected in kittiwake chicks. In
408 this species, the benefits of a larger MHC-IIB repertoire associated with increased resistance
409 to wider range of pathogens may outcompete the costs associated with increased risks of
410 autoimmune diseases and reduced T cell receptors diversity [35;34].

411 A previous study on the same population of black-legged kittiwakes showed that pairs
412 have a higher genetic dissimilarity (as measured by microsatellites) than expected under
413 random mating, and that the resulting increased heterozygosity of their chicks leads to higher
414 fitness [102]. Although MHC-IIB disassortative mate choice may also participate in limiting
415 inbreeding [79;103], in the present study we detected no significant correlation between
416 genome-wide relatedness and MHC-IIB dissimilarity. As observed in other species (e.g. [30]
417 [32;104]), these results suggest that the MHC-IIB dissimilarity-based mate choice in black-
418 legged kittiwakes is probably driven by the MHC rather than resulting from inbreeding
419 avoidance at the genome-wide scale.

420 In conclusion, we found evidence that black-legged kittiwakes choose partners based
421 on MHC-IIB dissimilarity. This finding supports the role of the benefits associated with high

MHC-IIB dissimilarity in the evolution of mating decisions in this population. It is consistent with a previous study showing that, in this species, sex-allocation strategy varies with the MHC-IIB dissimilarity between parents [48]. In addition, our study suggests that mate choice for MHC-IIB dissimilar partners varies with sex and age or experience of the chooser or the selected mate. When the relative benefits of MHC disassortative mate choice vary with sex and mate choice is mutual, a sexual conflict over mating strategies can arise and may select for a rich repertoire of sex-specific behavioural adaptations [105;8]. Our study paves the road for further studies of sex-specific mate choice in species with bi-parental care, a common trait in birds [106].

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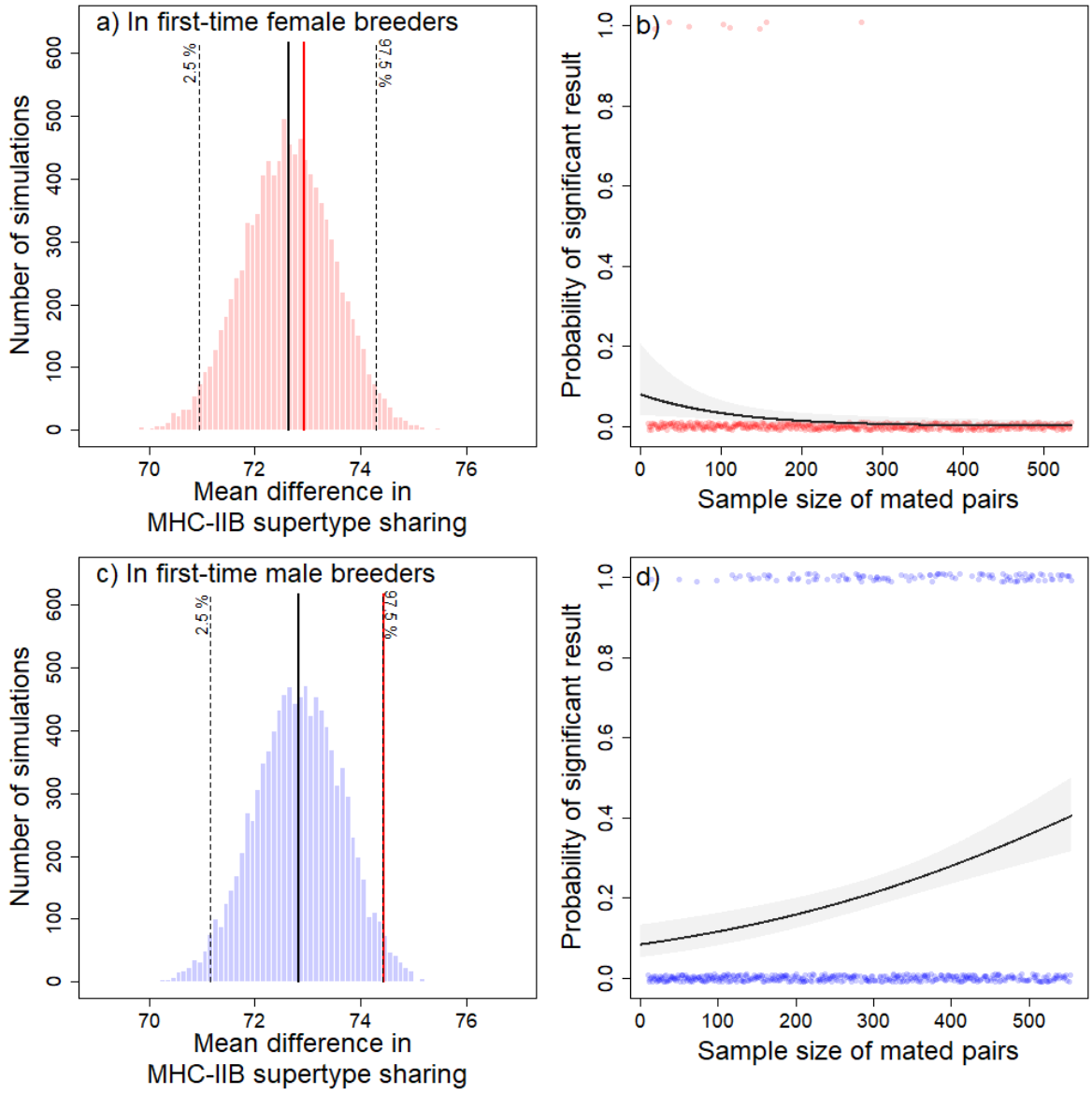
751 **Figures**

752 Figure 1: **a-c)** Frequency distribution of means of random difference in MHC-IIB supertypes
753 sharing between first-time breeders and their mate, calculated from 10000 bootstrap
754 randomizations of potential pairs: a) when considering females, c) when considering males.
755 The red vertical line is the observed mean of the difference in MHC-IIB supertypes sharing.
756 The black vertical line corresponds to the mean of the simulated distribution, the black
757 dashed lines are the 2.5 and 97.5 % quantiles of the simulated distribution showing the 95 %
758 confidence intervals. **b-d)** Likelihood of a type II error according to the sample size: a) when
759 considering females, b) when considering males. The grey dots represent the raw
760 observations from the simulations at each sample size, the line represents the predictions of
761 the model, the shaded area represents the 95 % confidence intervals around the predictions of
762 the model.

763

764 Figure 2: **a-c)** Frequency distribution of means of random difference in MHC-IIB supertypes
765 sharing between divorced breeders and their new mate, calculated from 10000 bootstrap
766 randomizations of potential pairs, when considering a) divorced females and c) divorced
767 males. The red vertical line is the observed mean of the difference in MHC-IIB supertypes
768 sharing between the focal divorced individuals and their real new partner. The black vertical
769 line corresponds to the mean of the simulated distribution, and the black dashed lines are the
770 2.5 and 97.5 % quantiles of the simulated distribution showing the 95 % confidence intervals.
771 **b-d)** Probability of obtaining a significant result according to the sample size, when
772 considering b) females and d) males. The grey dots represent the raw observations from the
773 simulations at each sample size, the line represents the predictions of the model, the shaded
774 area represents the 95 % confidence intervals around the predictions of the model.

775 Figure 1



776

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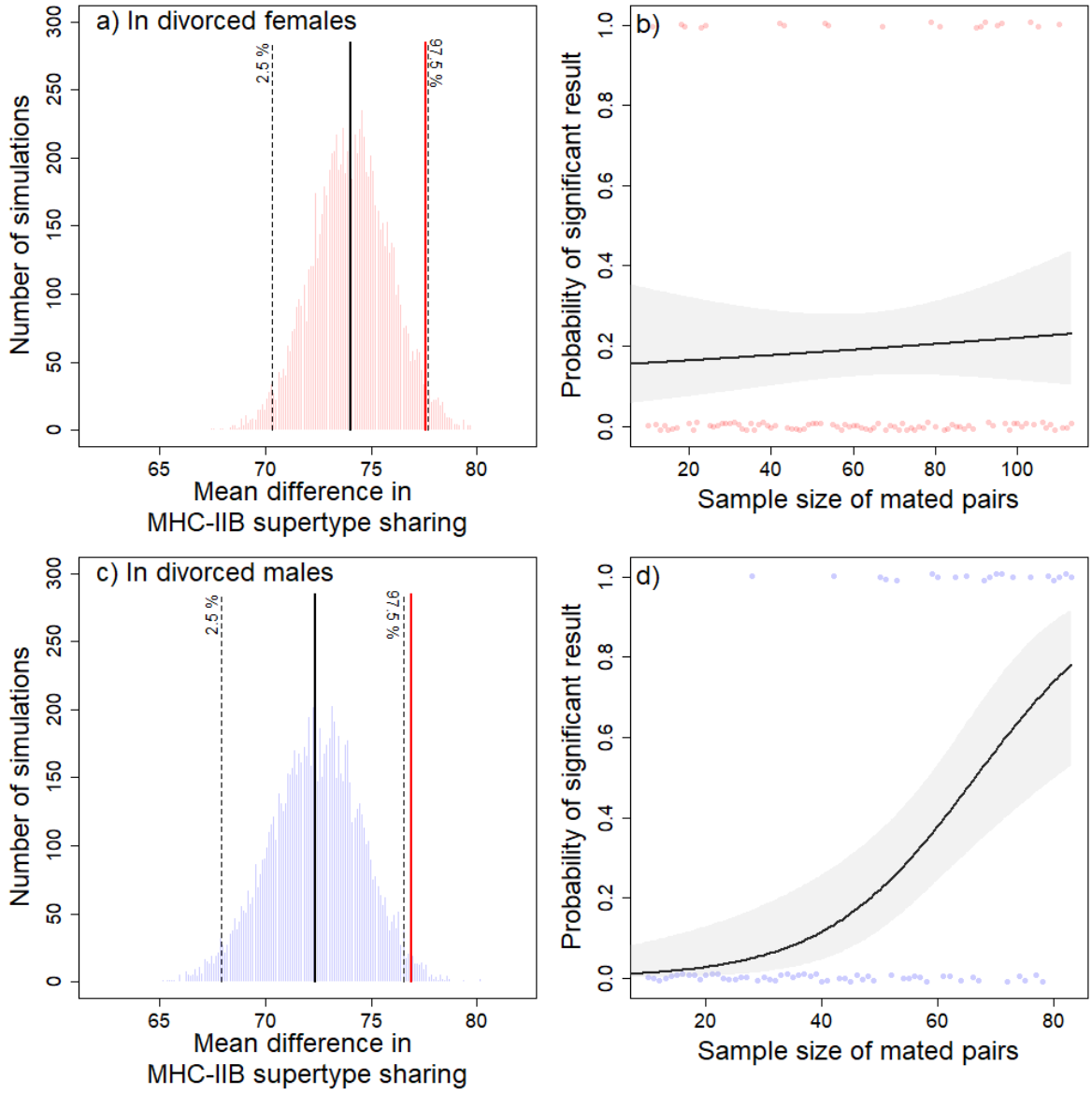
Figure 2


a) In divorced females
2.5 %
97.5 %
Number of simulations
0
50
100
150
200
250
300
65
70
75
80
Mean difference in
MHC-IIB supertype sharing
b)
Probability of significant result
0.0
0.2
0.4
0.6
0.8
1.0
20
40
60
80
100
Sample size of mated pairs
c) In divorced males
2.5 %
97.5 %
Number of simulations
0
50
100
150
200
250
300
65
70
75
80
Mean difference in
MHC-IIB supertype sharing
d)
Probability of significant result
0.0
0.2
0.4
0.6
0.8
1.0
20
40
60
80
Sample size of mated pairs