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MHC-II distance between parents predicts sex allocation decisions in a genetically monogamous bird

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HAL Authorization

1 **TITLE:** MHC-II distance between parents predicts sex allocation decisions in a genetically
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15 **ABSTRACT**

16 Theory predicts that parental heritable characteristics should shape sex allocation decisions
17 when their effects on reproduction or survival are offspring sex-dependent. Numerous studies
18 have questioned to what extent characteristics displayed by one of the parents matched
19 theoretical expectations. This contrasts with the handful of studies that investigated whether
20 compatibility between parents could also trigger selective pressures for sex allocation
21 adjustments. We studied the genetically monogamous black-legged kittiwake (*Rissa*
22 *tridactyla*), where previous data revealed that female chicks suffered higher fitness costs from
23 low diversity at genes of the major histocompatibility complex (MHC) than male chicks. We
24 predicted, and found in our dataset, that MHC-similar parents, producing low MHC-diverse
25 offspring, should avoid the production of females. The relation between MHC-distance
26 between parents (i.e. the functional distinctness of their MHC alleles) and offspring sex was
27 not linear, such that MHC-dissimilar parents also overproduced sons. Overall, our results
28 suggest that the genetically monogamous black-legged kittiwake parents flexibly adapt their
29 reproduction and circumvent the costs of suboptimal pairing by manipulating offspring sex.

30 *Keywords:* compatibility; heterozygote advantage; MHC; monogamy; sex allocation

31 **LAY SUMMARY**

32 When parental characteristics affect offspring reproduction or survival in a sex-dependent
33 way, parents should choose the right sex. In the black-legged kittiwake, a lack of diversity at
34 immune genes has strong negative effects in daughters, but not in sons. Accordingly, we show
35 that when kittiwake parents are at risk of producing low genetically diverse offspring, they
36 avoid producing daughters. Our results further suggest that it may be advantageous for parents
37 to produce high genetically diverse sons.

38 INTRODUCTION

39 Sex allocation theory predicts that parents should adjust their investment in daughters and
40 sons depending on the fitness costs and benefits associated with each sex (Trivers and Willard
41 1973; Charnov 1982; Frank 1990). Published data and theoretical models revealed that such
42 sex-specific costs-benefits ratios are shaped by diverse abiotic and biotic parameters
43 (reviewed in West 2009). These include parental heritable genetic or non-genetic
44 characteristics when their effects on reproduction or survival are offspring sex-dependent
45 (Cockburn et al. 2002; West 2009, chapter 6). One textbook example refers to situations
46 where sons inherit elaborate ornaments from their father (e.g. Burley 1981). When these
47 translate into increased reproductive success, such parents have been found to overproduce
48 sons (West 2009, chapter 6; Bowers et al. 2013; but see Booksmythe et al. 2017).

49 Besides individual parental characteristics, only a handful of studies investigated
50 whether compatibility between parents could also trigger selective pressures for sex allocation
51 adjustments (Pryke and Griffith 2009a; Pryke and Griffith 2009b; Brekke et al. 2010; Rioux-
52 Paquette et al. 2011; Sardell and DuVal 2014). This possibility was elegantly highlighted in
53 Gouldian finches (*Erythrura gouldiae*), where daughters suffer higher viability costs from a
54 Z-linked genetic incompatibility between red and black color morphs than sons (Pryke and
55 Griffith 2009a; Pryke and Griffith 2009b). As predicted by sex allocation theory, females
56 paired with a genetically incompatible male (i.e. an opposite-color morph) overproduced sons
57 (Pryke and Griffith 2009a).

58 The major histocompatibility complex (hereafter, MHC) is a key group of genes
59 involved in the activation of immune responses against parasites (Murphy and Weaver 2017).
60 Here also, compatibility between parents plays a pivotal role in an evolutionary context as
61 MHC-dissimilar mates are more likely to produce offspring carrying a higher diversity of
62 MHC-alleles (Setchell et al. 2013), thereby able to recognize and eliminate a broader range of

63 pathogens (Doherty and Zinkernagel 1975; Wakeland et al. 1990; Oliver et al. 2009). This
64 increased resistance to diseases ultimately translates into an overall higher reproductive
65 success and survival for more MHC-diverse individuals (Wedekind 1994; Brouwer et al.
66 2010; Thoss et al. 2011; Lenz et al. 2013). However, the fitness costs and benefits of MHC-
67 diversity may differ among individuals depending on their exposure and immune responses to
68 parasites (Roved et al. 2017; Whittingham et al. 2018, Pineaux et al. 2020), which are known
69 to vary according to key characteristics such as personality (Boyer et al. 2010), social status
70 (Habig and Archie 2015) or sex (Zuk 2009, Klein and Flanagan 2016). Some previous results
71 revealed that sex could modulate the association between MHC-diversity and fitness, with
72 males (Schaschl et al. 2012; Roved et al. 2018) or females (Hoover et al. 2018; Pineaux et al.
73 2020) suffering increased fitness costs from low MHC-diversity compared to the other sex.
74 For instance, in Alpine chamois, males may benefit from higher levels of MHC-diversity than
75 females because male-male contests increase males' risk of wounds and thus infections, and
76 deplete males' energetic reserves, thereby possibly leading to less energy available for
77 allocation to immune functions (Schaschl et al., 2012 and references therein). In a sex
78 allocation context, this predicts that parents able to adjust offspring sex in relation to the
79 expected fitness return of either sex given their MHC-compatibility should be advantaged.
80 Although the MHC has been a trending topic in evolutionary ecology for two decades
81 (Milinski 2006; Kamiya et al. 2014), no study has yet investigated whether MHC-
82 compatibility between parents could drive sex allocation decisions.

83 We investigated MHC-based sex allocation decision in the genetically monogamous
84 black-legged kittiwake (*Rissa tridactyla*), a species in which MHC-II diversity is associated
85 with survival and other fitness proxies in female offspring, but not male offspring (Pineaux et
86 al. 2020). This association also depends on female position in the laying sequence (two eggs
87 being the typical clutch size in kittiwakes). In males and first-laid females, the probability of

88 survival before fledging is 70%, irrespective of their MHC-II diversity. In second-laid female
89 offspring, the probability of survival is similarly around 70% in the most MHC-diverse half,
90 whereas it drops to 40% in the least MHC-diverse half (see Figure 1 in Pineaux et al. 2020).
91 This may result from condition-dependent parasite infections differentially affecting females
92 and males in relation to hatching order. We therefore predicted that MHC-II distance between
93 parents, by determining offspring MHC-II diversity, would influence offspring sex in relation
94 to laying position. Specifically, we expected a balanced sex ratio in MHC-dissimilar parents,
95 whereas relatively more sons should be produced by MHC-similar parents at the second
96 position of the laying sequence.

97 **MATERIALS AND METHODS**

98 *Study site*

99 The study was conducted during the 2009-2013 and 2016-2018 breeding seasons (May-
100 August) on a colony of black-legged kittiwakes nesting on an abandoned U.S. Air Force radar
101 tower on Middleton Island (59°26'N, 146°20'W), Gulf of Alaska. The nest sites created on
102 the upper walls of the tower can be observed from inside through sliding one-way mirrors and
103 birds can be individually identified using color and metal bands (Gill and Hatch 2002).

104 *General procedure*

105 We checked nest sites twice daily (9:00 and 18:00) to record laying and hatching events. On
106 the day of laying, we individually labeled A- and B-eggs (first- and second-laid eggs,
107 respectively) with a non-toxic marker. We determined offspring sex molecularly using DNA
108 extracted from a drop of blood from the metatarsal vein a few hours after hatching, or from
109 embryo tissues or blood vessels from eggshells when eggs did not hatch (see Merklings et al.,
110 2012 for a detailed sexing protocol). Regarding adults, we used DNA extracted from a blood

111 sample collected with a syringe or capillaries from the brachial vein to determine sex using
112 the same molecular method as for chicks.

113 *Molecular analysis of MHC-II*

114 The DNA samples were used to amplify 258 bp fragments (218 bp excluding primers) of the
115 exon 2 of the black-legged kittiwake MHC class-IIB. We used the MHC class-IIB specific
116 primers (forward: 5' GCACGAGCAGGGTATTTCCA and reverse: 5'
117 GTTCTGCCACACACTCACC) designed by Leclaire et al. (2014), which amplify at least
118 four MHC class-IIB loci (Pineaux et al. 2020). Samples were sequenced in two runs with an
119 Illumina MiSeq platform, using the 2 × 300 bp protocol (Fasteris SA, Plan-les-Ouates,
120 Switzerland; see Pineaux et al. 2020, for a detailed sequencing protocol). Amplicon sequences
121 were analyzed with ampliSAS, a three-step pipeline that consists of read demultiplexing,
122 unique sequence clustering, and erroneous sequence filtering (Sebastian et al. 2016). The
123 reproducibility of genotype between the two runs (n = 25 DNA samples that were split and
124 processed in independent PCRs) was 100%. We obtained 83 different MHC class II alleles
125 and, in the subsample used in this study, the mean number of alleles per individual was $3.29 \pm$
126 0.76 (\pm sd; range: 1-5).

127 We calculated the functional MHC-II distance between mates in pairs for which the
128 MHC class-IIB region was sequenced for both mates, using the approach described in Strandh
129 et al. (2012). To obtain functional alleles, we translated MHC-II DNA sequences into amino
130 acid sequences and considered DNA sequences as functionally identical if they had the same
131 amino-acids in the peptide-binding regions (PBRs; inferred from Leclaire et al. 2014). This
132 gives us a total of 68 functional alleles. To calculate functional distance, we first follow the
133 approach of Schwensow et al. (2007) to describe the chemical binding properties of each
134 amino acid in the PBRs using five physico-chemical descriptors (z-descriptors; Sandberg et
135 al. 1998). Then, following the approach of Strandh et al. (2012), the resulting Sandberg matrix

136 was used to construct an alternative maximum-likelihood phylogenetic tree with “Rcontml” in
137 the R package *Rphylip* (Revell and Chamberlain 2014). This tree represents clusters of
138 functionally-similar MHC sequences and was used as a reference to calculate the functional
139 distance between MHC-sequence repertoires of parents with unweighted UniFrac analyses
140 (“GUniFrac” package in R; Chen 2018). Functional MHC-II distance between parents varied
141 from 0 to 1 (mean \pm sd: 0.54 ± 0.19). A score of zero means that both parents have exactly the
142 same MHC alleles, whereas the closer to one their score is, the more functionally dissimilar
143 their MHC alleles are. MHC-distance did not significantly vary among years (Kruskal-Wallis,
144 $U = 7.15$, $df = 7$, $p = 0.41$).

145 The tree was also used to calculate the functional diversity of offspring. To calculate
146 functional MHC-II diversity, we used the minimum total length of all the branches required to
147 span an offspring’s MHC-II alleles (i.e., Faith’s phylogenetic diversity; Faith 1992) with the
148 R function “pd” in the *picante* R package (Kembel et al. 2010). In other words, for each
149 additional allele, only the part of the peptide-binding characteristics that is not shared with
150 other alleles is summed (Pineaux et al. 2020) The resulting score is thus positively correlated
151 to the range of peptides bound by all the alleles carried by an individual. Offspring functional
152 MHC-II diversity varied from 0.89 to 9.81 (mean \pm sd: 5.97 ± 1.12) and did not significantly
153 vary among years (Kruskal-Wallis, $U = 2.70$, $df = 6$, $p = 0.85$).

154 *Sample size*

155 We obtained MHC-II distance for 293 pairs that produced 548 two-eggs clutches, totaling 933
156 chicks and 163 unhatched eggs. Clutch size ranges from one to three eggs in this species, two-
157 eggs clutches being the most common clutch size in this population (Gill and Hatch 2002;
158 81% of the clutches in these study years). We did not include three-eggs clutches in the
159 analyses because they were too rare ($n = 9$), nor we included one-egg clutches ($n = 119$) as
160 our previous study shows no sex-specific effect of MHC-II diversity on fitness in offspring

161 raised alone (Pineaux et al. 2020). Still, including one egg-clutches in the main analysis
162 testing for effects of MHC-II distance on offspring sex produces the same results (not shown).
163 We sexed 913 out of the 933 chicks (97% of chicks) and 45 embryos out of the 163 unhatched
164 eggs (27% of unhatched eggs). We used these 958 sexed embryos or chicks in our main
165 analysis relating offspring sex to MHC-II distance between parents. Additionally, in order to
166 investigate the relationship between MHC-II distance between parents and chick MHC-II
167 diversity, we used a subsample of those offspring ($n = 471$) that had been sequenced for the
168 MHC-II as part of our previous study linking offspring MHC-II diversity to fitness (Pineaux
169 et al. 2020).

170 *Data analysis*

171 First, we tested whether MHC-II diversity of offspring was positively associated with MHC-II
172 distance between parents using a linear mixed model (LMM) built in the *lme4* package (Bates
173 et al. 2015) in R 4.0.1 (R Core Team 2020). Predictor variables included MHC-II distance
174 between parents, offspring sex, laying position and interactions between these variables.
175 Clutch ID and pair ID were included as random effects to consider the non-independence of
176 chicks born during the same breeding season (clutch ID) or born from the same parents in
177 different years (pair ID). However, variance estimates of the clutch ID random effect was
178 practically zero and was thus removed. We standardized fixed predictors by centering and
179 dividing them by two standard deviations using the *arm* package (Gelman and Su 2018).
180 Model selection followed a backward-stepwise approach using the “step” function with
181 Kenward-Roger’s approximation of denominator degrees of freedom in the R package
182 *lmerTest* (Kuznetsova et al. 2017). We checked for normality and homoscedasticity of
183 residuals and for normal distribution of random effects in the initial model.

184 Then, we used the same backward-stepwise approach to test the association between
185 offspring sex and MHC-II distance between parents according to laying position. We built a

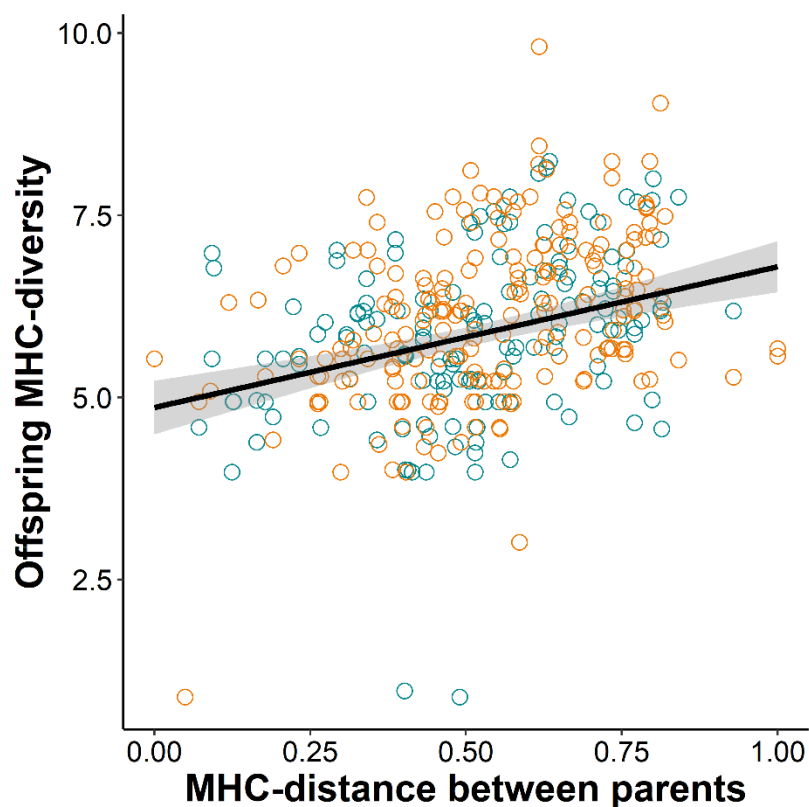
186 generalized linear mixed model (GLMM) with a binomial error distribution and a logit link
187 function (i.e. offspring sex was either 0 = female or 1 = male) in the *lme4* package (Bates et
188 al. 2015). Predictor variables included MHC-II distance between parents, laying position and
189 their interaction. We transformed the parental MHC-II distance using a restricted cubic spline
190 (RCS) because we did not expect the association between parental MHC-II distance and
191 offspring sex to be linear at the second position of the laying sequence (see predictions in the
192 introduction). RCS transforms an explanatory variable by dividing the range of values in
193 intervals, fits a separate curve in each interval but still results in a smooth and continuous
194 fitted curve. Intervals are delimited by knots. We used the default knot positions from the *rms*
195 R package (Harrell 2020) and we found the optimal number of knots to be three by comparing
196 the Akaike Information Criteria (AIC) of models with three, four or five knots. Year was
197 included as a continuous variable, given that an increase in the probability of producing sons
198 with time was found in this population during the period considered (Merkling et al. 2019).
199 We also included Clutch ID and pair ID as random effects and we checked for normal
200 distribution of these random effects. We standardized fixed variables using the *arm* package
201 (Gelman and Su 2018). We assessed significance of each predictor variable by the change in
202 deviance after removal of that variable (Likelihood-Ratio Test, LRT) using a chi-square test.
203 A variable was eliminated from the model if $p > 0.05$.

204 Following recommendations (Krackow and Neuhauser 2008), we performed the same
205 analyses on two datasets, an “unrestricted dataset” (N = 958) containing both complete (where
206 both offspring had been sexed) and incomplete clutches (where only one offspring had been
207 sexed), and a “restricted dataset” (N = 820) containing only complete broods. We also re-ran
208 analyses twice on a modified form of our unrestricted dataset by assuming that all unsexed
209 offspring (N = 138) were females or, alternatively, males. These additional analyses allowed

210 us to investigate whether the reported patterns could result from sex bias in mortality and/or
211 sexing success.

212 RESULTS

213 Offspring MHC-II diversity was positively associated with the MHC-II distance between
214 parents. All other explicative variables were lost in the backward-stepwise procedure (Figure
215 1; Table 1). This analysis may face collinearity issues since chick sex was related to MHC-II
216 distance between parents in our data (see the test of our main prediction below), both
217 parameters being included concomitantly into the initial model. However, variance inflated
218 factor (VIF) values were < 2 , indicating no such an issue (Zuur et al. 2010).



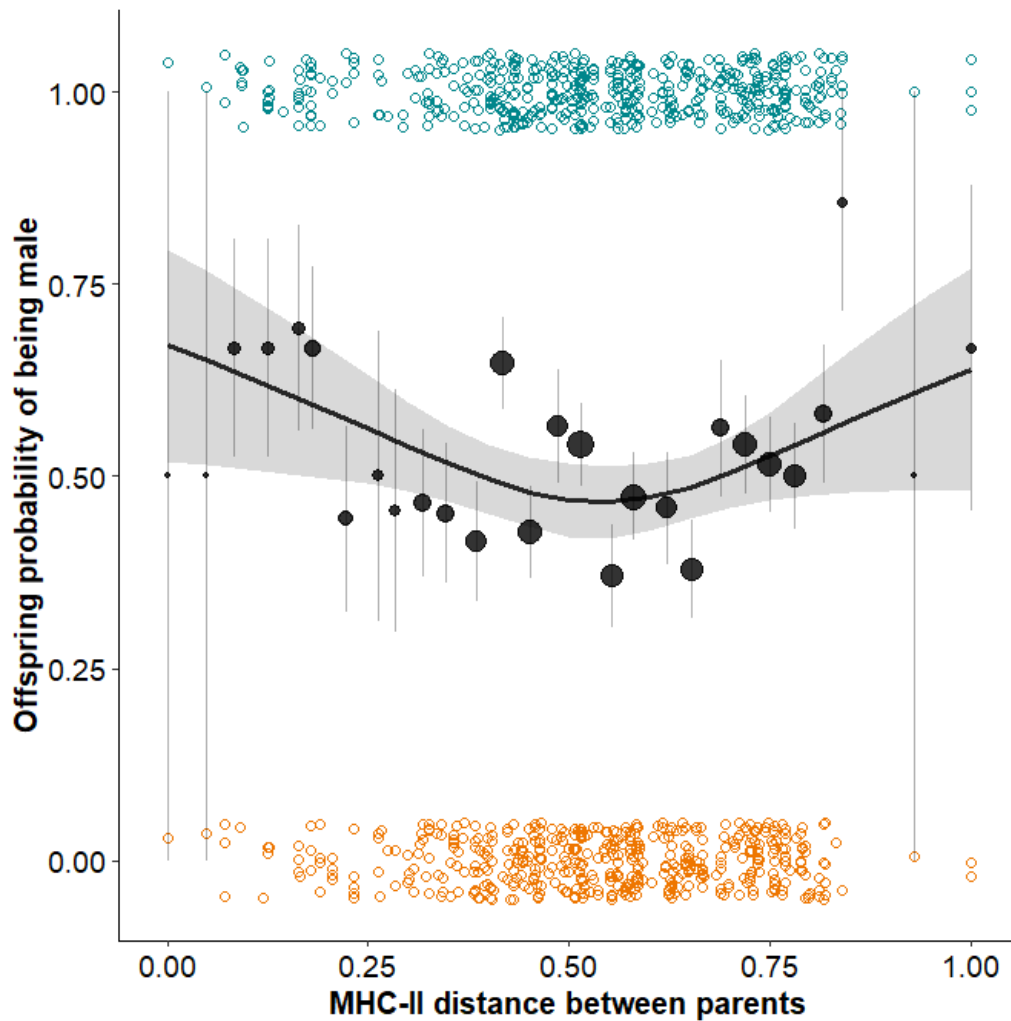
219

220 **Figure 1.** Offspring MHC-II diversity covaries with the MHC-II distance between parents in
221 both female (orange) and male (blue) offspring. The line shows the predictions from a LMM
222 including MHC-II distance between parents as a predictor variable. There was no significant

223 interaction between offspring sex and MHC-II distance (Table 1). The pair ID random effect
224 was not considered in the models used for graphic representations but was accounted for in
225 the analysis. Removing the three extremely low MHC-II diverse offspring did not change the
226 results. Shaded areas represent confidence intervals.

227

228 We then investigated our main prediction. Using the unrestricted dataset (containing
229 both complete and incomplete clutches), offspring sex was significantly associated with the
230 cubic spline transformed parental MHC-II distance while the other predictor variables were
231 eliminated in the backward-stepwise procedure (Table 2). The curve was “U” shaped, with
232 knots at an MHC-II distance of 0.30, 0.55 and 0.77 (Figure 2). More MHC-II similar pairs
233 overproduced sons independently of laying position (Figure 2). Among offspring produced by
234 the most MHC-II similar pairs (i.e. first of 30-quantiles), 26/39 (67%) were sons. Contrary as
235 expected, more MHC-II dissimilar pairs did not produce a balanced sex ratio. They produced
236 relatively more sons (Figure 2), with the most MHC-II dissimilar pairs (i.e. last of 30-
237 quantiles) having produced 19/31 (61%) sons.



238

239 **Figure 2.** Offspring probability of being male according to MHC-II distance between parents.
 240 Each colored dot represents a female chick (orange; n = 472) or a male chick (blue; n = 486).
 241 For illustrative purpose, parental MHC-II distance was divided into 30 categories of equal
 242 range (0.033), with the black dots representing the mean (\pm SE) sex ratio per category of
 243 parental MHC-II distance, and the size of the dots representing sample size per category. The
 244 curve represents predicted values derived from a model including parental MHC-II distance
 245 transformed with a restricted cubic spline (see methods). Shaded areas represent 95%
 246 confidence intervals. Random effects (pair ID and clutch ID) were not considered in this
 247 model used for graphic representation. Note: the vertical position of colored dots was

248 randomly rearranged to better appreciate the number of chicks in relation to parental MHC-II
249 distance.

250 The analyses performed on the restricted dataset (containing only complete clutches)
251 gave similar results (Table S1). Furthermore, assuming that all unsexed offspring were
252 females, or alternatively males, both lead to the same “U” shaped curve, indicating an
253 overproduction of sons in more MHC-II similar pairs and in more MHC-II dissimilar pairs
254 (Tables S2, S3).

255 **DISCUSSION**

256 Our data first confirmed that MHC-II similar kittiwake parents were more likely to
257 produce offspring with low MHC-II diversity. Previous results reported that such a low MHC-
258 II diversity in offspring was associated with increased mortality in daughters hatched in
259 second position as compared to other chick sex-rank categories (Pineaux et al. 2020).
260 Additionally, low MHC-II diversity negatively affected growth and tick resistance in
261 daughters only (Pineaux et al. 2020) In line with sex allocation theory (Cockburn et al. 2002;
262 West 2009, chapter 6), our data revealed that in such a context, MHC-II similar parents
263 avoided production of disadvantaged daughters. Contrary to our expectation, however, we did
264 not find hatching rank to further modulate the association between parental MHC-II distance
265 and offspring sex. The overall increased detrimental effect of low MHC-II diversity in
266 daughters as compared to sons (Pineaux et al. 2020) may have concealed more subtle patterns.

267 Unexpectedly, our data also revealed that MHC-dissimilar pairs overproduced sons.
268 This may lead to an increased fitness return if MHC-II diverse males have increased survival
269 or reproductive advantages compared to MHC-II diverse females later in life, as shown in
270 other species (Sauermann et al. 2001; Schaschl et al. 2012; Roved et al. 2018). Notably, given
271 that body mass and body size may be more important determinants of male than female

272 reproductive success in adult kittiwakes (Merkling et al. 2012), MHC-diverse males may
273 obtain a competitive and, *in fine*, reproductive advantage, if MHC-diversity is positively
274 associated with such morphological traits, as found in other species (Ditchkoff et al. 2001;
275 Lenz et al. 2009; Dunn et al. 2013). However, when testing this relationship using kittiwake
276 parents involved in this study, we found no such a positive association between MHC-
277 diversity and body mass in adult males (see supplementary material). More research is needed
278 to identify the possible fitness advantages of high MHC diversity in adult males in kittiwakes.

279 Previous studies investigated sex ratio patterns in this population (Merkling et al.
280 2012; Merkling et al. 2015; Merkling et al. 2019). Using 10 years of data from a long-term
281 feeding experiment, the most recent results suggested that offspring sex is not shaped by
282 pair's investment capacity (Merkling et al. 2019) despite higher reproductive costs for parents
283 raising sons (Merkling et al. 2015; Merkling et al. 2017). Here, our results indicate that
284 offspring genetic diversity may be a stronger driver of sex allocation decision in this
285 population than pair's ability to provide care.

286 Sex allocation based on MHC similarity between parents has been suggested in
287 humans, rats and mice because newborn males have been found to be more MHC-diverse than
288 newborn females in these species (Dorak et al. 2002, and references therein). However,
289 whether this result was caused by MHC-similar parents overproducing daughters was not
290 known. A study on humans found that parents sharing the same alleles at two MHC loci
291 produced a female-biased sex ratio whereas parents sharing no allele at these two MHC loci
292 produced a male-biased sex ratio (Astolfi et al. 1990). However, the potential adaptive value
293 of this pattern (e.g. whether males suffered more from low MHC-diversity than females) also
294 remains overlooked (Sauermann et al. 2001; Schaschl et al. 2012; Roved et al. 2018). Clearly,
295 important next steps should involve studies investigating potential fitness pathways and
296 proximate mechanisms underlying sex ratio departure from parity.

297 Proximate mechanisms of sex ratio adjustments are not well understood and how these
298 could depend on MHC is unknown. Regardless of the parent(s) biasing offspring sex, our
299 results may suggest that kittiwakes can assess the genetic characteristics of their mate (as
300 suggested by Mulard et al. 2009; Pineaux et al. 2019). The covariation between scent-gland
301 compounds and MHC in this species may suggest that odor cues might be used in MHC
302 recognition (Leclaire et al. 2014), as found in several taxa (Wedekind et al. 1995; Olsson et al.
303 2003; Radwan et al. 2008), including birds (Leclaire et al. 2017). Sex ratio adjustments may
304 also be the result of MHC-specific sperm-ova interactions (Wedekind 1994), in line with
305 previous studies reporting non-random production of blastocysts according to the MHC-
306 distance between gametes (Lenz et al. 2018; Zhu et al. 2019).

307 Because permanent or temporary constraints may force individuals to mate with suboptimal
308 partners (Stutchbury and Morton 1995; Tinghitella et al. 2013), tactics allowing to lessen
309 associated costs may have emerged. Such constraints are particularly likely to happen in
310 genetically monogamous species such as the kittiwake (Helfenstein et al. 2004). We
311 previously reported that breeding kittiwakes flexibly adapted their breeding timing and
312 copulatory behavior in response to within-pair genetic similarity (Pineaux et al. 2019). The
313 present study suggests another way for kittiwake parents to circumvent fitness costs
314 associated to suboptimal pairing.

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327 Institutional Animal Care and Use Committees, and the Institut Paul-Emile Victor Ethical
328 Committee, in accordance with United States laws and under permits from the U.S. Fish and
329 Wildlife Service and the State of Alaska. Any use of trade names is for descriptive purposes
330 only and does not imply endorsement by the U.S. Government.

331 **DATA ACCESSIBILITY**

332 Data will be accessible on a public repository (Open Science Framework).

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514

515

516 **Table 1:** a) Effect of predictor variables from the generalized linear mixed model built to
 517 explain chick MHC-II diversity and b) variance and standard deviation associated with
 518 random effects in the final model. Variables were eliminated following a backward-stepwise
 519 procedure. Step denotes the exclusion sequence of the non-significant terms of the model.
 520 Values for excluded variables refer to the step before their exclusion. Values included in the
 521 final model are in bold.

a)

Parameter	Estimate	SE	F	P	Step
MHC-II distance : Sex : Hatching order	0.027	0.333	0.641	0.423	1
MHC-II distance : Hatching order	0.028	0.143	0.040	0.842	2
Sex : Hatching order	0.043	0.162	0.071	0.790	3
Hatching order	0.014	0.071	0.036	0.849	4
MHC-II distance : Sex	0.070	0.162	0.183	0.669	5
Sex	-0.122	0.081	2.235	0.135	6
MHC-II distance	0.859	0.133	37.945	< 0.001	

522

b)

Random effect	Variance	SD
Pair ID	0.621	0.788

523

524

525 **Table 2:** a) Effect of predictor variables from the generalized linear mixed model built to
 526 explain chick sex and b) variance and standard deviation associated with random effects in the
 527 final model. Variables were eliminated following a backward-stepwise procedure. Step
 528 denotes the exclusion sequence of the non-significant terms of the model. Values for excluded
 529 variables refer to the step before their exclusion. Values included in the final model are in
 530 bold. When using a restricted cubic spline, one must include the untransformed predictor
 531 variable in the model to force (i.e. to restrict) the curve to be linear at the tails of the predictor
 532 variable (MHC-II distance here) to avoid unstable estimates. Note that the best way to
 533 interpret results from restricted cubic splines is to use a figure, not the estimated coefficients
 534 (Shepherd et al. 2017).

a)

Parameter	Estimate	SE	Chi ²	P	Step
Cubic spline (MHC-II distance) : Hatching order	0.133	0.680	0.038	0.845	1
MHC-II distance : Hatching order	-0.066	0.267	0.062	0.803	2
Year	-0.140	0.136	1.060	0.303	3
Hatching order	0.219	0.132	2.745	0.098	4
Cubic spline (MHC-II distance)	0.849	0.358	5.793	0.016	

535

b)

Random effect	Variance	SD
Clutch ID	0.162	0.127

Pair ID

0.090

0.300

536
