



**HAL**  
open science

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

Maxime Pineaux, Thomas Merklng, Etienne Danchin, Scott Hatch, Sarah Leclaire, Pierrick Blanchard

### ► To cite this version:

Maxime Pineaux, Thomas Merklng, Etienne Danchin, Scott Hatch, Sarah Leclaire, et al.. MHC-II distance between parents predicts sex allocation decisions in a genetically monogamous bird. *Behavioral Ecology*, 2022, 33 (1), pp.245-251. 10.1093/beheco/arab130 . hal-03770671

**HAL Id: hal-03770671**

**<https://cnrs.hal.science/hal-03770671>**

Submitted on 8 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **TITLE:** MHC-II distance between parents predicts sex allocation decisions in a genetically  
2 monogamous bird

3 **RUNNING TITLE:** MHC-based sex allocation

4 **AUTHORS**

5 Maxime Pineaux<sup>1‡</sup>, Thomas Merklings<sup>1</sup>, Etienne Danchin<sup>1</sup>, Scott Hatch<sup>2</sup>, Sarah Leclaire<sup>1†</sup> and  
6 Pierrick Blanchard<sup>1†</sup>

7 <sup>1</sup>Laboratoire Évolution & Diversité Biologique (EDB UMR 5174), Université Fédérale de  
8 Toulouse Midi-Pyrénées, CNRS, IRD, UPS. 118 route de Narbonne, Bât. 4R1, 31062  
9 Toulouse cedex 9 France

10 <sup>2</sup>Institute for Seabird Research and Conservation, 12850 Mountain Place, Anchorage AK  
11 99516, USA

12 <sup>‡</sup>corresponding author: m.pineaux@gmail.com

13 <sup>†</sup>co-last authors

14

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:  $0.54 \pm 0.19$ ). 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:  $5.97 \pm 1.12$ ) 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

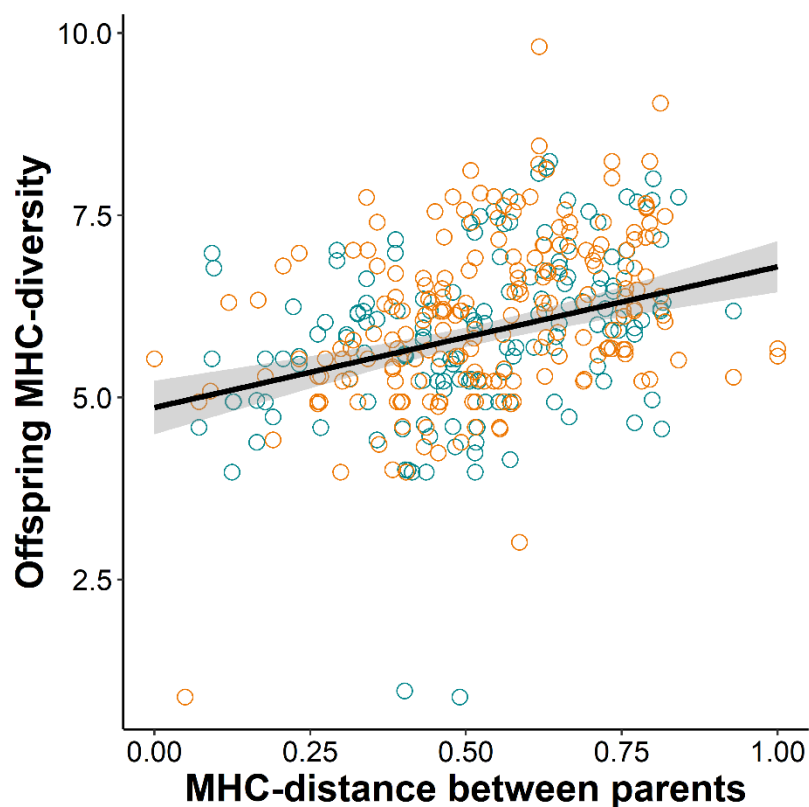
186 generalized linear mixed model (GLMM) with a binomial error distribution and a logit link  
187 function (i.e. offspring sex was either 0 = female or 1 = male) in the *lme4* package (Bates et  
188 al. 2015). Predictor variables included MHC-II distance between parents, laying position and  
189 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).



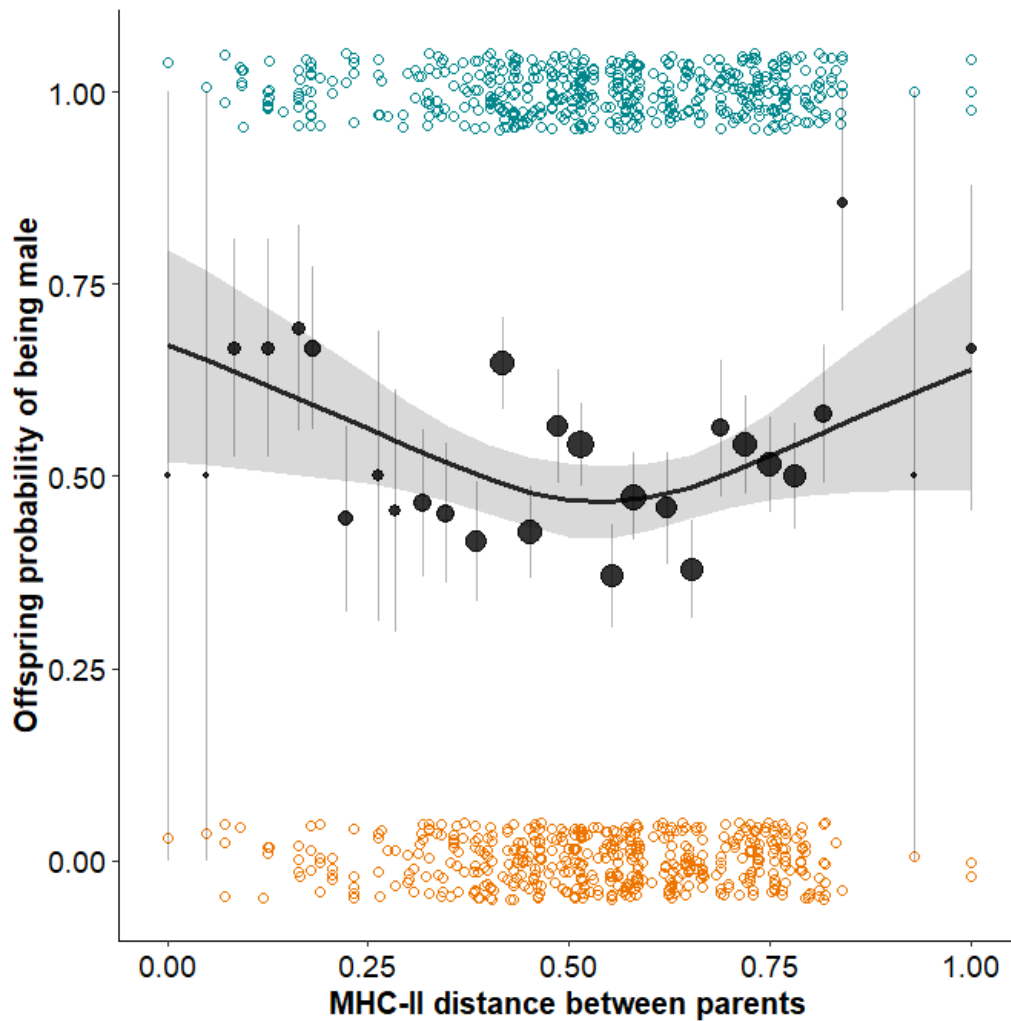
219

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.

## 315 **FUNDINGS**

316 Fieldwork was supported by a grant from the Institut Polaire Français Paul-Emile Victor  
317 (IPEV “Programme 1162 SexCoMonArc”) to S.L. and E.D. This work originated in the  
318 laboratory “Evolution et Diversité Biologique” (EDB) and was supported by the French  
319 Laboratory of Excellence Project “TULIP” (ANR-10-LABX-41; ANR-11-IDEX-0002-02),  
320 and the Agence Nationale de la Recherche Française (ANR grant "BactOdo", no. ANR-13-



321 PDOC-0002 to S.L.). M.P. was supported by a French doctoral scholarship. The authors  
322 declare no conflict of interest.

### 323 **ACKNOWLEDGEMENTS**

324 We are grateful to the Middleton fieldworkers who collected data for this study, as well as the  
325 undergraduate students who helped with chick sexing. The study was conducted under the  
326 approval of the U.S. Geological Survey Alaska Science Center, the University of Alaska  
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).

333 **REFERENCES**

- 334 Astolfi P, Martinetti M, Gigliberzolari F, Cuccia M (1990) The effect of parental and  
 335 maternal-fetal histocompatibility at MHC on sex-ratio in offspring. *Tissue Antigens*  
 336 35:172-177
- 337 Bates D, Machler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using  
 338 lme4. *Journal of Statistical Software* 67:1-48
- 339 Booksmythe I, Mautz B, Davis J, Nakagawa S, Jennions MD (2017) Facultative adjustment of  
 340 the offspring sex ratio and male attractiveness: a systematic review and meta-analysis.  
 341 *Biological Reviews* 92:108-134
- 342 Bowers EK, Munclinger P, Bures S, Kucerova L, Nadvornik P, Krist M (2013) Cross-  
 343 fostering eggs reveals that female collared flycatchers adjust clutch sex ratios  
 344 according to parental ability to invest in offspring. *Molecular Ecology* 22:215-228
- 345 Boyer N, Réale D, Marmet J, Pisanu B, Chapuis J-L (2010) Personality, space use and tick  
 346 load in an introduced population of Siberian chipmunks *Tamias sibiricus*. *Journal of*  
 347 *Animal Ecology* 79:538-547
- 348 Brekke P, Bennett PM, Wang JL, Pettorelli N, Ewen JG (2010) Sensitive males: inbreeding  
 349 depression in an endangered bird. *Proceedings of the Royal Society B-Biological*  
 350 *Sciences* 277:3677-3684
- 351 Brouwer L, Barr I, van de Pol M, Burke T, Komdeur J, Richardson DS (2010) MHC-  
 352 dependent survival in a wild population: evidence for hidden genetic benefits gained  
 353 through extra-pair fertilizations. *Molecular Ecology* 19:3444-3455
- 354 Burley N (1981) Sex-ratio manipulation and selection for attractiveness. *Science* 211:721-722
- 355 Charnov E (1982) *The theory of sex allocation*. Princeton University Press, Princeton
- 356 Chen J (2018) GUniFrac: Generalized UniFrac Distances. R package. In, 1.1 edn
- 357 Cockburn A, Legge S, Double M (2002) Sex ratios in birds and mammals: can the hypotheses  
 358 be disentangled? In: ICW H (ed) *Sex ratios: concept and research methods*. Cambridge  
 359 University Press, Cambridge (UK)
- 360 Ditchkoff SS, Lochmiller RL, Masters RE, Hooper SR, Van Den Bussche RA (2001) Major-  
 361 histocompatibility-complex-associated variation in secondary sexual traits of white-  
 362 tailed deer (*Odocoileus virginianus*): Evidence for good-genes advertisement.  
 363 *Evolution* 55:616-625
- 364 Doherty PC, Zinkernagel RM (1975) Enhanced immunological surveillance in mice  
 365 heterozygous at H-2 gene complex. *Nature* 256:50-52
- 366 Dorak MT, Lawson T, Machulla HKG, Mills KI, Burnett AK (2002) Increased heterozygosity  
 367 for MHC class II lineages in newborn males. *Genes and Immunity* 3:263-269
- 368 Dunn PO, Bollmer JL, Freeman-Gallant CR, Whittingham LA (2013) MHC variations is  
 369 related to a sexually selected ornament, survival, and parasite resistance in common  
 370 yellowthroats. *Evolution* 67:679-687
- 371 Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biological Conservation*  
 372 61:1-10
- 373 Frank SA (1990) Sex allocation theory for birds and mammals. *Annual Review of Ecology*  
 374 *and Systematics* 21:13-55
- 375 Gelman A, Su Y (2018) arm: Data analysis using regression and multilevel/hierarchical  
 376 models. In, 1.10-1 R package <https://CRAN.R-project.org/package=arm> edn
- 377 Gill VA, Hatch SA (2002) Components of productivity in black-legged kittiwakes *Rissa*  
 378 *tridactyla*: response to supplemental feeding. *J Avian Biol* 33:113-126
- 379 Habig B, Archie EA (2015) Social status, immune response and parasitism in males: a meta-  
 380 analysis. *Philosophical Transactions of the Royal Society B-Biological Sciences*  
 381 370:1669

382 Harrell FE (2020) rms: Regression Modeling Strategies. In, 6.0-1 R package [https://cran.r-](https://cran.r-project.org/package=rms)  
383 [project.org/package=rms](https://cran.r-project.org/package=rms) edn  
384 Helfenstein F, Tirard C, Danchin E, Wagner RH (2004) Low frequency of extra-pair paternity  
385 and high frequency of adoption in Black-legged Kittiwakes. *Condor* 106:149-155  
386 Hoover B, Alcaide M, Jennings S, Sin SYW, Edwards SV, Nevitt GA (2018) Ecology can  
387 inform genetics: Disassortative mating contributes to MHC polymorphism in Leach's  
388 storm-petrels (*Oceanodroma leucorhoa*). *Molecular Ecology* 27:3371-3385  
389 Kamiya T, O'Dwyer K, Westerdahl H, Senior A, Nakagawa S (2014) A quantitative review of  
390 MHC-based mating preference: the role of diversity and dissimilarity. *Molecular*  
391 *Ecology* 23:5151-5163  
392 Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP,  
393 Webb CO (2010) Picante: R tools for integrating phylogenies and ecology.  
394 *Bioinformatics* 26:1463-1464  
395 Krackow S, Neuhauser M (2008) Insights from complete-incomplete brood sex-ratio  
396 disparity. *Behavioral Ecology and Sociobiology* 62:469-477  
397 Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest Package: Tests in Linear  
398 Mixed Effects Models. *Journal of Statistical Software* 82  
399 Leclaire S, Strandh M, Mardon J, Westerdahl H, Bonadonna F (2017) Odour-based  
400 discrimination of similarity at the major histocompatibility complex in birds.  
401 *Proceedings of the Royal Society B-Biological Sciences* 284:5  
402 Leclaire S, van Dongen WFD, Voccia S, Merklings T, Ducamp C, Hatch SA, Blanchard P,  
403 Danchin E, Wagner RH (2014) Preen secretions encode information on MHC  
404 similarity in certain sex-dyads in a monogamous seabird. *Scientific Reports* 4:6  
405 Lenz TL, Hafer N, Samonte IE, Yeates SE, Milinski M (2018) Cryptic haplotype-specific  
406 gamete selection yields offspring with optimal MHC immune genes. *Evolution*  
407 72:2478-2490  
408 Lenz TL, Mueller B, Trillmich F, Wolf JBW (2013) Divergent allele advantage at MHC-DRB  
409 through direct and maternal genotypic effects and its consequences for allele pool  
410 composition and mating. *Proceedings of the Royal Society B-Biological Sciences*  
411 280:9  
412 Lenz TL, Wells K, Pfeiffer M, Sommer S (2009) Diverse MHC IIB allele repertoire increases  
413 parasite resistance and body condition in the Long-tailed giant rat (*Leopoldamys*  
414 *sabanus*). *Bmc Evolutionary Biology* 9:13  
415 Merklings T, Blanchard P, Chastel O, Glauser G, Vallat-Michel A, Hatch SA, Danchin E,  
416 Helfenstein F (2017) Reproductive effort and oxidative stress: effects of offspring sex  
417 and number on the physiological state of a long-lived bird. *Functional Ecology*  
418 31:1201-1209  
419 Merklings T, Hatch S, Leclaire S, Danchin E, Blanchard P (2019) Offspring sex-ratio and  
420 environmental conditions in a seabird with sex-specific rearing costs: a long-term  
421 experimental approach. *Evolutionary Ecology* 33:417-433  
422 Merklings T, Leclaire S, Danchin E, Lhuillier E, Wagner RH, White J, Hatch SA, Blanchard P  
423 (2012) Food availability and offspring sex in a monogamous seabird: insights from an  
424 experimental approach. *Behavioral Ecology* 23:751-758  
425 Merklings T, Welcker J, Hewison AJM, Hatch SA, Kitaysky AS, Speakman JR, Danchin E,  
426 Blanchard P (2015) Identifying the selective pressures underlying offspring sex-ratio  
427 adjustments: a case study in a wild seabird. *Behavioral Ecology* 26:916-925  
428 Milinski M (2006) The major histocompatibility complex, sexual selection, and mate choice.  
429 In: *Annual Review of Ecology Evolution and Systematics*. Annual Reviews, Palo  
430 Alto, pp 159-186

431 Mulard H, Danchin E, Talbot SL, Ramey AM, Hatch SA, White JF, Helfenstein F, Wagner  
432 RH (2009) Evidence that pairing with genetically similar mates is maladaptive in a  
433 monogamous bird. *Bmc Evolutionary Biology* 9:12

434 Murphy K, Weaver C (2017) *Janeway's immunobiology*, 9th edn. Garland Science, Taylor &  
435 Francis Group, LLC, New York, NY

436 Oliver MK, Telfer S, Piertney SB (2009) Major histocompatibility complex (MHC)  
437 heterozygote superiority to natural multi-parasite infections in the water vole (*Arvicola*  
438 *terrestris*). *Proceedings of the Royal Society B-Biological Sciences* 276:1119-1128

439 Olsson M, Madsen T, Nordby J, Wapstra E, Ujvari B, Wittsell H (2003) Major  
440 histocompatibility complex and mate choice in sand lizards. *Proceedings of the Royal*  
441 *Society B-Biological Sciences* 270:S254-S256

442 Pineaux M, Blanchard P, Danchin E, Hatch S, Helfenstein F, Mulard H, White J, Leclaire S,  
443 Wagner R (2019) Behavioural avoidance of sperm ageing depends on genetic  
444 similarity of mates in a monogamous seabird. *Biological Journal of the Linnean*  
445 *Society* 128:170-180

446 Pineaux M, Merkling T, Danchin E, Hatch S, Duneau D, Blanchard P, Leclaire S (2020) Sex  
447 and hatching order modulate the association between MHC-II diversity and fitness in  
448 early-life stages of a wild seabird. *Molecular Ecology* 29:3316 – 3329

449 Pryke SR, Griffith SC (2009a) Genetic incompatibility drives sex allocation and maternal  
450 investment in a polymorphic finch. *Science* 323:1605-1607

451 Pryke SR, Griffith SC (2009b) Postzygotic genetic incompatibility between sympatric color  
452 morphs. *Evolution* 63:793-798

453 R Core Team (2020) R: A language and environment for statistical computing. In. R  
454 Foundation for Statistical Computing, Vienna, Austria

455 Radwan J, Tkacz A, Kloch A (2008) MHC and preferences for male odour in the bank vole.  
456 *Ethology* 114:827-833

457 Revell LJ, Chamberlain SA (2014) Rphylip: an R interface for PHYLIP. *Methods in Ecology*  
458 *and Evolution* 5:976-981

459 Rioux-Paquette E, Festa-Bianchet M, Coltman DW (2011) Sex-differential effects of  
460 inbreeding on overwinter survival, birth date and mass of bighorn lambs. *Journal of*  
461 *Evolutionary Biology* 24:121-131

462 Roved J, Hansson B, Tarka M, Hasselquist D, Westerdahl H (2018) Evidence for sexual  
463 conflict over major histocompatibility complex diversity in a wild songbird.  
464 *Proceedings of the Royal Society B-Biological Sciences* 285:9

465 Sandberg M, Eriksson L, Jonsson J, Sjoström M, Wold S (1998) New chemical descriptors  
466 relevant for the design of biologically active peptides. A multivariate characterization  
467 of 87 amino acids. *Journal of Medicinal Chemistry* 41:2481-2491

468 Sardell RJ, DuVal EH (2014) Differential allocation in a lekking bird: females lay larger eggs  
469 and are more likely to have male chicks when they mate with less related males.  
470 *Proceedings of the Royal Society B-Biological Sciences* 281:7

471 Sauermann U, Nurnberg P, Bercovitch FB, Berard JD, Trefilov A, Widdig A, Kessler M,  
472 Schmidtke J, Krawczak M (2001) Increased reproductive success of MHC class II  
473 heterozygous males among free-ranging rhesus macaques. *Human Genetics* 108:249-  
474 254

475 Schaschl H, Suchentrunk F, Morris DL, Ben Slimen H, Smith S, Arnold W (2012) Sex-  
476 specific selection for MHC variability in Alpine chamois. *Bmc Evolutionary Biology*  
477 12:10

478 Schwensow N, Fietz J, Dausmann KH, Sommer S (2007) Neutral versus adaptive genetic  
479 variation in parasite resistance: importance of major histocompatibility complex  
480 supertypes in a free-ranging primate. *Heredity* 99:265-277

- 481 Setchell JM, Abbott KM, Gonzalez JP, Knapp LA (2013) Testing for post-copulatory  
482 selection for major histocompatibility complex genotype in a semi-free-ranging  
483 primate population. *American Journal of Primatology* 75:1021-1031
- 484 Shepherd BE, Rebeiro PF, Caribbean, Central and South America network for HIV  
485 epidemiology (2017) Brief Report: Assessing and Interpreting the Association  
486 Between Continuous Covariates and Outcomes in Observational Studies of HIV Using  
487 Splines. *Journal of acquired immune deficiency syndromes* 74:3
- 488 Strandh M, Westerdahl H, Pontarp M, Canback B, Dubois MP, Miquel C, Taberlet P,  
489 Bonadonna F (2012) Major histocompatibility complex class II compatibility, but not  
490 class I, predicts mate choice in a bird with highly developed olfaction. *Proceedings of*  
491 *the Royal Society B-Biological Sciences* 279:4457-4463
- 492 Stutchbury BJ, Morton ES (1995) The effect of breeding synchrony on extra-pair mating  
493 systems in songbirds. *Behaviour* 132:675-690
- 494 Thoss M, Ilmonen P, Musolf K, Penn DJ (2011) Major histocompatibility complex  
495 heterozygosity enhances reproductive success. *Mol Ecol* 20:1546-1557
- 496 Tinghitella RM, Weigel EG, Head M, Boughman JW (2013) Flexible mate choice when  
497 mates are rare and time is short. *Ecology and Evolution* 3:2820-2831
- 498 Trivers R, Willard D (1973) Natural selection of parental ability to vary sex-ratio of offspring.  
499 *Science* 179:90-92
- 500 Wakeland EK, Boehme S, She JX, Lu CC, McIndoe RA, Cheng I, Ye Y, Potts WK (1990)  
501 Ancestral polymorphisms of MHC class-II genes: divergent allele advantage.  
502 *Immunologic Research* 9:115-122
- 503 Wedekind C (1994) Mate choice and maternal selection for specific parasite resistances  
504 before, during and after fertilization. *Philosophical Transactions of the Royal Society*  
505 *B-Biological Sciences* 346:303-311
- 506 Wedekind C, Seebeck T, Bettens F, Paepke AJ (1995) MHC-dependent mate preferences in  
507 humans. *Proceedings of the Royal Society B-Biological Sciences* 260:245-249
- 508 West SA (2009) *Sex allocation*. Princeton University Press, Princeton
- 509 Zhu Y, Wan QH, Zhang HM, Fang SG (2019) Reproductive strategy inferred from major  
510 histocompatibility complex-based inter-individual, sperm-egg, and mother-fetus  
511 recognitions in giant pandas (*Ailuropoda melanoleuca*). *Cells* 8:21
- 512 Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common  
513 statistical problems. *Methods in Ecology and Evolution* 1:3-14

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
<b>MHC-II distance</b>	<b>0.859</b>	<b>0.133</b>	<b>37.945</b>	<b>&lt; 0.001</b>	

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 <sup>2</sup>	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
<b>Cubic spline (MHC-II distance)</b>	<b>0.849</b>	<b>0.358</b>	<b>5.793</b>	<b>0.016</b>	

535

b)

Random effect	Variance	SD
Clutch ID	0.162	0.127

Pair ID

0.090

0.300

536

---