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A GULL SPECIES RECOGNIZES MHC-II DIVERSITY AND DISSIMILARITY USING ODOR CUES

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Abstract

The major histocompatibility complex (MHC) plays a crucial role in resistance to parasite, and is thus often suggested to be an important force driving social interactions, including mating preference, in vertebrates. However, the phenotypic cues used by individuals to assess the MHC characteristics of conspecifics is generally unknown. Here we used behavioral tests to investigate whether, in black-legged kittiwakes, females use odor cues to distinguish male MHC-II diversity and MHC-II dissimilarity. We found that females took less time to peck at the odor sample when it came from a male with high MHC-II dissimilarity. They also took less time to peck at an odor sample coming from a male with high or low MHC-II diversity compared to intermediate MHC-II diversity. However, this result is due to the single individual who has only one MHC-II allele. When excluding this individual, females took less time to peck at an odor sample coming from a male with high MHC-II diversity. Altogether, our study adds evidence for olfactory recognition of MHC characteristics in birds, but further studies are required to determine whether kittiwakes use this ability to optimize fitness.

Keywords: Birds; Olfaction; Sexual selection; Intraspecific communication; Major histocompatibility complex; Black-legged kittiwake; *Rissa tridactyla*

27 1. Introduction

28 Most birds do not display any apparent olfactory behavior, such as sniffing or scent-marking. Therefore, it
29 was long thought that birds had little or no sense of smell and relied primarily on visual or acoustic cues.
30 However there is now abundant evidence that birds have a functional olfactory system (Bang and Cobb
31 1968; Steiger et al. 2008) and use olfaction in various non-social contexts, including foraging, navigation or
32 nest sanitation (Abankwah et al. 2020; Caro and Balthazart 2010; Potier 2020; Bonadonna and Gagliardo
33 2021). In contrast, the role of olfactory cues in avian social life is still poorly known (Caro et al. 2015), albeit
34 being a rapidly growing research area (Whittaker and Hagelin 2021).

35 As in other vertebrates, an important force driving mate choice in birds is the major
36 histocompatibility complex (MHC) (Zelano and Edwards 2012; Løvlie et al. 2013), a polymorphic cluster of
37 genes that plays a critical role in immunity. In general, higher MHC diversity is considered to provide
38 resistance to a wider range of parasites and to increase fitness (Milinski 2006). Accordingly, species from
39 diverse taxa have therefore developed MHC-based mate choice, such as mate choice for MHC-dissimilar
40 partners (i.e., MHC-compatible partners), thereby avoiding the production of offspring with low MHC
41 diversity (Strandh et al. 2012; Kamiya et al. 2014; Hoover et al. 2018) or mate choice for MHC-diverse
42 partners, which may provide both genetic and non-genetic benefits (Richardson et al. 2005; Griggio et al.
43 2011). However, high MHC diversity may also be associated with costs, such as an increased risk of
44 autoimmune disorders or a reduction in the potential for inducing an immune reaction due to negative
45 selection of T-cells (Nowak et al. 1992; Simmonds and Gough 2007; Woelfing et al. 2009; Migalska et al.
46 2019). There is therefore increasing support for an intermediate optimum of MHC-diversity (Madsen and
47 Ujvari 2006; Kalbe et al. 2009; Rekdal et al. 2019).

48
49 Whatever the optimum in MHC-diversity, MHC characteristics needs to be encoded in phenotypic
50 cues for animals to assess the MHC characteristics of potential partners during mate choice. However,
51 while several mammals, lizards or fish have been shown to assess MHC characteristics using odor cues
52 (reviewed in Schubert et al. 2021), this ability in birds has been poorly explored. To the best of our
53 knowledge, only the blue petrel (*Halobaena caerulea*), a Procellariiform seabird, and the song sparrow
54 (*Melospiza melodia*) and the house sparrow (*Passer domesticus*), two Passeriform birds, have been tested.
55 While blue petrels and song sparrows were shown to discriminate MHC-II characteristics using odor cues
56 (Leclaire et al. 2017; Grieves et al. 2019), house sparrows did not discriminate MHC-I characteristics using
57 olfaction (Amo et al. 2022). To estimate the taxonomic range of olfactory assessment of MHC in birds, more
58 avian taxa need to be studied.

59 The black-legged kittiwake (*Rissa tridactyla*) is a genetically monogamous Charadriiform seabird
60 which has a functional sense of smell (Leclaire et al. 2009). In this species, preen gland secretions encode

information about MHC dissimilarity (Leclaire et al. 2014) and offspring sex-ratio varied with the MHC dissimilarity between parents (Pineaux et al. 2022), suggesting that kittiwakes might be able to recognize the MHC characteristics of conspecifics. Here, we used behavioral tests to investigate whether female kittiwakes can use odor cues to distinguish the MHC characteristics of males. We used a protocol inspired by Leclaire et al. (2009), which showed that kittiwakes discriminate between several odors deposited in their nest. Our test is based on the pecking behavioral response of incubating females to an odor cue put in their nest. Results cannot therefore be interpreted as female preference. However, a differential response to different odors will indicate recognition. Because the MHC-based mating pattern of kittiwake is unknown, we tested recognition of both MHC diversity and MHC dissimilarity, and always included both the linear and the quadratic term of MHC diversity.

2. Materials and methods

2.1 Study site

The study was conducted during the 2018 incubating period (May-June) in a population of black-legged kittiwakes nesting on an abandoned US Air Force radar tower on Middleton Island, Alaska (59°26'N, 146°20'W). Nests on artificial ledges were visible from inside the building through sliding one-way glass windows (Gill and Hatch 2002).

2.2 Behavioral tests

We assessed female's response to male odor samples placed on the edge of the nest bowl. Odor samples were a mixture of cloacal samples (potentially related to fecal odor) and nest materials (potentially related to fecal odor deposited on the nest during defecation and fecal, skin, plumage and preen gland odor passively deposited on the nest when birds are sitting). We collected odor samples from 43 incubating males. After capture, we collected cloacal samples as in Leclaire et al. (2022). Briefly, we injected 1 mL of sterile phosphate-buffered saline solution into male's cloaca with a sterile needleless syringe and drew the solution out. At the time of capture, a piece of nest material (i.e., a blend of mosses and grasses of ca. 4 cm diameter) was also collected from the nest of the captured male, using clean nitrile gloves. The piece of nest material was immediately mixed with the cloacal sample and stored in plastic bags at -20°C until the test. Odor samples were stored on average for 17 ± 1 days (range: 8 – 24 days).

Fifty two incubating females were each tested once. They were never tested with the odor of their partner. All odor samples ($n = 43$) were used at least once. Just before the test, a small piece (ca. 2 cm diameter) of a random odor sample was defrosted for 15 minutes. The remaining odor sample was kept frozen. Nine of these remaining odor samples were used in tests later on. The odor sample was placed on the edge of the nest bowl, just under the beak of an incubating female. When placing the odor sample on

95 the nest, most females took off. Behavioral observations started when the female landed back on her nest.
96 Females returned to their nest on average \pm se: 14 ± 5 sec after the odor sample was placed on the nest
97 (range: 0-462 sec). For each test, we recorded whether the female pecked at the odor sample and the time
98 she took to peck at the odor sample for the first time. Observation lasted until the female pecked at the
99 odor sample or for 15 min when she did not peck at the odor sample. Most females who pecked at the
100 odor sample took the sample with their bill and moved it along the nest bowl, as they often do with nest
101 material when maintaining the nest structure. Tests were carried out by 3 observers who were blind to
102 MHC-characteristics of the males concerned.

103

104 *2.3 MHC analyses*

105 Details about MHC analyses are given in Pineaux et al. (2020). Briefly, DNA was extracted from blood
106 samples, and 258 pb fragments of exon 2 of MHC class-II β were amplified using PCR and sequenced with an
107 Illumina MiSeq platform. Amplicon sequences were analyzed with AmpliSAS (Sebastian et al. 2016). We
108 focused on the amino acid sequences of the peptide binding region (Leclaire et al. 2014). MHC-II diversity
109 was estimated using the number of MHC-II alleles. MHC-II dissimilarity between the female and the
110 sampled male was estimated on a pure shared/non-shared allele basis. MHC-II dissimilarity was calculated
111 as $TOT_{FM} / (N_F + N_M)$, where TOT_{FM} = total number of variable alleles present in the male and the female
112 (shared allele count as 1) and N = number of alleles within an individual, which represents the percent
113 difference in allele sharing (Strandh et al. 2012). Because five females were not genotyped at the MHC-II,
114 we could not calculate the MHC-II dissimilarity between the female and the sampled male for five tests.

115

116 *2.4 Statistics*

117 To test for difference in females' behavioral response according to the MHC-II diversity of the male odor
118 donor and to the MHC-II dissimilarity between the female and the male odor donor, first we used a
119 generalized linear mixed model (GLMM) with the probability to peck at the odor sample (0 when the
120 female did not peck at the sample or 1 when the female pecked at the sample) as the binomial response
121 variable. Fixed effects included MHC-II dissimilarity, male MHC-II diversity and the quadratic term of male
122 MHC-II diversity. Although the MHC-II diversity of the male odor donor was negatively related to the MHC-II
123 dissimilarity to the female (Spearman rank test: $S = 22305$, $r = -0.29$, $P = 0.048$, $n = 47$ tests), we included
124 the two variables together in the models as the variance inflation factors were < 1.5 (Zuur et al. 2010).
125 Fixed effects also included the difference between the date of the test and the laying date, as female
126 reaction to male MHC-II characteristics might potentially change across the breeding season (Maning et al.
127 1992; Leclaire et al. 2017). The identity of the male odor donor was included as a random effect, while the
128 observer was not included as a random effect because its variance estimate was 0 and it caused

129 convergence issues. Second, for females who pecked at the samples, we used a linear mixed model (LMM)
130 with the time taken to peck at the odor sample as the response variable (log-transformed to meet
131 normality), and the same fixed effects as above. Both the observer and the identity of the male odor
132 donors were included as random effects. The time the odor sample was kept in the freezer tended to have
133 a negative effect on the time taken to peck at the sample (Spearman's correlation test: $S = 13678$, $P =$
134 0.077 , $r = -0.28$, $n = 40$ tests). However, we decided not to include this variable in the final models to avoid
135 over-parameterization of the models and to increase the sample size (we did not record the date of sample
136 collection for 4 tests). Including this variable in the models did not change the results.

137 We performed all statistical analyses using the R statistical software (R Core Team 2019). In
138 GLMMs, we used Wald's tests to assess the significance of variables. In LMMs, significance of each
139 predictor variable was calculated using the Satterthwaite approximation as implemented in the package
140 *lmerTest* (Kuznetsova et al. 2017). Normality and homogeneity of variance were checked visually (Zuur et
141 al. 2010).

142

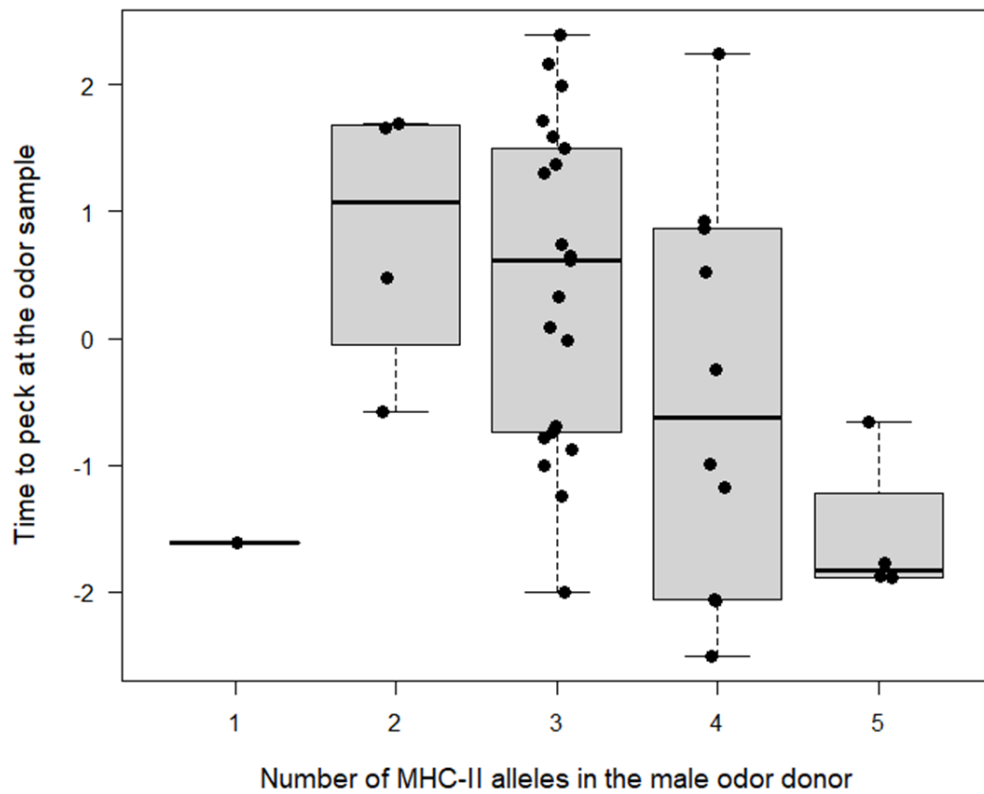
143

144 3. Results

145 8 females (18 %) did not peck at the odor sample. The probability of pecking at the sample did not vary with
146 male MHC-II diversity (quadratic term: $\chi^2_1 = 0.01$, $P = 0.94$ and linear term: $\chi^2_1 = 0.17$, $P = 0.68$) or MHC-II
147 dissimilarity ($\chi^2_1 = 0.06$, $P = 0.80$).

148 Among the females that pecked at the sample, females took more time to peck at the odor sample
149 when the male had an intermediate number of MHC-II alleles (quadratic term: $\chi^2_1 = 5.49$, $P = 0.025$ and
150 linear term: $\chi^2_1 = 6.60$, $P = 0.015$; Fig. 1). When excluding the test where the single male odor donor had
151 only one MHC-II allele (Fig. 1), females took less time to peck at the odor sample when the male had a
152 higher number of functional MHC-II alleles (linear term: $\chi^2_1 = 13.73$, $P < 0.001$ and quadratic term: $\chi^2_1 =$
153 0.36 , $P = 0.55$; Fig. 1). This result suggests that this male carrying a single MHC-II allele drives the quadratic
154 relationship between the time taken to peck at the sample and the MHC-II diversity. Similar results were
155 obtained when excluding the MHC-II dissimilarity between the female and the male odor donor from the
156 models, thereby increasing the sample size by 4 tests ($n = 44$ tests). Females that pecked at the sample took
157 also less time to peck at the odor sample when they were more MHC-II dissimilar to the male odor donor
158 ($\chi^2_1 = 6.08$, $P = 0.019$; Fig. 2).

159



160

161

162 **Fig. 1:** Time taken by females to peck at the odor sample according to the number of MHC-II alleles of the
 163 male odor donor (i.e., the MHC-II diversity of the male). The 8 females that did not peck at the odor sample
 164 during the 15-minutes test were excluded. The time to peck at the sample was represented by the residuals
 165 of a linear mixed model in which the time to peck at the sample was explained by all variables except the
 166 MHC-II diversity of the male.

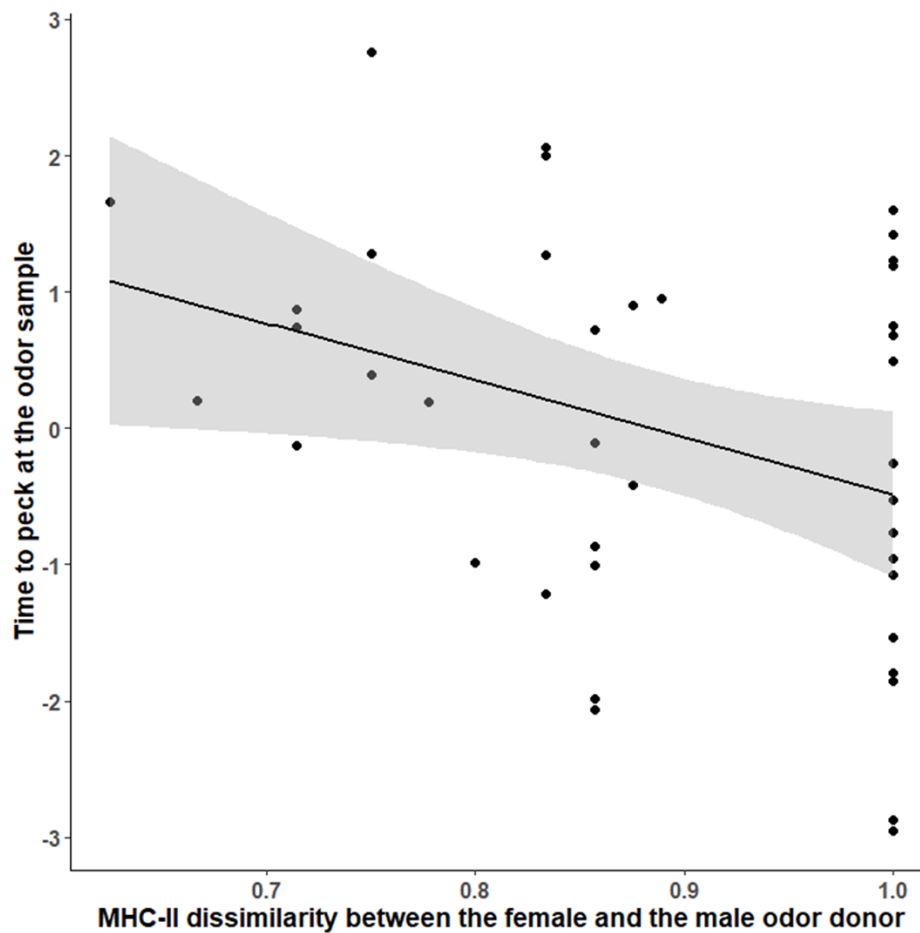


Fig. 2: Time taken by females to peck at the odor sample according to the MHC-II dissimilarity to the male odor donor. The 8 females that did not peck at the odor sample during the 15-minutes test were excluded. The time to peck at the sample was represented by the residuals of a linear mixed model in which the time to peck at the sample was explained by all variables except the MHC-II dissimilarity. Similar results were obtained when the MHC-II dissimilarity was coded as a binomial variable (i.e., 0 for pairs that shared at least one allele and 1 for pairs that shared no allele). The black line and the grey shadow represent prediction line and 95% confidence interval.

5. Discussion

Using behavioral tests, we investigated whether, in black-legged kittiwakes, females recognized the MHC-II characteristics of males. First, we found that females took more time to peck at the odor sample when it came from a male with intermediate MHC-II diversity. However, the quadratic relationship between MHC-II diversity and the time taken to peck at the sample was only due to the single male that had only one MHC-II allele (Fig. 1). When excluding this male, the relationship between male MHC-II diversity and female behavior became linear. In this kittiwake population, very few individuals carry a single MHC-II alleles (< 1 %

184 of the individuals; unpublished data), potentially suggesting that carrying a single MHC-II allele is counter-
185 selected. More tests including male odor donors carrying a single MHC-II allele would have been needed to
186 ascertain the quadratic nature of the relationship between male MHC-II diversity and the female's reaction
187 to the odor sample. Recently, several studies in fish, snakes and birds have tested the "golden mean
188 hypothesis" and have shown that individuals with intermediate MHC-diversity have higher reproductive
189 success (Kalbe et al. 2009), better immunity (Rekdal et al. 2021) and lower parasite load (Wegner et al.
190 2004; Madsen and Ujvari 2006). In kittiwakes, the optimum in MHC-II diversity has been tested in chicks
191 only, and was found to be maximum in females (Pineaux et al. 2020). However, the optima in MHC-
192 diversity can vary according to individual traits that affect the trade-off between the benefits and costs
193 associated with high MHC-diversity (Roved et al. 2018; Pineaux et al. 2020). Therefore, the optimum in
194 MHC-II diversity may differ between adults and chicks, because of age-differences in exposure to parasites,
195 acquired immunity and predisposition to infection (Benskin et al. 2009). Studies are needed to determine
196 whether, in kittiwakes, intermediate or maximum MHC-II diversity is optimum in adult males.

197 We also found that females took less time to peck at an odor sample coming from a male with
198 higher MHC-II dissimilarity. Although female pecking response cannot be interpreted as preference for
199 certain odors, our results show that female kittiwakes can use odor cue to recognize MHC-II diversity and
200 MHC-II dissimilarity. Further studies are needed to determine the contexts in which females use this ability
201 (e.g., mate choice, adjustment of parental investment, offspring sex-ratio distortion). Although the role of
202 olfaction in the recognition of MHC dissimilarity has been shown in several vertebrate species (reviewed in
203 Schubert et al. 2021), its role in the recognition of MHC diversity has been less studied, probably because
204 MHC diversity, by influencing health, can be reflected in any condition-dependent traits (Lie et al. 2008;
205 Dunn et al. 2013; Slade et al. 2017). However, three-spined sticklebacks (*Gasterosteus aculeatus*), ring-
206 tailed lemurs (*Lemur catta*) and song sparrows (*Melospiza melodia*) can discriminate MHC diversity using
207 odor cues (Milinski et al. 2005; Grieves et al. 2019; Grogan et al. 2019).

208 In birds, the sources of the body odors used in social communication are unknown. Here, we tested
209 whether females could discriminate male MHC-II characteristics using a mixture of odor sources (*i.e.*,
210 cloacal samples mixed with nest material), thereby preventing us to identify precisely the sources of MHC-
211 related odors. In kittiwakes, preen secretion encode information about MHC-II dissimilarity (Leclaire et al.
212 2014), but studies are required to determine the role of other sources such as feces or plumage microbiota
213 (Leclaire et al. 2019; Schubert et al. 2021). In addition, while our results are consistent with odor-based
214 discrimination of MHC-II characteristics, our methods do not allow us to determine whether discrimination
215 is based on broader genome-wide genetic variation or on other polymorphic genes whose variations covary
216 with those in the MHC, such as the major urinary protein (MUP) genes in natural populations of mice
217 (Sherborne et al. 2007).

218

219 Altogether, our results, by adding evidence for olfactory discrimination of MHC characteristics in
220 birds, suggest that this capacity may be widespread in this taxa. However, whether birds use it in mate
221 choice or more generally to optimize fitness has still to be demonstrated.

222

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227

228

229 **References**

230 Abankwah, V., Deeming, D.C., Pike, T.W. (2020). Avian Olfaction: A Review of the Recent Literature.
231 *Comparative Cognition & Behavior Reviews*. 15.

232 Amo, L., Amo de Paz, G., Kabbert, J., Machordom, A. (2022). House sparrows do not exhibit a preference for
233 the scent of potential partners with different MHC-I diversity and genetic distances. *Plos one*. 17:
234 e0278892. doi: 10.1371/journal.pone.0278892

235 Bang, B.G., Cobb, S. (1968). The Size of the Olfactory Bulb in 108 Species of Birds. *The Auk*. 85:55–61.

236 Benskin, C.M.W.H., Wilson, K., Jones, K., Hartley, I.R. (2009). Bacterial pathogens in wild birds: a review of
237 the frequency and effects of infection. *Biological Reviews*. 84:349–373. doi:10.1111/j.1469-
238 185X.2008.00076.x.

239 Bonadonna F, Gagliardo A. 2021. Not only pigeons: avian olfactory navigation studied by satellite telemetry.
240 *Ethology, Ecology & Evolution*. 33(3):273–289.

241 Caro SP, Balthazart J. 2010. Pheromones in birds: myth or reality ? *Journal of Comparative Physiology A*.
242 196(10):751-766. doi: 10.1007/s00359-010-0534-4

243 Caro SP, Balthazart J, Bonadonna F. 2015. The perfume of reproduction in birds: Chemosignaling in avian
244 social life. *Hormones and Behavior*. 68(0):25–42. doi:10.1016/j.yhbeh.2014.06.001.

245 Dunn PO, Bollmer JL, Freeman-Gallant CR, Whittingham LA. 2013. MHC variation is related to a sexually
246 selected ornament, survival, and parasite resistance in common yellowthroats. *Evolution: International*
247 *Journal of Organic Evolution*. 67(3):679–687.

248 Gill VA, Hatch SA. 2002. Components of productivity in black-legged kittiwakes *Rissa tridactyla*: response to
249 supplemental feeding. *Journal of Avian Biology*. 33(2):113–126.

250 Grieves LA, Gloor GB, Bernards MA, MacDougall-Shackleton EA. 2019. Songbirds show odour-based
251 discrimination of similarity and diversity at the major histocompatibility complex. *Animal Behaviour*.
252 158:131–138.

253 Griggio M, Biard C, Penn DJ, Hoi H. 2011. Female house sparrows "count on" male genes: experimental
254 evidence for MHC-dependent mate preference in birds. *BMC Evolutionary Biology*. 11(44).
255 doi:10.1186/1471-2148-11-44.

256 Grogan KE, Harris RL, Boulet M, Drea CM. 2019. Genetic variation at MHC class II loci influences both
257 olfactory signals and scent discrimination in ring-tailed lemurs. *BMC Evolutionary Biology*. 19(1):1–16.

258 Hoover B, Alcaide M, Jennings S, Sin SYW, Edwards SV, Nevitt GA. 2018. Ecology can inform genetics:
259 Disassortative mating contributes to MHC polymorphism in Leach's storm-petrels (*Oceanodroma*
260 *leucorhoa*). *Molecular Ecology*. 27(16):3371–3385.

261 Kalbe M, Eizaguirre C, Dankert I, Reusch TBH, Sommerfeld RD, Wegner KM, Milinski M. 2009. Lifetime
262 reproductive success is maximized with optimal major histocompatibility complex diversity. *Proceedings of*
263 *the Royal Society B*. 276(1658):925–934. doi:10.1098/rspb.2008.1466.

264 Kamiya T, O'Dwyer K, Westerdahl H, Senior A, Nakagawa S. 2014. A quantitative review of MHC-based
265 mating preference: the role of diversity and dissimilarity. *Molecular Ecology*. 23(21):5151–5163.

266 Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. lmerTest Package: Tests in linear mixed effects models.
267 *Journal of Statistical Software*. 82(1):1–26. <https://doi.org/10.18637/jss.v082.i13>

268 Leclaire S, van Dongen WF, Voccia S, Merckling T, Ducamp C, Hatch SA, Blanchard P, Danchin É, Wagner RH.
269 2014. Preen secretions encode information on MHC similarity in certain sex-dyads in a monogamous
270 seabird. *Scientific Reports*. 4:6920.

271 Leclaire S, Mulard H, Wagner RH, Hatch SA, Danchin E. 2009. Can Kittiwakes smell? Experimental evidence
272 in a Larid species. *Ibis*. 151(3):584–587.

273 Leclaire S, Pineaux M, Blanchard P, White J, Hatch SA. 2022. Microbiota composition and diversity of
274 multiple body sites vary according to reproductive performance in a seabird. *Molecular Ecology*. Published
275 online. doi:10.1111/mec.16398

276 Leclaire S, Strandh M, Dell’Ariccia G, Gabirot M, Westerdahl H, Bonadonna F. 2019. Plumage microbiota
 277 covaries with the major histocompatibility complex in blue petrels. *Molecular Ecology*. 28(4):833–846.

278 Leclaire S, Strandh M, Mardon J, Westerdahl H, Bonadonna F. 2017. Odour-based discrimination of
 279 similarity at the major histocompatibility complex in birds. *Proceedings of the Royal Society B*.
 280 284(1846):20162466.

281 Lie HC, Rhodes G, Simmons LW. 2008. Genetic diversity revealed in human faces. *Evolution*. 62(10):2473–
 282 2486. doi:10.1111/j.1558-5646.2008.00478.x.

283 Løvlie H, Gillingham MA, Worley K, Pizzari T, Richardson DS. 2013. Cryptic female choice favours sperm
 284 from major histocompatibility complex-dissimilar males. *Proceedings of the Royal Society London B*.
 285 280(1769):20131296.

286 Madsen T, Ujvari B. 2006. MHC class I variation associates with parasite resistance and longevity in tropical
 287 pythons. *Journal of Evolutionary Biology*. 19(6):1973–1978. doi:10.1111/j.1420-9101.2006.01158.x.

288 Manning C, Wakeland E, Potts W. 1992. Communal nesting patterns in mice implicate MHC genes in kin
 289 recognition. *Nature*. 360:581–583. doi: 10.1038/360581a0

290 Migalska M, Sebastian A, Radwan J. 2019. Major histocompatibility complex class I diversity limits the
 291 repertoire of T cell receptors. *Proceedings of the National Academy of Sciences*. 116(11):5021–5026.

292 Milinski M. 2006. The major histocompatibility complex, sexual selection, and mate choice. *Annual Review*
 293 *of Ecology Evolution and Systematics*. 37:159–186.

294 Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, Boehm T. 2005. Mate choice decisions
 295 of stickleback females predictably modified by MHC peptide ligands. *Proceedings of the National Academy*
 296 *of Sciences USA*. 102(12):4414–4418. doi:10.1073/pnas.0408264102.

297 Nowak MA, Tarczy-Hornoch K, Austyn JM. 1992. The optimal number of major histocompatibility complex
 298 molecules in an individual. *Proceedings of the National Academy of Sciences USA*. 89(22):10896–10899.
 299 doi:10.1073/pnas.89.22.10896.

300 Pineaux M, Merklings T, Danchin E, Hatch S, Duneau D, Blanchard P, Leclaire S. 2020. Sex and hatching order
 301 modulate the association between MHC-II diversity and fitness in early-life stages of a wild seabird.
 302 *Molecular Ecology*. 29(17):3316–3329.

303 Pineaux M, Merkling T, Danchin E, Hatch SA, Leclaire S, Blanchard P. 2022. MHC-II distance between
 304 parents predicts sex allocation decisions in a genetically monogamous bird. *Behavioral Ecology*. 33(1):245–
 305 251.

306 Potier S. 2020. Olfaction in raptors. *Zoological Journal of the Linnean Society*. 189(3):713–721.
 307 doi:10.1093/zoolinnean/zl121.

308 R Core Team. 2019. R: A language and environment for statistical computing.

309 Rekdal SL, Anmarkrud JA, Lifjeld JT, Johnsen A. 2019. Extra-pair mating in a passerine bird with highly
 310 duplicated major histocompatibility complex class II: Preference for the golden mean. *Molecular Ecology*.
 311 28(23):5133–5144.

312 Rekdal SL, Anmarkrud JA, Lifjeld JT, Johnsen A. 2021. Elevated phytohaemagglutinin-induced skin-swelling
 313 response at an intermediate number of MHC class II alleles in bluethroat nestlings. *Journal of Avian Biology*.
 314 52(5). doi:10.1111/jav.02734.

315 Richardson DS, Komdeur J, Burke T, von Schantz T. 2005. MHC-based patterns of social and extra-pair mate
 316 choice in the Seychelles warbler. *Proceedings of the Royal Society B*. 272:759–767. doi:
 317 10.1098/rspb.2004.3028

318 Roved J, Hansson B, Tarka M, Hasselquist D, Westerdahl H. 2018. Evidence for sexual conflict over major
 319 histocompatibility complex diversity in a wild songbird. *Proceedings of the Royal Society B*.
 320 285(1884):20180841.

321 Schubert N, Nichols HJ, Winternitz JC. 2021. How can the MHC mediate social odor via the microbiota
 322 community? A deep dive into mechanisms. *Behavioral Ecology*. 32(3):359–373.

323 Sebastian A, Herdegen M, Migalska M, Radwan J. 2016. amplisas: A web server for multilocus genotyping
 324 using next-generation amplicon sequencing data. *Molecular Ecology Resources*. 16(2):498–510.

325 Sherborne AL, Thom MD, Paterson S, Jury F, Ollier WE, Stockley P, Beynon RJ, Hurst JL. 2007. The genetic
 326 basis of inbreeding avoidance in house mice. *Current biology*. 17(23): 2061-2066.

327 Simmonds MJ, Gough SCL. 2007. The HLA Region and Autoimmune Disease: Associations and Mechanisms
 328 of Action. *Current Genomics*. 8(7):453–465. doi:10.2174/138920207783591690.

329 Slade JWG, Watson MJ, MacDougall-Shackleton EA. 2017. Birdsong signals individual diversity at the major
 330 histocompatibility complex. *Biology Letters*. 13(11):20170430. doi:10.1098/rsbl.2017.0430.

331 Steiger SS, Fidler AE, Valcu M, Kempenaers B. 2008. Avian olfactory receptor gene repertoires: evidence for
 332 a well-developed sense of smell in birds? *Proceedings of the Royal Society B*. 275(1649):2309–2317.

333 Strandh M, Westerdahl H, Pontarp M, Canback B, Dubois M-P, Miquel C, Taberlet P, Bonadonna F. 2012.
 334 Major histocompatibility complex class II compatibility, but not class I, predicts mate choice in a bird with
 335 highly developed olfaction. *Proceedings of the Royal Society B*. 279(1746):4457–4463.
 336 doi:10.1098/rspb.2012.1562.

337 Wegner KM, Kalbe M, Schaschl H, Reusch TBH. 2004. Parasites and individual major histocompatibility
 338 complex diversity—an optimal choice? *Microbes and Infection*. 6(12):1110–1116.
 339 doi:10.1016/j.micinf.2004.05.025.

340 Whittaker DJ, Hagelin JC. 2021. Female-Based Patterns and Social Function in Avian Chemical
 341 Communication. *Journal of Chemical Ecology*. 47(1):43–62. doi:10.1007/s10886-020-01230-1.

342 Woelfing B, Traulsen A, Milinski M, Boehm T. 2009. Does intra-individual major histocompatibility complex
 343 diversity keep a golden mean? *Philosophical Transactions of the Royal Society B*. 364(1513):117–128.
 344 doi:10.1098/rstb.2008.0174.

345 Zelano B, Edwards SV. 2002. An MHC component to kin recognition and mate choice in birds: predictions,
 346 progress, and prospects. *American Naturalist*. 160(S6): S225-S237.

347 Zuur AF, Ieno EN, Elphick CS. 2010. A protocol for data exploration to avoid common statistical problems.
 348 *Methods in Ecology and Evolution*. 1(1):3-14. doi: 10.1111/j.2041-210X.2009.00001.x