House sparrows do not exhibit a preference for the scent of potential partners with different MHC-I allele numbers and genetic distances

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Running headline: Lack of preference for the scent of MHC class I dissimilar potential partners in house sparrows

24 **Abstract**

25 MHC genes play a fundamental role in immune recognition of pathogens and parasites.
 26 Therefore, females may increase offspring heterozygosity and genetic diversity by
 27 selecting MHC genetically compatible or heterozygous males. In birds, several studies
 28 suggest that MHC genes play a role in mate choice, and recent evidence suggest that
 29 olfaction may play a role in such discrimination. Previous studies indicated that house
 30 sparrow females with low allelic diversity prefer males with higher diversity in MHC-I
 31 alleles. Here, we directly explored whether both house sparrow females and males could
 32 estimate by scent the number in MHC amino acid and functional variants as well as the
 33 level of MHC-I similarity or dissimilarity of potential partners. Our results show that
 34 neither females nor males exhibit a preference related to the number of MHC-I amino
 35 acid variants or functional variants or in relation to MHC amino acid or functional
 36 similarity of potential partners, suggesting that MHC-I is not detected through olfaction.
 37 Further studies are needed to understand the mechanisms responsible for MHC-I based
 38 mate discrimination in birds.

39

40

41 **Keywords**

42 avian olfaction, major histocompatibility complex (MHC), mate choice, *Passer*
 43 *domesticus*, sexual selection

44 **Introduction**

45 When selecting partners for mating, females may choose those that will provide the best
 46 resources, genes or both in order to increase offspring fitness [1, 2]. Most experimental
 47 studies addressing sexual selection in birds have focused on male quality to explain
 48 adaptive mate choice, considering females to assess potential mates by using male
 49 ornamental traits, such as plumage coloration [3, 4], or acoustic traits such as male
 50 songs [5], that are condition-dependent signals of male quality and therefore, may allow
 51 them to obtain resources and/or good genes for their offspring. In fact, in many
 52 passerine species, male parental care of offspring is needed to ensure nestling survival.
 53 In contrast, studies addressing the mate choice of females in relation to genetic
 54 dissimilarity are less well described. However, the increased use of molecular
 55 techniques for assessing paternity and genetic compatibility are providing examples for
 56 female mate choice that is not exclusively based on genes reflecting quality, but indeed
 57 suggest additional mechanism critical to female choosiness. Females may thus use
 58 alternative mechanisms for choosing mates such as extra-pair copulations [6, 7], or
 59 post-copulatory choice [8].

60 The study of alternative sexual selection is providing examples of major
 61 histocompatibility complex (MHC) dissimilar selection in birds ([7, 9, 10], but see
 62 [11]), pointing out that clutch sizes and the probability to extrapair relationships that can
 63 be affected by the partner's MHC diversity [7, 9, 10]. For example, although blue tit
 64 *Cyanistes caeruleus* females are known to use song [12] and plumage coloration [13]
 65 when selecting mates, there is evidence that they select extrapair partners in based on
 66 their genetic dissimilarity, as extra-pair nestlings showed higher degree of
 67 heterozygosity compared to within-pair nestlings [14]. Since nestlings with higher
 68 degree of heterozygosity were more likely to survive [14], females may increase their

69 fitness by selecting genetically dissimilar mates when engaging in extrapair copulations.
70 Although, females may ensure all nestling survival by choosing the best parental male
71 based on phenotypical characteristics such as plumage coloration [13], they may indeed
72 use other characteristic male signals such as genetic heterozygosity [15, 16].

73 In order to provide the best genetic benefits to their offspring females can select
74 males with “good genes”, that will consequently be inherited by their offspring and may
75 increase their fitness [17] or select more heterozygous or dissimilar males to increase
76 the genetic diversity of offspring [18]. When this heterozygosity is exhibited in genes of
77 the immune system, the benefits for offspring fitness are obvious in terms of resistance
78 to a wide range of infections [19]. The MHC is a large chromosomal region containing
79 several highly polymorphic genes (MHC class I and II loci) that play a central role in
80 controlling immunological self/non-self recognition [20, 21] and encode cell-surface
81 glycoproteins that mediate antigen presentation to T-lymphocytes. Therefore, MHC
82 genes play a fundamental role in immune recognition of pathogens and parasites.
83 Specifically, MHC I genes are involved in recognition of intracellular pathogens
84 whereas MHC II genes are involved in the recognition of extracellular parasites. For
85 example, in house sparrows, *Passer domesticus*, specific MHC class I alleles are
86 associated with stronger immune T cell responses to MHC I presented antigen [22] and
87 increased resistance to malaria parasites [23, 24]. Furthermore, house sparrow females
88 exhibiting lower numbers of functional MHC I alleles lay eggs with more eggshell
89 bacteria [25], and some MHC genes have been found to partly explain early survival in
90 this species [26]. Consequently, both pathogen-mediated selection [27] and sexual
91 selection [28-31] may explain the maintenance of MHC polymorphism.

92 Although, the evidence is not as well described as in mammals [32, 33],
93 including humans [34], evidence for MHC-associated mate choice has been found in

94 fishes [35] and reptiles [36]. However, several observational studies in birds suggest
 95 that MHC genes play a role in mate choice ([7, 9,], but see [11, 37]. While olfaction has
 96 been ascribed to account for MHC recognition in other taxa, sexual selection based on
 97 olfaction has formerly been neglected in avian studies. However, the study by Leclaire
 98 and collaborators [15] showed first evidence that birds can use olfactory cues to assess
 99 MHC similarity. Here incubating males preferred the scent of MHC II-dissimilar
 100 females, while incubating females preferred the scent of MHC-similar males [15].
 101 Conversely, a study assessing the scent preferences in song sparrows *Melospiza melodia*
 102 showed that during breeding season both female and males spent longer time periods
 103 close to odours from MHC II-dissimilar partners [16]. Given these controversial results,
 104 further studies are needed to disentangle the MHC scent preferences of birds in the
 105 context of sexual selection during mating season. To our knowledge, MHC class I scent
 106 preferences of birds in the context of sexual selection has not been previously assessed.

107 In this study we have experimentally explored whether olfactory signals play a
 108 role in the preference for potential partners with greater MHC I dissimilarity and/or
 109 diversity in house sparrows, *Passer domesticus*.

110

111 **Materials and methods**

112

113 **Study Species**

114 The house sparrow was chosen as a model species as their olfactory capabilities have
 115 been previously described in the context of roosting cavity assessment [38] and social
 116 interactions [39]. Moreover, in the house sparrow, MHC class I allele diversity is
 117 associated with reproductive success [40]. It has been shown that males with low MHC
 118 diversity or too dissimilar MHC alleles fail to form breeding pairs [41]. Therefore,

female house sparrows likely assess their male mate preferences based on their own MHC allelic diversity. Females with low MHC allelic diversity choose males with relatively higher MHC allelic diversity, whereas no preference is exhibited by females with high MHC diversity ([42], but see [43]).

We captured with mist nets 100 male and 51 female adult house sparrows *Passer domesticus* at the Madrid Zoo (Spain) during February and March 2015. Immediately after capturing, we measured birds with a dial calliper to the nearest 0.01 cm and birds were weighed with a spring balance to the nearest 0.1 g. We measured the length of the visible badge of males with a digital calliper to the nearest mm from the base of the bill to the point on the black breast at which the black feathers finished. This measurement was used instead of the area of the badge, because measurement of the width across the whole black area is less accurate, and it has been used as a measure of badge size in previous studies [44]. All birds were individually banded with numbered aluminium and PVC rings. We obtained blood (80-100 μ l) from the brachial vein with the aid of a needle and a capillary tube. Blood samples were centrifuged ($2000 \times g$, 5 min) with a portable centrifuge (Nahita 2507-01). Serum and cellular fractions were separated, and the cellular fraction was conserved in ethanol and maintained for later analysis.

Birds were housed at the FIEB, Foundation for the Research and the Study of Ethology and Biodiversity (Casarrubios del Monte, Toledo), in outdoor aviaries, until two weeks before the experiments, when birds were individually housed in cages inside the aviaries, so they were maintained at outdoor temperature and photoperiod. Bird weight and badge size was again measured at this time point.

MHC Characterization

143 For DNA metabarcoding library preparation, PCRs were carried out in a final volume of
144 25 μ l, containing 2.5 μ l of template DNA, 0.5 μ M of the primers (GCA21M, 5' CGT
145 ACA GCG GCT TGT TGG CTG TGA, and fA23M, 5' GCG CTC CAG CTC CTT
146 CTG CCC ATA, primers to which the Illumina sequencing primer sequences were
147 attached to their 5' ends), 12.5 μ l of Phusion DNA polymerase mix (Thermo Fisher
148 Scientific), and ultrapure water up to 25 μ l. The reaction mixture was incubated as
149 follows: an initial denaturation at 98 °C for 30 s, followed by 35 cycles of 98 °C for 10
150 s, 60 °C for 20 s, 72 °C for 20 s, and a final extension step at 72 °C for 10 minutes. The
151 index sequences which are required for multiplexing different libraries in the same
152 sequencing pool were attached in a second PCR round with identical conditions and
153 only 5 cycles. The libraries were run on a 1 % agarose gel stained with REAL Safe
154 (Durviz), and imaged under UV light. Negative controls that contained no DNA were
155 included to check for contamination during library preparation. Libraries were pooled in
156 equimolar amounts according to band intensity. The pool was purified using the Mag-
157 Bind RXNPure Plus magnetics beads (Omega Biotek), following the instructions
158 provided by the manufacturer. The pool was sequenced in a MiSeq PE300 run
159 (Illumina).

160 The data were filtered to remove low-quality sequences, any reads representing
161 artefactual MHC alleles or putatively non-functional sequences. Firstly, a 1%
162 bioinformatics filter was applied. After that, a minimum total sequence
163 abundance/individual was set to 200, accordingly to [37]. After data processing, we
164 obtained 117 different MHC class I alleles in 151 individuals. The average ($\pm$ SE)
165 number of alleles per individual was 4.62 ± 0.15 (range: 1–10).

166

167 **Amino acid and functional distance between birds**

168 Amino acid and functional distances between individual genotypes were used to
169 describe MHC similarity between individuals and were calculated following the
170 approaches described in [15, 16, 37]. To calculate amino acid distances, a maximum-
171 likelihood tree was inferred for all translated MHC sequences, using PhyML (v. 3.0)
172 [45] in the ATGC platform with a JTT substitution model, selected automatically by
173 SMS [46], following the Bayesian Information Criterion. Because this metric can be
174 sensitive to cutoff thresholds and other methodological decisions [47, 48], in addition to
175 calculating distances for the full data set, we also generated four additional phylogenies,
176 each removing one of the four alleles with the longest branch lengths. We calculated
177 unweighted UniFrac distances for each of the 5 phylogenies, then used the average of
178 all analyses [16].

179 To calculate functional distances, we obtained the functional MHC alleles from
180 Lukasch et al. [26], that are based on the chemical binding properties of the amino acids
181 in the peptide binding regions (PBRs), described by five physio-chemical descriptor
182 variables (z-descriptors) for each amino acid [49]. We obtained 113 functional MHC
183 class I alleles. The average ($\pm$ SE) number of functional alleles per individual was 4.48
184 $\pm$ 0.15 (range: 1–10). The functional MHC alleles obtained are shown in Table 2
185 (supplementary material). The resulting matrix of allele frequencies was used to
186 construct an alternative maximum-likelihood tree with contml in the PHYLIP-package,
187 v. 3.695. This tree represents clusters of functionally-similar MHC sequences rather
188 than clusters of evolutionary-similar MHC sequences. The amino acid and functional
189 trees were used as references from which the amino acid and functional distances
190 between MHC-sequence repertoires were calculated, using unweighted UniFrac
191 analyses (Phyloseq v. 1.22.3 package in R). We also generated seven additional
192 phylogenies, each removing one of the seven functional alleles with the longest branch

lengths, and calculated mean unweighted UniFrac distances across all eight functional trees. Amino acid distances were positively correlated with functional distances among the 151 individuals used in this study (Mantel test: $r = 0.87$, $P = 0.01$; calculated using Vegan v. 2.4-6 package in R).

Behavioural Experiment

The experiment was performed indoors in May and June 2015 using an olfactometry chamber (Fig 1). The experimental device was composed of a small central plastic box (15 x 25 x 25 cm) where the experimental bird was placed. The chamber contained a small 12 V PC fan that extracted the air from the device creating a low-noise-controlled airflow (Fig 1). In each test, a bird was introduced into the central box and maintained in the dark for 5 min after that the doors were opened. Each choice chamber was divided into two sectors with screens. The distal sectors of the choice chambers (15 x 25 x 25 cm) contained two little cages where the scent donor birds were situated. Both, the doors connecting the central chamber with the choice chambers and the screens creating the sectors, were made with a dense plastic mesh that allowed air flow but prevented birds from seeing through them. The device was sealed and only openings at the farthest walls of the choice chambers allowed air flow. The fan created two constant air flows, each one entering across the openings located at the farthest walls of each choice chamber, passing by the donor birds and crossing the central chamber, and going outside from the device through the fan. Thus, the focal bird received two separate air flows, carrying the scent of the corresponding donor bird. Donor birds were temporarily kept in darkness and reduced space, preventing them from moving or calling. Therefore, the experimental bird only perceived the scent of the donor birds without visual or acoustics contact. The experimental room was to the greatest possible extend sealed

218 from exterior noise, enabling the experimenter to perceive any acoustic signals from any
219 of the birds in the device. The device and the methodology have been successfully used
220 in social contexts before [50, 51].

221 We noted down the choice chamber that was first approached by each tested
222 focal bird after opening the central chamber. The assessment of “first choice” as a
223 preference measure of birds to chemical stimuli has been previously demonstrated [15,
224 50, 51]. To minimize the experimental time duration of the trials and to release the birds
225 as soon as possible, birds that did not show any choice preference after 1 min, were
226 motivated to make an active choice by gently knocking on the middle of the entry door
227 of the central chamber to motivate the bird to move to any of the choice chambers. The
228 knocking on the door did not influence the preference of birds (see results).

229 We selected donor birds of the same sex, similar body size and body condition.
230 When using male donor birds, we chose birds with similar badge size. Therefore, we
231 offered focal birds the scent of two potential mates of similar body condition and
232 dominance status in the case of males. There were no significant differences in the body
233 condition (repeated measured ANOVA, $F_{1,127} = 0.60$, $P = 0.44$), or the badge size ($F_{1,48}$
234 $= 0.09$, $P = 0.77$) between both scent donor birds. The MHC characterization was done
235 a posteriori. We tested 72 males and 48 females. Only one female bird did not move to
236 any chamber even after knocking on the door, and was therefore excluded from the
237 analysis. We used 68 different pairs of scent-donor birds, each pair was used on average
238 $1.76 (\pm 0.8)$ times. Focal birds made 61 % of their first choices to the left and 39 % of
239 the choices to the right side. Birds were returned to their cages as soon as they were
240 tested. The olfactometry device was carefully cleaned with alcohol in between trials and
241 experiments continued after the alcohol was completely evaporated.

242

243 **Data Analysis**

244 To analyse whether birds could detect amino acid variant numbers of potential mates by
 245 using chemical cues alone, we performed a generalized linear mixed model with
 246 binomial errors and a logit link function (GLMM). We modelled the probability that
 247 birds chose the scent of the conspecific of the opposite sex with greater number of
 248 MHC-I amino acid variants (as a dichotomous variable: greater number of MHC amino
 249 acid variants (yes) vs. lower number of MHC amino acid variants (not)) in relation to
 250 the sex and the number of MHC amino acid variants of the focal bird. We included the
 251 interaction between the sex and number of MHC amino acids of the focal bird in the
 252 model to test whether males and females differed in their preferences for partners with
 253 different numbers of MHC amino acid variants in relation to their own number of MHC
 254 amino acid variants. We also included the pair of donor birds in the model as a random
 255 factor to control for the fact that pairs of donors could be used more than once. We
 256 included in the initial model a variable reflecting whether the experimental bird left the
 257 chamber when we opened the doors or after 1 min as fixed factor.

258 We performed a similar analysis to assess whether birds chose the scent of the
 259 conspecific of the opposite sex with greater number of MHC-I functional alleles,
 260 including in number of MHC-I functional alleles of the focal bird in the model, the sex
 261 and the interaction between the number of MHC-I functional alleles and the sex in the
 262 model. We also included the pair of donor birds in the model as a random factor. We
 263 included in the initial model a variable reflecting whether the experimental bird left the
 264 chamber when we opened the doors or after 1 min as fixed factor.

265 We also performed two generalized linear mixed models with binomial errors
 266 and a logit link function (GLMM) to model the probability that birds chose the scent of
 267 the conspecific of the opposite sex with greater a) MHC-I amino acid distance and b)

268 MHC-I functional distance (as a dichotomous variable: greater MHC-I amino acid or
269 functional distance (yes) vs. lower amino acid or functional distance (not)) in relation to
270 the sex of the focal bird. We included in the initial model a variable reflecting whether
271 the experimental bird left the chamber when we opened the doors or after 1 min as fixed
272 factor. We also included the pair of donor birds in the model as a random factor. Data
273 analyses were performed with R program [52].

274

275 **Ethical Note**

276 After capture, birds were housed in ten aviaries (2.5 × 2.5 × 2.5 m), separated by
277 sex (10 females per aviary and 16 males per aviary). Aviaries contained vegetation
278 (bamboo branches) that birds could use as perches, and grass and sand on the ground.
279 Commercial food for granivorous passerines and water were provided *ad libitum*. In
280 order to reduce the time of capturing individual birds used within the trials, two weeks
281 before the experiments, birds were individually housed in cages (60 x 40 x 40 cm)
282 inside the aviaries, so they were maintained at outdoor temperature and photoperiod.
283 After the behavioural tests were completed, birds were released again in the aviaries for
284 two weeks and thereafter released at their capture site. Birds maintained healthy
285 throughout the experiments. Experiments were carried out under license of the Ethical
286 Committee of the CSIC (268/2015), the Animal Experimental Committee of the Junta
287 de Castilla la Mancha (202121) and the department of Flora and Fauna of Comunidad
288 de Madrid (10/238509.9/14).

289

290

291 **Results**

292

Our results do not show any preference of birds for the scent of potential partners having a greater number of MHC-I amino acid variants ($Z = 1.70$, $P = 0.09$, Table 1, Fig 2a) or MHC functional alleles ($Z = 1.60$, $P = 0.11$, Table 2, Fig 2b). There was not effect of sex in this lack of preferences ($Z = -0.99$, $P = 0.32$, and $Z = -0.21$, $P = 0.83$, respectively), and the interaction between the sex and the number of amino acid variants or functional alleles was not significant in either analysis ($Z = 0.83$, $P = 0.41$ and $Z = 0.37$, $P = 0.71$, respectively). Neither the number of MHC amino acid variants ($Z = -1.59$, $P = 0.11$) nor the number of functional alleles ($Z = -1.47$, $P = 0.14$) influenced bird choice. Whether the experimental bird left the chamber when opened or after 1 min as fixed factor did not affect the choice of the respective bird (see Table 1 and 2).

Our results do not show any preference of birds for the scent of potential partners having a greater MHC-I amino acid distance ($Z = 0.27$, $P = 0.64$, Table 3, Fig 3a) or functional distance ($Z = 0.28$, $P = 0.35$, Table 4, Fig 3b). Neither the sex ($Z = 0.38$, $P = 0.70$, and $Z = 0.96$, $P = 0.33$, respectively) nor whether the bird made the choice within the first minute or afterwards ($Z = 0.42$, $P = 0.74$, and $Z = -1.08$, $P = 0.28$, respectively) influenced their choice.

Discussion

Our experimentally obtained results on house sparrows do not provide evidence of MHC-I scent discrimination in a passerine bird species. Although olfaction has been demonstrated to play a major role in assessing genetic dissimilarity in birds [15, 16] as well as in other taxa [53], our results, however, show that neither females, nor males,

318 exhibited any preference for the scent of conspecifics with greater allelic diversity or
 319 more MHC-dissimilarity. This is contrary to previous studies showing that female blue
 320 petrels *Halobaena caerulea*, preferred the scent of MHC-similar males [15], and female
 321 song sparrow *Melospiza melodia* preferred the scent of MHC-dissimilar and more
 322 MHC-diverse males during the mating period [16]. Differences between our results and
 323 those of Grieves and collaborators could be due to the different methodology used to
 324 test bird scent preferences as well as to the level of sociality of both study species.
 325 Grieves and collaborators analysed the percentage of time spent close to the two stimuli
 326 after 5 minutes from the beginning of the trial. Neither the first choice nor the
 327 preference of birds during the first 5 minutes of the trial was reported. We in contrast,
 328 analysed the first choice of birds. The validity of first choice as a measure of the interest
 329 of birds in particular chemical stimuli has been previously demonstrated [50, 51, 54, 55,
 330 56], including MHC related scents [15]. Furthermore, our results are robust because we
 331 used a large sample size used and because we used scent-donor birds of similar body
 332 condition in each trial to exclude any potential effect of body condition of scent-donor
 333 birds in the choice of focal birds.

334 While there is no reason to expect that all bird species use the same type of
 335 information to evaluate potential partners, nevertheless our results are difficult to
 336 explain in a sexual context, because in house sparrows MHC class I diversity is
 337 associated with reproductive success [40]. It is also known that males with low degree
 338 of MHC class I diversity or too dissimilar MHC alleles fail to form breeding pairs [41].
 339 Furthermore, results of a previous study found that house sparrow females with low
 340 MHC allelic diversity choose males with high MHC allelic diversity [42], suggesting
 341 that house sparrow females show mate preferences based on the MHC of potential
 342 partners ([42], but see [43]). First choice is a good measure of the spontaneous interest

343 of an animal to a particular cue, whereas time spent close to the stimulus [57] may
344 rather be related to the behaviour shown later on in the series of events to a certain cue.
345 Using life birds as scent donors, the first choice was a valid measure of the response of
346 birds to scent in our study. To analyse whether the lack of preferences for the scent of
347 most MHC class I dissimilar conspecifics is maintained over the time, more studies are
348 needed to assess the subsequent response of birds to the scent of potential partners, for
349 instance using uropygial gland secretions as scent sources.

350 Although several hypotheses have been proposed to explain the underlying
351 mechanisms [53] is still not completely understood how MHC genes influence scent
352 [58, 59]. As MHC proteins appear in urine and sweat, it was proposed that these
353 molecules may constitute odorants [60]. However, since MHC proteins are non-
354 volatiles this hypothesis is rather weak [61]. Another hypothesis proposes that the
355 peptides bound by MHC molecules are the precursors of volatile odorants [62], or that
356 those peptides can be detected by the vomeronasal organ [63]. Furthermore, it has been
357 proposed that MHC genes may influence the bacterial profile that influences the scent
358 of an individual [64, 65, 66, 67]. In many avian species, the source of scent that birds
359 are detecting usually comes from externally applied secretions of the uropygial gland
360 [68]. The quantity and composition of this secretion varies between species, sexes, ages,
361 seasons, and is dependent on diet and hormonal levels [69]. This secretion conveys
362 information on genetic compatibility [15,16], which may play a role during kin
363 recognition [70] and mate choice [15, 16, 37].

364 Evidence that birds are using the volatile compound of uropygial gland
365 secretions for detecting the MHC characteristics of conspecifics [16] comes from
366 studies in song sparrows that were able to assess MHC similarity when exposed only to
367 the uropygial gland secretion. However, MHC-related scent could also be produced by

368 symbiotic bacteria [71] whose diversity is associated with MHC-II diversity [66].

369 Further research is needed to elucidate the source of MHC-related scent in birds.

370 In conclusion, the observed lack of preference for the scent of MHC dissimilar

371 potential partners suggests that the assessment of genetic diversity and dissimilarity in

372 MHC-I is not based on olfaction in our study population of house sparrows. However,

373 our results do not fully exclude the possibility that mate choice in house sparrows

374 includes the selection of partners based on the degree of similarity or dissimilarity in

375 MHC genes. Here, increased MHC allele diversity in the progeny, resulting in higher

376 resistance to pathogens [22] such as malaria parasites [23, 24, 72] may add to increased

377 reproductive success [40]. We suggest that different methodological approaches in

378 future investigation are needed to further the understanding of the mechanisms

379 responsible for MHC-I based mate discrimination in birds.

380

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386

387 **Data Accessibility**

388 All data shown/used are presented in this manuscript.

389

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618 **Figures legend**

619 **Fig 1. Olfactometry chamber. The solid arrows indicate the direction of air flow**
 620 **within the chamber, whereas the dashed lines indicate the direction of opening of**
 621 **the two doors connected to the two choice plastic chambers.**

622

623 **Fig 2. Number of birds that chose the side of the olfactometry chamber containing**
 624 **the scent of a conspecific of the opposite sex with greater (black) or lower (grey) a)**
 625 **number of MHC-I amino acid variants or b) number of MHC-I functional**
 626 **variants.**

627

628 **Fig 3. Number of birds that chose the side of the olfactometry chamber containing**
 629 **the scent of a conspecific of the opposite sex with greater (black) or lower (grey) a)**
 630 **MHC-I amino acid distance or b) MHC-I functional distance.**

631

632

633 **Fig 1**

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637 **Fig 2**

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640 **Fig 3.**

641

642 Tables

643

644 **Table 1. Model for bird choice in relation to the number of MHC- I amino acid**
 645 **variants of a potential partner (as a dichotomous variable: greater number of**
 646 **MHC amino acid variants (yes) vs. lower number of MHC amino acid variants), in**
 647 **relation to the sex and the number of MHC amino acid variants of the focal bird,**
 648 **the interaction between the sex and number of MHC amino acids of the focal bird,**
 649 **the variable reflecting whether the experimental bird left the chamber when we**
 650 **opened the doors or after 1 min as fixed factor and the pair of donor birds as a**
 651 **random factor.**

	Estimate	SE	Z	P
Intercept	1.44	0.85	1.70	0.09
Sex	-1.30	1.32	-0.99	0.32
Choice within 1 min or after	-0.06	0.54	-0.11	0.91
Number of MHC amino acid variants	-0.28	0.17	-1.59	0.11
Sex x Number of MHC amino acid variants	0.21	0.26	0.83	0.41

652

653

654 **Table 2. Model for bird choice in relation to the number of MHC-I functional**
 655 **alleles of a potential partner (as a dichotomous variable: greater number of**
 656 **functional alleles (yes) vs. lower number of functional alleles), in relation to the sex**
 657 **and the number of MHC functional alleles of the focal bird, the interaction**
 658 **between the sex and number of functional alleles of the focal bird, the variable**
 659 **reflecting whether the experimental bird left the chamber when we opened the**
 660 **doors or after 1 min as fixed factor and the pair of donor birds as a random factor.**

	Estimate	SE	Z	P
Intercept	1.44	0.90	1.60	0.11
Sex	-0.31	1.44	-0.21	0.83
Choice within 1 min or after	-0.22	0.54	-0.41	0.68

Number of MHC functional alleles	-0.28	0.19	-1.47	0.14
Sex x Number of MHC functional alleles	0.10	0.28	0.37	0.71

661

662 **Table 3. Model for bird choice in relation to the difference in the MHC-I amino**
663 **acid distance between the focal birds and the two scent donor birds of the opposite**
664 **sex (as a dichotomous variable: greater MHC-I amino acid distance (yes) vs. lower**
665 **MHC-I amino acid distance (no)) in relation to the sex of the focal bird, the**
666 **variable reflecting whether the experimental bird left the chamber when we**
667 **opened the doors or after 1 min as fixed factor and the pair of donor birds as a**
668 **random factor.**

	Estimate	SE	Z	P
Intercept	-0.12	0.27	-0.46	0.65
Sex	-0.15	0.38	-0.39	0.70
Choice within 1 minute or after	-0.14	0.42	-0.33	0.74

669

670

671 **Table 4. Model for bird choice in relation to the difference in the MHC-I**
672 **functional distance between the focal birds and the two scent donor birds of the**
673 **opposite sex (as a dichotomous variable: greater MHC-I functional distance (yes)**
674 **vs. lower MHC-I functional distance (no)) in relation to the sex of the focal bird,**
675 **the variable reflecting whether the experimental bird left the chamber when we**
676 **opened the doors or after 1 min as fixed factor and the pair of donor birds as a**
677 **random factor.**

	Estimate	SE	Z	P
Intercept	0.26	0.27	0.94	0.35
Sex	-0.38	0.38	-0.99	0.33
Choice within 1 minute or after	-0.45	0.42	-1.08	0.28

678