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2 Male quality and conspecific scent preferences in the house finch *Carpodacus*

3 *mexicanus*

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20 Running title: Male quality and olfactory sex preference in birds

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22 There is increasing evidence that birds use chemical cues in different contexts, and this is
23 changing the traditional view that placed birds alone as the only largely olfaction-free
24 vertebrates. We performed a choice experiment to examine whether male house finches (*C.*
25 *mexicanus*) exhibit any preferences for the sex of conspecifics when only their chemical
26 cues are available. When exposed during the breeding season to the scent of a male or a
27 female, males appeared to respond indiscriminately to both odours. However, when
28 analysing *a posteriori* the choices of males in relation to their relative quality, males with
29 worse quality than scent-donor males avoided the male-scented area, whereas males with
30 better quality moved towards the male-scented area. Our results suggest that in the context
31 of mate-choice/competition for mates, house finches may obtain information via olfaction
32 to assess the quality of rival males.

33

34 *Keywords:* Avian olfaction, *Carpodacus mexicanus*, Chemical ecology, Male quality,
35 Intrasexual interaction, Sex-recognition, Mate Choice

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39 The role of chemical communication in different contexts of avian life is receiving
40 growing attention (for reviews see Caro & Balthazart 2010; Hagelin & Jones 2007;
41 Hagelin 2007a), and this is changing the traditional view that beyond mechanical
42 interactions, birds mainly respond to visual and acoustic cues in their environment (albeit
43 restricted roles have been recognised for magnetic and thermal cues as well; Hagelin &
44 Jones 2007). However, our understanding of the role of chemical cues in intra-specific
45 relationships is still much scarcer in birds than in other taxa. It has been shown that birds
46 can recognize their nest using chemical cues (e.g. Bonadonna et al. 2004, Caspers &
47 Krause 2011, Krause & Caspers 2012) and discriminate the scent of their partners from the
48 scent of other conspecifics (e.g. Bonadonna & Nevitt 2004), as well as use scent for kin
49 recognition (Bonadonna & Sanz-Aguilar in press). It has also been shown that chemical
50 signals play a role in the social behaviour of the crested auklet (Hagelin 2007b), and Hirao
51 and collaborators (2009) found that mate preferences in domestic cockerels involve
52 olfactory responsiveness to the female's uropygial secretions.

53 Birds possess several odour sources, such as feathers, skin or scales, and most have
54 an uropygial gland which secretes both volatile and non volatile compounds in the form of
55 waxy fluids that birds collect and spread on their feathers (Jacob & Zisweiler 1982). The
56 amount and composition of this secretion has been shown to vary among seasons (e.g.
57 Reneerkens et al. 2002; Amo et al. 2012), sexes (e.g. Whitaker et al. 2010; Amo et al.
58 2012), age classes (Amo et al. 2012), diets (e.g. Sandilands et al. 2004a,b), hormone levels
59 (e.g. Whelan et al. 2010) and individuals (Mardon et al. 2010; Whittaker et al. 2010;
60 Leclaire et al. 2011a), suggesting that it may convey potentially useful information during
61 intra-specific interactions such as sex recognition. Furthermore, recent findings show that
62 semiochemical profiles correlate with heterozygosity both in male and female black-legged
63 kittiwakes *Rissa tridactyla* (Leclaire et al. 2011b), opening the possibility that avian

64 chemical cues may also convey information on genetic compatibility (Leclaire et al.
65 2011b) which may be useful during kin recognition (Coffin et al. 2011; Krause et al. 2012)
66 and mate choice, as recently demonstrated by Bonadonna and Sanz-Aguilar (in press).

67 A first step to determine whether chemical signals play a role in pair formation in
68 birds would be to analyse whether they discriminate the sex of conspecifics based on
69 chemical cues only. The first study that examined this hypothesis found no evidence of
70 conspecific sex recognition in the Antarctic prion (*Pachyptila desolata*) (Bonadonna et al.
71 2009), although this species shows olfactory partner recognition (Bonadonna & Nevitt
72 2004). By contrast, Zhang et al. (2010) demonstrated that female budgerigars
73 (*Melopsittacus undulatus*) can distinguish the odour of male and female conspecifics, but
74 reported that males did not make a choice when confronted with the scent of both sexes
75 (Zhang 2011). Whittaker et al. (2011a) exposed male and female dark-eyed juncos (*Junco*
76 *hyemalis*) to the scent of the uropygial gland secretion of male and female conspecifics and
77 found that both sexes exhibit a preference for the scent of males. Amo et al. (2012)
78 corroborated such preference of males and females for male scent in spotless starlings
79 (*Sturnus unicolor*), using living birds as scent sources. Both studies were performed during
80 the mating period of the focal species, and in both cases, the attraction of males to male
81 scent was explained in terms of intraspecific aggression. While there is no reason to expect
82 that all bird species use the same type of information in social contexts, it is clearly
83 necessary to increase the number of bird species in which chemical sex recognition is
84 investigated, as well as to increase our knowledge of male preferences for the scent of
85 males and females.

86 In a context of intrasexual aggression, asymmetries between rivals are frequently
87 used to decide whether to get involved or to what extent to escalate a fight (Maynard Smith
88 & Parker 1976). For example, males with better body condition, and hence greater fighting

89 ability, are usually dominant over worse males. Therefore, assessing the quality of the rival
90 is useful for individuals to reduce the costs of aggression. Chemical cues of conspecifics
91 are known to be useful in many species to evaluate the quality of rivals (e.g. Arakawa et al.
92 2008; Mason & Parker 2010). In birds, it has been recently shown that the chemical
93 composition of the uropygial gland is related to the body size of males (Whittaker et al.
94 2011a). This evidence opens the possibility that birds may use chemical cues to assess the
95 quality of conspecifics, which may be particularly useful in male-male competition and
96 mate choice.

97 We performed an experiment aimed at examining the ability of male house finches,
98 *Carpodacus mexicanus*, to use chemical cues in sex recognition. The house finch is a small
99 socially monogamous and sexually dichromatic passerine that breeds both in cavities and
100 open nests (Thompson 1960). We offered males the choice between the scents of a male
101 and a female during their mating period. If they were able to discriminate between sexes by
102 scent, we would expect them to be either attracted to the scent of conspecific females, or,
103 as previously observed, to move towards the scent of other males due to intrasexual
104 aggression. In the event of males not making a consistent selection on the basis of sex, we
105 decided to analyse the intrasexual aspect of male choice behaviour by exploring *a*
106 *posteriori* whether differences in quality between the focal male and the scent-donor male
107 affected the choice made by the birds. We expected that differences in quality between
108 males would determine the response of focal males to the female and male scents, with
109 focal males of superior quality moving towards the scent of the lower quality male, while
110 males of worse quality moving away from the scent of the superior male.

111

112 **METHODS**

113

114 *Study Species*

115

116 We used 31 adult house finches (24 males and 7 females) captured with nets by
117 professional bird catchers authorized by Secretaría del Medio Ambiente y Recursos
118 Naturales (SEMARNAT). Birds were captured at the State of San Luis Potosí, México in
119 February 2009, outside the breeding period, but when they normally begin to search for
120 mates. Birds were taken by car in cages to the Instituto de Ecología, Universidad Nacional
121 Autónoma de México (UNAM; México City), where they were housed individually in 33 x
122 27 x 31 cm mesh cages containing three perches that they readily used to roost. Birds cages
123 where located inside an aviary with a roof to protect them from the sun and the rain, but
124 they were otherwise maintained in outdoor conditions, at ambient temperature and under a
125 natural photoperiod. Between adjacent cages, dense polycarbonate separations where
126 located to hamper visual and chemical communication amongst adjacent neighbours. A
127 total of 59 house finches were held in the aviary in individual cages and experimental birds
128 were randomly located within the aviary. The rest of the birds (24 males and 4 females)
129 were used in other studies and therefore were not available for our tests. Although birds
130 were kept in individual cages, they were maintained inside the same aviary and thus we
131 could not completely prevent that some visual or acoustic communication took place
132 between them. Consequently we assume that all birds were similarly familiar to each other
133 in terms of visual and acoustic cues. Birds were provided with a commercial dry mixture of
134 seeds for granivorous birds and water ad libitum. The experiments were performed after
135 two months of acclimatization.

136 As a standard procedure, one week before the experiment, birds were weighted with
137 a Pesola spring balance to the nearest 0.05 g. We measured the tarsus length with a digital
138 calliper to the nearest 0.01 mm and calculated body condition as the ratio weight/tarsus

139 length. We also measured the T-cell mediated immune response of birds by injecting 0.02
140 mg of phytohemagglutinin (PHA) dissolved in 0.04 ml of phosphate-buffered saline (PBS)
141 in the web (patagium) of the right wing. We measured the patagium thickness before and
142 24 h after the injection with a pressure sensitive spessimeter with an accuracy of 0.01 mm.
143 The cellular immune response index was calculated as the difference between the pre- and
144 post-injection measures (Smits et al. 1999). No adverse effects of the PHA injection were
145 observed.

146 Colour was measured using a MINOLTA handheld spectrophotometer (MINOLTA
147 CR-200, Minolta Co. Ltd, Osaka, Japan) that measures the reflectance from 360 to 700 nm
148 in intervals of 10 nm. Although it does not measure across the whole UV spectrum
149 available to birds (some birds are sensitive down to 320 nm), this limitation may not be a
150 problem because the house finch has little plumage reflectance below 400 nm (Hill 2002).
151 Reference calibrations against zero and a white standard tablet associated with the
152 apparatus were performed according to the instructions provided by the maker. Reflectance
153 spectra for each individual were automatically obtained by means of three sequential
154 measurements per individual, each taken at a slightly different place of the bird's breast.
155 The SPECTRAMAGIC software (Minolta Co. Ltd) was used to analyse spectra. We
156 calculated the yellow–red chroma saturation (YRC) as the proportion of total reflectance
157 traceable to the yellow–red region ($R_{560-700}/R_{360-700}$) of the spectrum. We used YRC
158 to describe feather reflectance because this region corresponds to the colour range that is
159 subject to mate choice in this species (Hill 2002).

160 On completion of each trial, birds were returned to their cages where they were
161 seen to resume their normal behaviour. Birds were healthy during the tests and did not
162 exhibit signals of stress due to the manipulation. After the experiment, the birds were
163 placed in large aviaries during two weeks before being released by their capture site.

164 Aviaries were located indoors, with mesh in the open windows to protect them from the
165 sun and the rain, and they were kept at ambient temperature under a natural photoperiod.
166 Aviaries contained several 1.5 - 2 m (height) tree branches in order to provide perches and
167 places to sleep to the birds. They also contained food and water ad libitum. We did not
168 observe any overt aggression among the birds in these facilities and all were healthy during
169 their stay in the aviaries. Birds were kept in captivity 3 months in total, and then released at
170 their capture location at the beginning of May. The night before the release, birds were
171 introduced in small individual cardboard boxes specifically designed. Inside these boxes,
172 birds were in darkness to keep them quiet and we did not observe any sign of stress during
173 the trip. They were transported by car during the night and released early in the morning.
174 Although there was plenty of natural food in the sites where the birds were released, we
175 placed their seed food in several locations in order to guarantee that they could easily find
176 food the first day of release.

177

178 *Experimental Design*

179

180 The experiments were carried out during the reproductive period of *Carpodacus*
181 *mexicanus*, in April 2009. We performed the experiments in an olfactometric chamber
182 similar to that previously used to successfully test bird scent preferences (Hagelin et al.
183 2003; Amo et al. 2012; see Fig. 1) in indoor conditions. The device was composed by a
184 small central plastic box (19 x 19 x 17.5 cm high) where the experimental bird was
185 introduced. The central box contained a compartment (14 x 19 x 17.5 cm) fitted with a fan
186 that drew the air from the device, thus, creating a low-noise, controlled airflow (Fig. 1).
187 The central box was flanked by two lateral choice chambers where chemical stimuli were
188 located (see below). For each test, a focal bird was introduced in the central box and

189 maintained in darkness for 5 minutes. Then the lateral doors of the central chamber (9 x 9
190 cm) were simultaneously opened giving the bird access to the two choice chambers (43 x
191 23 x 16 cm). Each choice chamber was divided into two sectors with screens (Fig. 1). The
192 farther sectors of the choice chambers contained two small cages where donor birds of the
193 corresponding sex were situated. Both the doors communicating the central chamber with
194 the choice chambers and the screens creating the sectors were made of a dense plastic
195 mesh that allows air flow but prevents the birds from seeing through them. The device was
196 only opened at the farthest walls of the choice chambers to allow air flow. The fan created
197 one constant air flow through each of the donor birds' cages and into the central chamber,
198 so the focal bird received two separate draughts, each one with the scent of the
199 corresponding donor bird. Donor birds were in darkness and in a reduced space, so they
200 did not move or call. Therefore, the experimental bird received the smell of the donor birds
201 without watching or hearing them. The room where the experiment was performed was in
202 complete silence so the experimenter could perceive any noise coming from the device. In
203 all the experiments the side of the chamber where the stimuli were presented was balanced.

204 We recorded as chosen the chamber into which the focal bird first entered after
205 remotely opening the doors. The validity of first choice as a measure of the interest of birds
206 to particular chemical stimuli has been previously demonstrated (e.g. Bonadonna & Nevitt
207 2004; Bonadonna et al. 2006; Amo et al. 2012). In order to minimize the length of the
208 trials, if the test bird did not leave the central chamber after one minute, we gently knocked
209 on the middle of the entry door of the central chamber to prompt it to move to one of the
210 choice chambers. Our records indicate that before knocking at the door, all birds were
211 looking towards the chamber they subsequently entered. The knocking on the door did not
212 affect the preference of birds neither in previous studies (Amo et al. 2012) nor in the
213 present experiment (see Results). The mean duration of the trials was 5 min 53 s (\pm 4 s).

214 The device carefully cleaned with a commercial antibacterial pH neutral soap and
215 allowed to air dry between trials; we used new stimuli on each trial and only one trial per
216 bird was performed each day. We tested the preferences of 18 focal males for the scent of a
217 conspecific male or female. Most males were first used as focal individuals and
218 subsequently as scent donors. We used 18 different pairs of scent donors (17 males and 7
219 females), thus on average females were used 2.6 times, whereas most males were used as
220 scent donors only once. In each trial, scent donor birds were located inside the small cages
221 a maximum of 15 minutes and focal birds were located in the olfactometric chamber a
222 maximum of 7 minutes. Inside the small cages, scent-donor birds were kept in darkness so
223 they were quiet and did not move or call during the trial. Also, during the first 5 minutes of
224 the trial, focal birds were also kept in darkness so they never called or fluttered. Birds did
225 not show signs of stress during the trials since they remained quiet and never fluttered. As
226 soon as the trial was over birds were returned to their cages where they resumed their
227 normal behaviour. The experiment was performed under licence of Secretaría del Medio
228 Ambiente y Recursos Naturales).

229

230 *Data Analysis*

231

232 We used STATISTICA 8.0 to build a generalized linear model with binomial errors and a
233 logit link function (GLZ) to analyse whether birds chose the side of the chamber
234 containing a male vs. a female (as a dichotomous variable (male [yes] vs. female [no]) in
235 relation to the side of the chamber where a particular sex was placed. Since some birds
236 entered one experimental chamber within one minute of the door being opened, but most
237 entered only after the door was gently tapped at the end of the first minute, we included
238 this period as a factor in a previous model in order to test whether there were differences in

239 the preferences of birds that made spontaneous choices versus those that needed
240 prompting.

241 *A posteriori*, we performed a Principal Component Analysis with three variables
242 indicating the condition of birds: body condition (weight/tarsus length), T-cell mediated
243 immune response, and YRC. We obtained a factor that combined the three variables (see
244 below) and we referred to it as quality. We calculated the difference in PC1 scores between
245 the focal and the scent-donor males (Δ quality; see below) and evaluated whether this
246 measure of the difference in quality affected the decision of the focal male to move
247 towards the source of male or female odour. The dichotomous (male = yes, female = no)
248 variable was analysed through a generalized linear model with binomial errors and a logit
249 link function (GLZ).

250

251 **RESULTS**

252

253 *Discrimination of the Sex of Conspecifics*

254

255 A similar number of focal males chose the side of the chamber containing the male ($N =$
256 10) and the female ($N = 8$) scent (*Wald Stat* = 0.22, *df* = 1, $P = 0.64$), a result that was not
257 influenced by the location in which the contrasting stimuli were presented (*Wald Stat* =
258 1.13, *df* = 1, $P = 0.29$). Only four birds readily entered an experimental chamber within one
259 minute of the door being opened, the rest entering after one minute had passed (mean time
260 before choosing \pm SE: 53 \pm 4 s). There were no differences in choice between birds that
261 responded within one minute of the door being opened and birds that took longer to
262 respond (*Wald Stat* < 0.001, *df* = 1, $P = 0.99$).

263

264 *A Posteriori Test: Relationship between Difference in Quality and Choice*

265

266 Body condition (loading = 0.68), T-cell mediated immune response (loading = 0.78) and
267 plumage colouration (loading = 0.62) were significantly correlated and combined in a
268 Principal Component Analysis that resulted in one axis explaining 48% of the variance
269 (eigenvalue = 1.45) and representing a gradient of individual quality, varying from low
270 quality individuals (in the positive end) to high quality individuals (in the negative end).
271 According to this gradient, high quality individuals had better body condition, redder
272 plumage colouration, and greater T-cell mediated immune response than low quality
273 individuals (see supplementary online material). Therefore, the difference (Δ quality)
274 between the scores of the focal male and the scent donor male was negative when the focal
275 male had better quality than the scent donor male, and positive when the focal male had
276 worse quality than the scent donor male.

277 The choice of males in the olfactometric chamber was significantly related to Δ
278 quality (*Wald Stat* = 4.53, *df* = 1, *P* = 0.03). When the focal male had better quality than
279 the scent donor male, it generally chose the side of the chamber containing the male,
280 whereas when the focal male had worse quality than the scent donor male, the focal male
281 usually chose the side of the chamber containing the female (Fig. 2). Similar results were
282 obtained when analysing separately each variable included in the quality index (body
283 condition and T-cell mediated immune response), except for red coloration (see
284 supplementary online material).

285

286 **DISCUSSION**

287

288 Results did not show a clear preference of male house finches for the scent of females or

289 males, because a similar number of birds chose the chamber containing birds of either sex.
290 However, this lack of preference does not seem to be due to a methodological artefact,
291 since results of another study using the same apparatus and experimental setup indicate
292 that *C. mexicanus* may use chemical information to detect a predator (Amo, L., López-
293 Rull, I., Pagán, I. & Macías Garcia, C., unpublished data), as was previously shown in this
294 (Roth et al. 2008) and other species (Amo et al. 2008, 2011; but see Johnson et al. 2011).
295 Those findings also show that our procedure of knocking at the door did not affect the
296 choice made by the birds, but merely prompted them to act according to whatever
297 information they had already gathered during the previous minutes (see also Amo et al.
298 2012 for another demonstration that knocking at the door prompts undecided starlings to
299 make the same choice that spontaneous males do).

300 Interpreting social scent preferences in a choice arena is difficult because, although
301 the study was performed during the reproductive period of the species when we may
302 expect a preference for the scent of potential partners, odour preferences may be related to
303 other behaviours that also take place during this period, such as territorial defence or
304 dominance interactions. This is in accordance with previous studies that have shown that,
305 in a context of mate-choice, male competition alters mating preferences (Wong &
306 Candolin 2005) both in birds (e.g. Holveck et al. 2011) and in other taxa (e.g. Makowicz et
307 al. 2010; Mautz & Jennions 2011). For example, intrasexual interactions may explain male
308 scent preferences found in previous studies. Whittaker et al. (2011a) found that dark eyed
309 junco males (*J. hyemalis*) were attracted to the scent of other males rather than to the
310 female scent, probably because juncos form socially monogamous territorial pairs, but
311 frequently engage in extra-pair copulations (25% of all offspring, Ketterson et al. 1997),
312 thus males may be more motivated to expel potential rivals than to search for a wandering
313 female. Similarly, the results of Amo et al. (2012) showing an attraction of spotless starling

314 (*S. unicolor*) males to the scent of other males (when offered both a male and a female
315 scent) tally with the fact that in the season when they were tested, males often engage in
316 aggressive intrasexual encounters to obtain a cavity for breeding. Therefore, the preference
317 of males for the scent of another male may be explained in terms of intrasexual
318 competition. Male interest in the scent of other males has also been demonstrated in
319 crested auklets (Jones et al. 2004) where both sexes are significantly attracted to scented
320 male models, but the response of males, that fight for nest sites (Hagelin 2007b), is
321 stronger than that of females. Thus male interest in male scents seems common in birds
322 (and indeed in other organisms such as mice, where males are also attracted to the scent
323 marks of other males; Arakawa et al. 2008). Yet this is not the rule; male budgerigars did
324 not exhibit any preference when exposed to the scent of males and females (Zhang 2011),
325 and Bonadonna et al. (2009) found that Antarctic prions cannot distinguish the sex of a
326 conspecific from its odour during the incubation period, despite the fact that they are able
327 to recognize the scent of their partner (Bonadonna & Nevitt 2004). Therefore, previous
328 studies of sex discrimination in birds via olfaction have found either that males are
329 attracted to the scent of other males, or that they do not exhibit a chemical preference for
330 either sex, but no study has found bird male attraction to female scent. In contrast, studies
331 that have analysed mating preferences in birds based on other kind of stimuli, such as
332 visual or auditory, have found no male preference for other males, but a clear preference
333 for females (e.g. Holveck et al. 2011). Differences in the preferences expressed by males in
334 studies examining olfactory, visual and/or auditory cues may greatly depend on the
335 experimental protocol, including the criterion to define preference; in olfactory studies this
336 is normally determined by the first choice (e.g. Whittaker et al. 2011a; Amo et al. 2012).
337 First choice is a good measure of the spontaneous interest of an animal for a particular cue,
338 whereas time spent close to the stimulus (e.g. Holveck et al. 2011) may be related to the

339 behaviour that takes place later on in the series of events triggered by the exposure to the
340 scent. Indeed, males exposed to male and female odours in an olfactometric device may
341 first approach the male and attempt to expel it, and only afterwards (when they realize that
342 they cannot expel the male or that the male may not interact with the female) they may
343 spend more time close to the female stimulus. Due to the use of living birds as scent
344 donors, the first choice was the best measure of the response of birds to the scents in our
345 study. However, more studies are needed to assess the subsequent response of birds to the
346 scent of males and females, using for instance uropygial gland secretions as scent sources.

347 We performed the experiment in April, within the species' breeding period, and
348 although pair formation in *C. mexicanus* can take place earlier in the USA (Thompson
349 1960), a previous study has found mating preferences during a long period of the breeding
350 season (from March to June; Hill 1990), and nest building in Mexico City continues
351 throughout July (Suárez-Rodríguez, M., López-Rull, I. & Macías Garcia, C., unpublished
352 data). Therefore, we expected that birds were in full breeding condition, and motivated to
353 look for partners. However, males were not attracted to the scent of females, nor did they
354 choose the scent of males, as other birds do (Whittaker et al. 2011a; Amo et al. 2012).
355 Instead, the response of males to the chemical cues of conspecifics appears to depend on
356 the breeding system. *Carpodacus mexicanus* is socially monogamous, and although house
357 finches do not defend territories during the breeding season, they may incur in agonistic
358 interactions with conspecifics to defend their mate from potential rivals (Thompson 1960).
359 This may explain why not all males moved towards the male scent; our focal subjects
360 rather behaved as if the chemical cues from conspecifics provided more information to
361 decide a course of action than merely the gender of the individual.

362 In an *a posteriori* analysis we investigated the choice of males in terms of their
363 quality relative to the quality of the scent-donor, and thus, potentially rival male. Results of

364 the PCA showed that males with redder coloration have better body condition and T-cell
365 mediated immune response than less red males. The difference in quality between focal
366 and scent donor males influenced the choice of focal males, with unpaired males with
367 better quality than scent donor males approaching rival males, and focal males in worse
368 condition approaching the female. An extensive body of research has shown that plumage
369 coloration is an honest signal of quality in male house finches, and that it is selected by
370 females (Hill 1990, 1991). Plumage coloration in this species is a condition-dependent trait
371 and only males with access to large quantities of carotenoids, which avoid disease and are
372 in good nutritional condition, can produce the reddest and more saturated plumage
373 coloration (Hill 2002). Previous studies have shown that during the non-breeding period,
374 drab males are dominant over redder males in a foraging context. Yet motivation to
375 compete for food may be higher in the drab males precisely because they are in bad
376 condition; they cannot overcome that situation and eventually compete for breeding
377 opportunities unless they improve –through foraging- their current condition. This
378 dominance of drab males may not be maintained during the breeding period, as colourful
379 males, with redder plumage, are also more likely to acquire mates, begin breeding earlier,
380 and fledge more offspring in a year (reviewed in Hill 2002). However, because birds grow
381 feathers during periodic moults, plumage coloration may reflect more accurately the
382 condition of males during the moulting period than afterwards, and thus it may not reflect
383 actual body condition and quality of males so precisely as other cues that are constantly
384 produced, such as chemicals. While the factor obtained by the PCA combined all our
385 indexes of individual quality (body condition, immune response and coloration), red
386 coloration made the smallest (but still large) contribution. In fact, this variable did not
387 seem to influence the choice of males when analysing separately how each condition-
388 related variable affected the choice of males (see supplementary material). By contrast,

389 differences in the two real-time indexes of condition; T-cell mediated immune response
390 and body condition, seem to influence the choice of the focal male (see supplementary
391 online material). Therefore, unpaired males, at least in the presence of a female, were more
392 willing to be involved in competition with other males when those potential rivals were of
393 worse quality, especially in terms of body condition and immune response. However, when
394 the experimental male was of worse quality than the scent donor male, it opted to approach
395 the female instead of moving towards a potential rival. Our results suggest that males may
396 obtain information via olfaction about the quality of potentially rival males and use it to
397 decide whether to be involved in an intraspecific and probably aggressive interaction or to
398 avoid it.

399 We used live birds as source of odour, but it is likely that the uropygial gland
400 secretions were responsible for our results, as these are the main source of bird odour, and
401 previous studies suggest that they have the potential to contain information about a variety
402 of attributes (e.g. Whittaker et al. 2011a). Several reports have indicated that both the
403 endocrine activity of the uropygial gland (considered the main odour source in most bird
404 species, Hagelin & Jones 2007) and the chemistry of its secretions (reviewed in Campagna
405 et al. 2012) vary among ages, sexes and seasons (e.g. Amo et al. 2012). It is also related to
406 the diet (e.g. Sandilands et al. 2004a, b) and to hormonal levels (e.g. Whelan et al. 2010;
407 Whittaker et al. 2011b). Therefore a bird's odour may be signalling not only its sex, but
408 also its age, reproductive status and body condition. Bird scent also differs between
409 populations (Whittaker et al. 2010) and individuals; for example, individual dark-eyed
410 juncos (*J. hyemalis*) differ quantitatively in the relative concentrations of the volatile
411 compounds of the uropygial gland secretion (Soini et al. 2007; Whittaker et al. 2010,
412 2011a). More recently it has been shown that semiochemical profiles are correlated with
413 heterozygosity both in male and female black-legged kittiwakes (*Rissa tridactyla*), setting

414 the scenario for the existence of odour-based mate-choice in birds (Leclaire et al. 2011b).
415 Indeed in other taxa such as mammals and fish, scents have been shown to vary between
416 individuals and to reveal body condition, parasite load, health state and even genetic
417 compatibility (e.g. major histocompatibility complex, Brennan & Keverne 2004; Milinski
418 2006). Therefore, odours may be used in intrasexual interactions to assess the dominance
419 status of rivals (e.g. Arakawa et al. 2008; Mason & Parker 2010) and / or to select potential
420 partners (Johansson & Jones 2007; Thomas 2011). Given that this occurs in other
421 vertebrate classes, it is likely that scent might also be used in birds for individual
422 recognition and assessment. In the case of dark eyed juncos mentioned above, individual
423 differences in the composition of the uropygial gland secretion were related to male size
424 (Whittaker et al. 2011a), and females exhibited a preference for the scent of smaller males
425 over that of larger males. Condition-dependent mate choice has also been reported in zebra
426 finch (*Taeniopygia guttata*) females exposed to acoustic male cues (Holveck & Riebel
427 2010).

428 A recent study by López-Rull et al. (2010) showed that the uropygial secretion
429 plays a role in plumage signalling by updating the signal value of the house finch male red
430 feathers. This may relate to the fact that the uropygial secretion inhibits the growth of
431 feather-degrading bacteria (Shawkey et al. 2003), thus males in better condition would
432 have a tighter control of the bacterial populations. Assuming that the same secretion is
433 responsible for the scent of *C. mexicanus*, our results now also suggest that the products of
434 the uropygial gland may not only be useful in updating feather coloration or protecting
435 against feather degrading bacteria (an attribute which might also be ascertained from their
436 odour), but additionally may indicate other aspects of the current quality of males. This is
437 consistent with our knowledge of the chemical complexity of the secretion in this species
438 (Haribal et al. 2005), although we also need to know how much (and how) it varies

439 between individual house finches before we can evaluate this hypothesis.

440 In summary, our results suggest that chemical cues in house finches may be related
441 to the quality of the individuals, as male scent preferences are related to body condition
442 and immune response of the bird used as source of odour, and may be useful in the
443 assessment of potential competitors. Further experiments are needed to corroborate our
444 correlative study and to demonstrate a role of bird scent in signalling quality.

445

446

447 **Acknowledgements**

448

449 We specially thank Simon Griffith and two anonymous referees for their useful comments.

450 We thank E. Ávila and Roxana Torres for logistic support, and Frida Somohano for
451 carefully reviewing the English. L.A. was supported by a DGAPA-UNAM postdoctoral
452 fellowship and by the JdC programme while writing, and I.L.R was supported by a
453 postdoctoral fellowship from CSIC-UNAM (Spain-Mexico).

454

455 **References**

456

457 **Amo, L., Galván, I., Tomás, G. & Sanz, J. J.** 2008. Predator odour recognition and
458 avoidance in a songbird. *Functional Ecology*, **22**, 289–293.

459 **Amo, L., Visser, M. E. & van Oers, K.** 2011. Smelling out predators is innate in birds.
460 *Ardea*, **99**, 177–184.

461 **Amo, L., Avilés, J. M., Parejo, D., Peña, A., Rodríguez, J. & Tomás, G.** 2012. Sex
462 recognition by odour and variation in the uropygial gland secretion in starlings.
463 *Journal of Animal Ecology*, **81**, 695–613.

- 464 **Arakawa, H., Blanchard, D. C., Arakawa, K., Dunlap, C. & Blanchard, R. J.**
465 2008. Scent marking behavior as an odorant communication in mice. *Neuroscience*
466 & *Biobehavioral Reviews*, **32**, 1236–1248.
- 467 **Bonadonna, F. & Nevitt, G. A.** 2004. Partner-specific odour recognition in an Antarctic
468 seabird. *Science*, **306**, 835.
- 469 **Bonadonna, F., Villafane, M., Bajzak, C. & Jouventin, P.** 2004. Recognition of burrow's
470 "olfactory signature" in blue petrels, *Halobaena caerulea*: an efficient
471 discrimination mechanism in the dark. *Animal Behaviour*, **67**, 893–898.
- 472 **Bonadonna, F., Caro, S., Jouventin, P. & Nevitt, G. A.** 2006. Evidence that blue petrel,
473 *Halobaena caerulea*, fledglings can detect and orient to dimethyl sulfide. *Journal of*
474 *Experimental Biology*, **209**, 2165–2169.
- 475 **Bonadonna, F., Caro, S. P. & Brooke, M. de L.** 2009. Olfactory Sex Recognition
476 Investigated in Antarctic Prions. *PLoS ONE*, **4**, e4148.
- 477 **Bonadonna, F. & Sanz-Aguilar, A.** In press. Kin recognition and inbreeding avoidance in
478 wild birds: the first evidence for individual kin-related odour recognition. *Animal*
479 *Behaviour* (2012) <http://dx.doi.org/10.1016/j.anbehav.2012.06.014>.
- 480 **Brennan, P. A. & Keverne, E. B.** 2004. Something in the air? New insights into
481 mammalian pheromones. *Current Biology*, **14**, R81–R89.
- 482 **Campagna, S., Mardon, J. A., Celerier, A. & Bonadonna, F.** 2012. Potential
483 semiochemical molecules from birds: a practical and comprehensive compilation of
484 the last 20 years studies. *Chemical Senses*, **37**, 3–25.
- 485 **Caro, S. P. & Balthazart, J.** 2010. Pheromones in birds: myth or reality? *Journal of*
486 *Comparative Physiology A*, **196**, 751–766.
- 487 **Caspers, B. A. & Krause, E. T.** 2011. Odour-based natal nest recognition in the zebra
488 finch (*Taeniopygia guttata*), a colony-breeding songbird. *Biology Letters*, **7**, 184–

- 489 186.
- 490 **Coffin, H., Watters, J. & Mateo, J.** 2011. Odor-based recognition of familiar and related
491 conspecifics: A first test conducted on captive Humboldt penguins (*Spheniscus*
492 *humboldti*). *PLoS ONE*, **6(9)**, e25002.
- 493 **Hagelin, J. C., Jones, I. L. & Rasmussen, L. E.** 2003. A tangerine-scented social odour
494 in a monogamous seabird. *Proceedings of the Royal Society of London, Series B*,
495 **270**, 1323–1329.
- 496 **Hagelin, J. C.** 2007a. Odors and chemical signaling. In: *Reproductive Biology and*
497 *Phylogeny of Birds* (Ed. by B. G. M. Jamieson), pp. 75–120. Enfield, NH: Science
498 Publishers.
- 499 **Hagelin, J. C.** 2007b. The citrus-like scent of crested auklets: reviewing the evidence for
500 an avian olfactory ornament. *Journal of Ornithology*, **148**, S195–S201.
- 501 **Hagelin, J. C. & Jones, I. L.** 2007. Bird odors and other chemical substances: a defense
502 mechanism or overlooked mode of intraspecific communication? *TheAuk*, **124**, 741–
503 61.
- 504 **Haribal, M., Dhondt, A. A., Rosane, D. & Rodriguez, E.** 2005. Chemistry of preen
505 gland secretions of passerines: different pathways to same goal? Why?
506 *Chemoecology*, **15**, 251–260.
- 507 **Hill, G. E.** 1990. Female house finches prefer colourful males: sexual selection for a
508 condition-dependent trait. *Animal Behaviour*, **40**, 563–572.
- 509 **Hill, G. E.** 1991. Plumage coloration is a sexually selected indicator of male quality.
510 *Nature*, **350**, 337–339.
- 511 **Hill, G. E.** 2002. *A red bird in a brown bag. The function and evolution of colourful*
512 *plumage in the house finch*. Oxford: Oxford University Press.
- 513 **Hirao, A., Aoyama, M. & Sugita, S.** 2009. The role of uropygial gland on sexual behavior

- 514 in domestic chicken *Gallus gallus domesticus*. *Behavioral Processes*, **80**, 115–120.
- 515 **Holveck, M. J. & Riebel, K.** 2010. Low-quality females prefer low-quality males when
516 choosing a mate. *Proceedings of the Royal Society of London, Series B*, **277**, 153–
517 160.
- 518 **Holveck, M. J., Geberzahn, N. & Riebel, K.** 2011. An experimental test of condition-
519 dependent male and female mate choice in zebra finches. *PLoS ONE* **6**(8), e23974.
- 520 **Jacob, J. & Zisweiler, V.** 1982. The uropygial gland. In: *Avian Biology*, vol. 6. (Ed. by D.
521 S. Farner, J. R. King & K. C. Parkes), pp. 199–314. New York: Academic Press.
- 522 **Johansson, B. G. & Jones, T. M.** 2007. The role of chemical communication in mate
523 choice. *Biological Reviews*, **82**, 265–289.
- 524 **Johnson, L. S., Murphy, S. M. & Parrish, G. W.** 2011. Lack of predator-odor detection
525 and avoidance by a songbird, the House Wren. *Journal of Field Ornithology*, **82**,
526 150–157.
- 527 **Jones, I. L., Hagelin, J. C., Major, H. L. & Rasmussen, L. E. L.** 2004. An experimental
528 field study of the function of crested auklet feather odor. *The Condor*, **106**, 71–78.
- 529 **Ketterson, E. D., Parker, P. G., Raouf, S. A., Nolan, V. Jr., Ziegenfus, C. & Chandler,**
530 **C. R.** 1997. The relative impact of extra-pair fertilizations on variation in male and
531 female reproductive success in dark-eyed juncos (*Junco hyemalis*). *Ornithological*
532 *Monographs*, **1997**, 81–101.
- 533 **Krause, E. T. & Caspers, B. A.** 2012. Are olfactory cues involved in nest recognition in
534 two social species of Estrildid finches? *PLoS ONE*, **7**(5), e36615.
- 535 **Krause, E. T., Krüger, O., Kohlmeier, P. & Caspers, B. A.** 2012. Olfactory kin
536 recognition in a songbird. *Biology Letters*, **8**, 327–329.
- 537 **Leclaire, S., Merkling, T., Raynaud, C., Giacinti, G., Bessièrè, J. M., Hatch, S. A. &**
538 **Danchin, E.** 2011a. An individual and a sex odor signature in kittiwakes? Study of

- 539 the semiochemical composition of preen secretion and preen down feathers.
540 *Naturwissenschaften*, **98**, 615–624.
- 541 **Leclaire, S., Merklings, T., Raynaud, C., Mulard, H., Bessière, J. M., Lhuillier, E.,**
542 **Hatch, S. A. & Danchin, E.** 2011b. Semiochemical compounds of preen secretion
543 reflect genetic make-up in a seabird species. *Proceedings of the Royal Society of*
544 *London, Series B*, **279**, 1185–1193.
- 545 **López-Rull, I., Pagán, I. & Macías Garcia, C.** 2010. Cosmetic enhancement of signal
546 coloration: experimental evidence in the house finch. *Behavioral Ecology*, **21**, 781–
547 787.
- 548 **Makowicz, A. M., Plath, M. & Schlupp, I.** 2010. Male guppies (*Poecilia reticulata*)
549 adjust their mate choice behaviour to the presence of an audience. *Behaviour*, **147**,
550 1657–1674.
- 551 **Mardon, J., Saunders, S. M., Anderson, M. J., Couchoux, C. & Bonadonna, F.** 2010.
552 Species, gender, and identity: cracking petrels' sociochemical code. *Chemical*
553 *Senses*, **35**, 309–321.
- 554 **Mason, R. T. & Parker, M. R.** 2010. Social behavior and pheromonal communication in
555 reptiles. *Journal of Comparative Physiology A*, **196**, 729–749.
- 556 **Mautz, B. S. & Jennions, M. D.** 2011. The effect of competitor presence and relative
557 competitive ability on male mate choice. *Behavioral Ecology*, **22**, 769–775.
- 558 **Maynard Smith, J. & Parker, G. A.** 1976. The logic of asymmetrical contests. *Animal*
559 *Behaviour*, **32**, 564–578.
- 560 **Milinski, M.** 2006. The major histocompatibility complex, sexual selection, and mate
561 choice. *Annual Review of Ecology, Evolution and Systematics*, **37**, 159–186.
- 562 **Reneerkens, J., Piersma, T. & Sinninghe Damsté, J. S.** 2002. Sandpipers (Scolopacidae)
563 switch from monoester to diester preen waxes during courtship and incubation, but

- 564 why? *Proceedings of the Royal Society of London, Series B*, **269**, 2135–2139.
- 565 **Roth, T. C. II, Cox, J. G. & Lima, S. L.** 2008. Can foraging birds assess predation risk by
566 scent? *Animal Behaviour*, **76**, 2021–2027.
- 567 **Sandilands, V., Powell, K., Keeling, L. & Savory, C. J.** 2004a. Preen gland function in
568 layer fowls: factors affecting preen oil fatty acid composition. *British Poultry*
569 *Science*, **45**, 109–115.
- 570 **Sandilands, V., Savory, J. & Powell, K.** 2004b. Preen gland function in layer fowls:
571 factors affecting morphology and feather lipid levels. *Comparative & Biochemical*
572 *Physiology A*, **137**, 217–225.
- 573 **Shawkey, M. D., Pillai, S. R. & Hill, G. E.** 2003. Chemical warfare? Effects of uropygial
574 oil on feather-degrading bacteria. *Journal of Avian Biology*, **34**, 345–349.
- 575 **Smits, J. E., Bortolotti, G. R. & Tella, J. L.** 1999. Simplifying the phytohaemagglutinin
576 skin-testing technique in studies of avian immunocompetence. *Functional Ecology*,
577 **13**, 567–572.
- 578 **Soini, H. A., Schrock, S. E., Bruce, K. E., Wiesler, D., Ketterson, E. D. & Novotny, M.**
579 **V.** 2007. Seasonal variation in volatile compound profiles of preen gland secretions
580 of the dark-eyed junco (*Junco hyemalis*). *Journal of Chemical Ecology*, **33**, 183–
581 198.
- 582 **Thomas, M. L.** 2011. Detection of female mating status using chemical signals and cues.
583 *Biological Reviews*, **86**, 1–13.
- 584 **Thompson, W. L.** 1960. Agonistic behaviour in the house finch. Part I: Annual cycle and
585 display patterns. *Condor*, **62**, 245–271.
- 586 **Whelan, R. J., Levin, T. C., Owen, J. C. & Garvin, M. C.** 2010. Short-chain carboxylic
587 acids from gray catbird (*Dumetella carolinensis*) uropygial secretions vary with
588 testosterone levels and photoperiod. *Comparative Biochemical & Physiology B*, **156**,

589 183–188.

590 **Whittaker, D. J., Soini, H. A., Atwell, J. W., Hollars, C., Novotny, M. V. &**

591 **Ketterson, E. D.** 2010. Songbird chemosignals: volatile compounds in preen gland

592 secretions vary among individuals, sexes, and populations. *Behavioral Ecology*, **21**,

593 608–614.

594 **Whittaker, D. J., Richmond, K. M., Miller, A. K., Kiley, R., Burns, C. B., Atwell, J. W.**

595 **& Ketterson, E. D.** 2011a. Intraspecific preen oil odor preferences in dark-eyed

596 juncos (*Junco hyemalis*). *Behavioral Ecology*, **22**, 1256–1263.

597 **Whittaker, D. J., Soini, H. A., Gerlach, N. M., Posto, A. L., Novotny, M. V. &**

598 **Ketterson, E. D.** 2011b. Role of testosterone in stimulating seasonal changes in a

599 potential avian chemosignal. *Journal of Chemical Ecology*, **37**, 1349–1357.

600 **Wong, B. B. M. & Candolin, U.** 2005. How is female mate choice affected by male

601 competition? *Biological Reviews*, **80**, 559–571.

602 **Zhang, J. X., Wei, W., Zhang, J. H. & Yang, W. H.** 2010. Uropygial gland-secreted

603 alkanols contribute to olfactory sex signals in budgerigars. *Chemical Senses*, **35**,

604 375–382.

605 **Zhang, J. X.** 2011. An approach to search for putative pheromones in birds via chemical

606 analysis - A reply to Mardon J, Saunders SM, and Bonadonna F. *Chemical Senses*,

607 **36**, 5–7.

608

609

610 **Figure legend**

611 **Figure 1.** Schematic representation of the olfactometric device used. A focal bird was
612 introduced into the central chamber and exposed to scent-carrying air flowing in (arrows)
613 from the choice chambers. Odour donors were hidden from the focal bird's view behind a
614 dense plastic mesh (fine cross hatching) and kept in darkened cages to soothe them. A
615 choice was scored when the focal bird entered one of the choice chambers.

616

617 **Figure 2.** Relationship between the difference in quality between the focal male and the
618 scent-donor male and its choice of the scent of a conspecific male or a female.

619

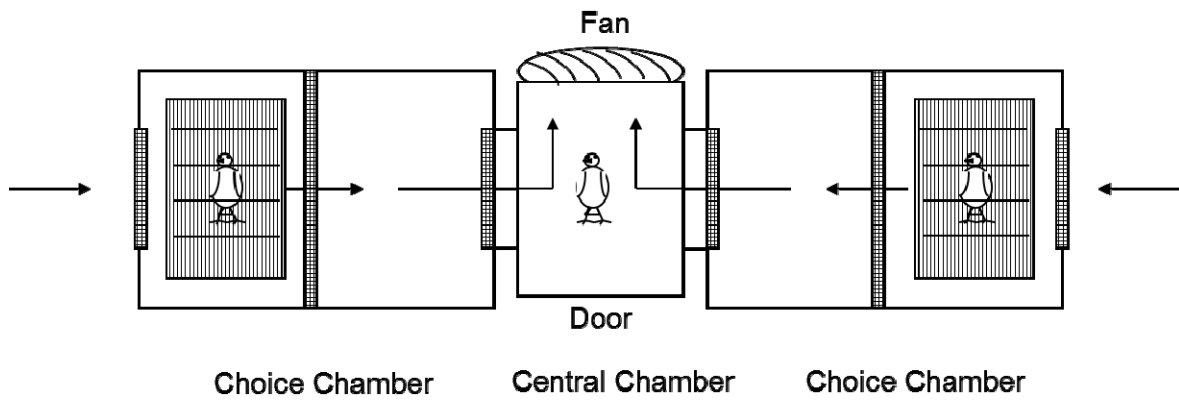
620 **Figure A1.** Relationship between the difference in a) body condition, b) T-cell mediated
621 immune response and c) red coloration between the focal male and the scent-donor male
622 and its choice of the scent of a male or a female conspecific.

623

624

625 Fig. 1.

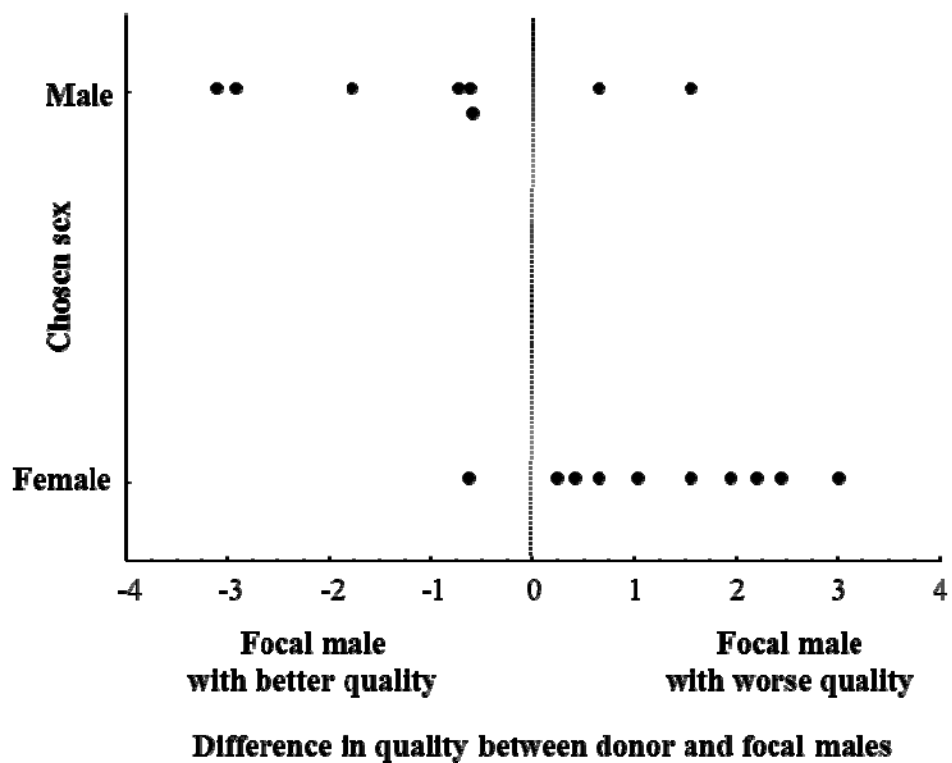
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629 Fig. 2

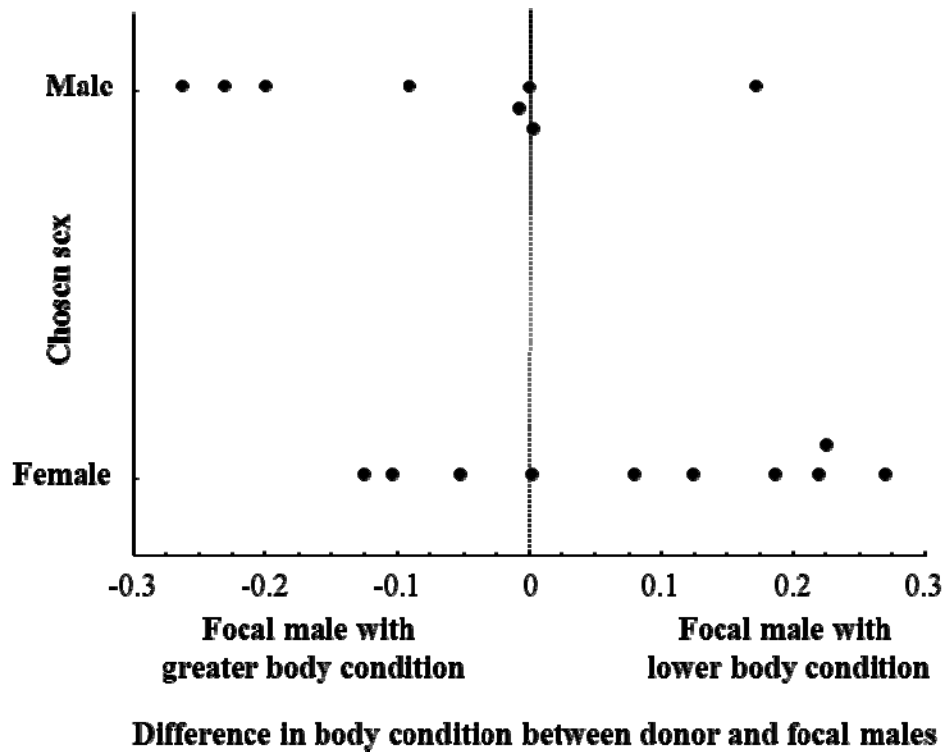


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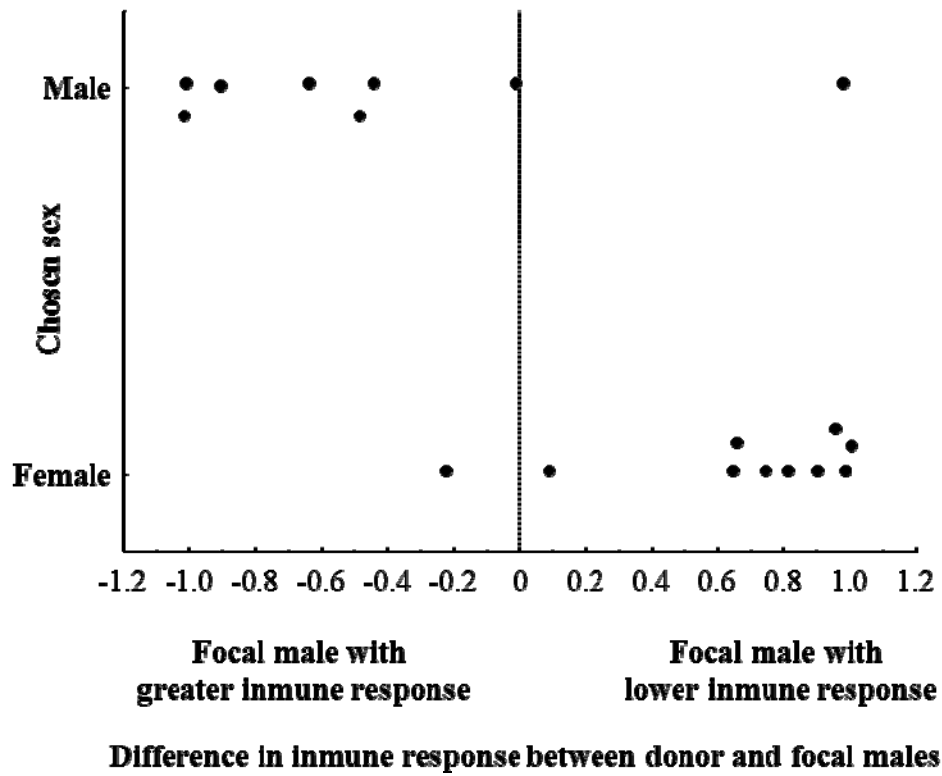
632 Fig. A1

633 a)



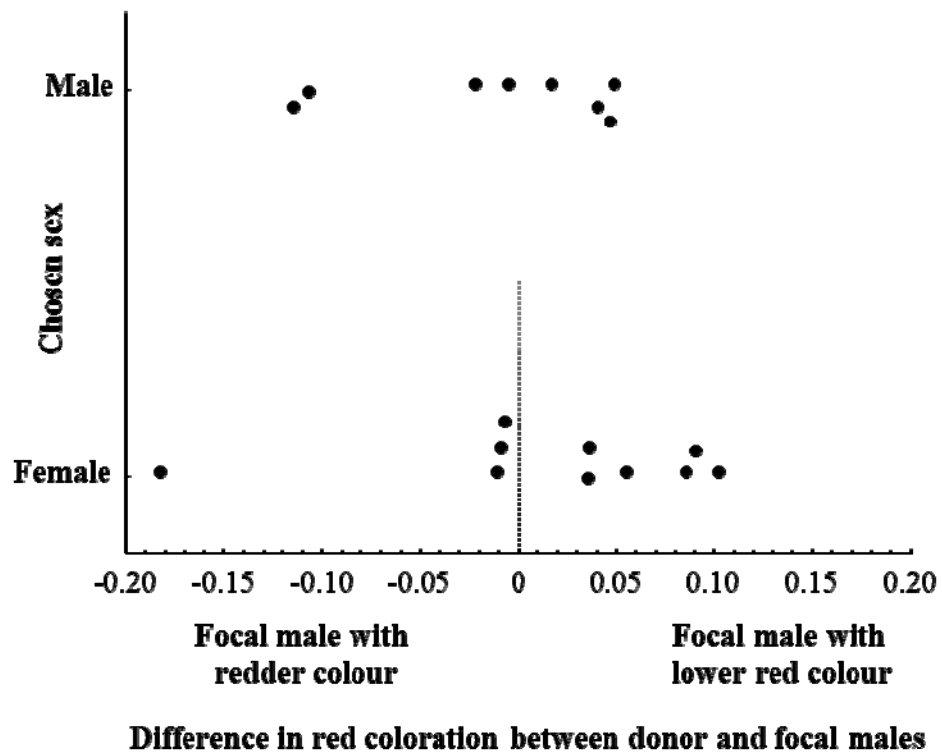
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635 b)



636

637 c)



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 639
 640

641 **Appendix: Supplementary material**

642

643 Data Analysis

644 We analysed separately whether the difference between the focal male and the scent donor
645 male in the variables indicating male quality (body condition, T-cell mediated immune
646 response and red coloration) influenced the decision of the focal male to move towards the
647 source of male or female odour. We used STATISTICA 8.0 to build a generalized linear
648 model with binomial errors and a logit link function (GLZ) to analyse whether males chose
649 the side of the chamber containing a male vs. a female (as a dichotomous variable (male
650 (yes) vs. female (no)) in relation to the difference between the focal and the scent-donor
651 males in a) body condition, b) T-cell mediated immune response and c) red coloration.

652

653 Results

654 Results of the relationship between the difference in quality between males and the choice
655 of the focal male were similar when analysing separately the variables indicating male
656 quality, except for red coloration, and when analysing the PCA factor combining such
657 variables.

658 The choice of males in the olfactometric chamber was related to the Δ body
659 condition, although differences only approached significance levels (*Wald Stat* = 3.62, *df* =
660 1, *P* = 0.057; Fig. A1a). When the focal male had better body condition than the scent
661 donor male, it generally chose the side of the chamber containing the male, whereas when
662 the focal male had worse body condition than the scent donor male, the focal male
663 normally chose the side of the chamber containing the female.

664 The choice of males was significantly related to the Δ T-cell mediated immune
665 response (*Wald Stat* = 6.16, *df* = 1, *P* = 0.01; Fig. A1b). When the focal male exhibited a

666 greater response to the injection of an antigen than the scent donor male, it generally chose
667 the side of the chamber containing the male, whereas when the focal male had lower T-cell
668 mediated immune response than the scent donor male, the focal male normally chose the
669 side of the chamber containing the female.

670 The choice made by males in the olfactometric chamber was not related to the Δ red
671 coloration (*Wald Stat* = 0.77, *df* = 1, *P* = 0.38; Fig. A1c).

672

673