

# The role of genetic constraints and social environment in explaining female extra-pair mating

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## 1 **Abstract**

2 Why females of socially monogamous species copulate with males other than their partner has  
3 been a long-standing, unresolved puzzle. We previously reported that female promiscuity  
4 appears to be a genetic corollary of male promiscuity (intersexual pleiotropy hypothesis). Here  
5 we put this earlier finding to a critical test using the same population of zebra finches  
6 *Taeniopygia guttata*. After three generations of artificial selection on male courtship rate, a  
7 correlate of extra-pair mating, we assess whether female promiscuity changed by indirect  
8 selection and we re-examine the crucial genetic correlations. Our new analyses with  
9 substantially increased statistical power clearly reject the hypothesis that male and female  
10 promiscuity are genetically homologous traits. Our study highlights that individual females show  
11 low repeatability in extra-pair mating behavior across different social environments. This  
12 emphasizes the potential importance of pair bond strength and the availability of favored extra-  
13 pair males as factors explaining variation in patterns of female promiscuity.

14

## 15 **Introduction**

16 Why females in socially monogamous species actively engage in matings outside the pair bond  
17 is a long-standing, intriguing question [1-5]. Mating outside the pair bond is obviously adaptive  
18 for males (i.e. benefits from this behavior will typically outweigh costs), because it leads to  
19 additional offspring that are raised by another pair, and hence directly increases male fitness [6,  
20 7]. However, why females engage in extra-pair copulations is more puzzling: promiscuous  
21 behavior does not increase the number of offspring females can produce and is associated with  
22 costs such as increased predation risk, increased risk of contracting sexually transmitted  
23 diseases, reduced paternal care and punishment by the social mate [1, 4]. In birds, more than 90%  
24 of species breed in socially monogamous pairs, but extra-pair paternity is common [2, 8]. Birds  
25 have served as paragons for studying the evolution of female promiscuity, because males  
26 typically cannot force copulations and females often actively seek extra-pair copulations [9-11].  
27 The majority of studies tried to explain the occurrence of female extra-pair mating behavior by  
28 highlighting the potential benefits [1, 2, 12]. These included indirect genetic [13-15] as well as  
29 direct ecological benefits [8, 16, 17]. Yet, despite much empirical work, the general support for  
30 these adaptive scenarios remains limited [4, 18-21]. Therefore, alternative, non-adaptive  
31 explanations deserve attention [12].

32 Several hypotheses of 'genetic constraint' have been proposed to solve the evolutionary puzzle  
33 of apparent non-adaptive female extra-pair behavior [22, 23]. These hypotheses assume that  
34 promiscuous behavior is heritable and state that the alleles underlying female promiscuity are  
35 maintained in the population, because they have additional pleiotropic effects that are

36 beneficial to at least one sex. Depending on whether the pleiotropic effect is expressed in males  
37 or females, two types of hypotheses can be distinguished.

38 (1) The hypothesis of ‘intersexual pleiotropy’ proposes that female and male promiscuity are  
39 homologous traits that are affected by the same sets of genes [22]. Alleles that increase  
40 promiscuity will be maintained in the population due to positive selection in males. When  
41 inherited to a daughter, these alleles will cause female promiscuity even if this behavior is not  
42 adaptive for females. This hypothesis requires a positive genetic correlation between measures  
43 of female and male promiscuity (i.e. positive cross-sex genetic covariance).

44 (2) The hypothesis of ‘intrasexual pleiotropy’ posits that female promiscuity is maintained  
45 because its causal alleles have pleiotropic effects on other female traits that are under positive  
46 selection [11, 23]. For example, female responsiveness to male courtship might be genetically  
47 linked to female fecundity, because courtship may proximately stimulate egg production [24].  
48 Alternatively, genetic variants underlying increased female sexual responsiveness towards her  
49 social mate may be favored by selection because low responsiveness can lead to infertility and  
50 hence reduced fitness [23]. Positive selection on alleles for increased responsiveness towards  
51 the social mate could then lead to increased female responsiveness towards extra-pair males as  
52 well. This hypothesis requires that female promiscuity is positively genetically correlated to  
53 either female fecundity or to female responsiveness towards her social mate (i.e. within-sex  
54 genetic covariance).

55 Empirical testing of these hypotheses using field data on extra-pair paternity is difficult, because  
56 heritability of male and female promiscuity is low [25-28]. The main problem is that the realized

57 patterns of paternity also depend on factors other than the intrinsic inclination of an individual  
58 to seek extra-pair copulations, such as sperm competition and mate guarding.

59 In an earlier study on captive zebra finches [29], we combined data on realized levels of extra-  
60 pair paternity with detailed observations on behaviors that reflect an individual's propensity to  
61 engage in extra-pair mating. We found strong, positive genetic correlations between male and  
62 female measures of extra-pair mating behavior, supporting the 'intersexual pleiotropy'  
63 hypothesis. We rejected the 'intrasexual pleiotropy' hypothesis, because the genetic correlation  
64 between responsiveness to the partner and responsiveness to extra-pair males did not differ  
65 from zero. Our study thus suggested that female promiscuity can be changed indirectly by  
66 artificially selecting males for increased or reduced courtship rate, a genetic correlate of male  
67 extra-pair siring success and of female promiscuity.

68 The present study reports on the results of such an artificial selection experiment. Using the  
69 birds from the initial study, we set up two replicate lines for high male courtship rate, two  
70 replicate lines for low courtship rate and two unselected control lines. Increasing the genetic  
71 variance in male courtship rate allowed us to test with increased statistical power whether  
72 female extra-pair mating behavior is indeed genetically linked to male courtship rate. Based on  
73 our previous results, we predicted that the level of female promiscuity would change indirectly  
74 by selection imposed on male behavior only.

75 This study also amends a weakness of the initial study: previously, we measured the behavior of  
76 a female only once, in the context of being paired to the partner she had chosen in an  
77 experiment. The observed behavior was then assumed to be representative for that female.  
78 However, extra-pair behavior might also have been a property of the female's social

79 environment (e.g. strength of the social pair bond, characteristics of the available extra-pair  
80 males). To resolve this, we here measured extra-pair behavior of each female with two  
81 successive partners. This allows to assess the amount of variation that is due to the female and  
82 to the environment. Thus, we examine the repeatability of female promiscuity and quantify its  
83 heritability and genetic covariance with other traits.

84 To examine the ‘intersexual pleiotropy’ hypothesis, we quantified the sign and strength of the  
85 genetic correlations between measures of female promiscuity and two measures of male sexual  
86 behavior, namely (1) male courtship rate (under artificial selection), and (2) male success in  
87 siring extra-pair eggs. To test the ‘intrasexual pleiotropy’ hypothesis, we quantified the  
88 correlations between female promiscuity and (3) female responsiveness towards her social  
89 mate, and (4) measures of total female fecundity.

## 90 **Results**

### 91 **Selection lines for male courtship rate**

92 We established six selection lines and bred them over three consecutive generations: two lines  
93 selected for high male courtship rate, two for low courtship rate, and two unselected control  
94 lines. Figure 1 shows, for each generation, the actual phenotypes (courtship rate) of all male  
95 offspring that were bred as a function of the mean breeding value of their parents (i.e. as a  
96 function of the predicted offspring phenotypes based on a genetic model that includes the  
97 observed phenotypes of parents and their relatives). The slope of the regression lines is close to  
98 unity, indicating that the offspring generations behaved as predicted by the genetic model. With  
99 each generation, we chose parents with even more extreme breeding values, as reflected by the  
100 outward movement of the high and low lines along the x-axis over progressive generations

101 (Figure 1A-C). In consequence, the offspring phenotypes became progressively differentiated  
102 along the y-axis between the selection lines. After three generations of selection, the average  
103 difference between the high and the low lines reached 2.4 phenotypic standard deviations  
104 (Cohen's  $d$ ; [30]). The two replicates of each type of line behaved almost identically (Figure 1,  
105 Table S3).

## 106 **Indirect response to selection**

107 We assessed whether the successful selection on male courtship rate resulted in correlated  
108 changes in levels of extra-pair paternity in both sexes. To this end, we put equal numbers of  
109 males and females from the three types of selection lines in communal aviaries, noted pair  
110 formation and let the birds breed for two breeding rounds, each lasting seven weeks during  
111 which females laid up to three clutches. We then quantified for each individual the level of  
112 extra-pair paternity.

113 During the time they were monogamously paired, 190 females produced 2,951 fertile eggs, 726  
114 of which (24.6%) were sired by extra-pair males. Levels of extra-pair paternity (% of extra-pair  
115 young in all broods) ranged from on average 37.4% in line 1 'high' to 15.8% in line 2 'low', with  
116 the other four lines showing intermediate levels (Figure 2). An analysis of individual levels of  
117 extra-pair paternity with selection regime (coded as a continuous variable: 1df; low = -1, control  
118 = 0, high = 1) as the predictor of interest, showed a significant effect ( $\beta = 0.698$ ,  $z = 3.1$ ,  $p =$   
119  $0.002$ ,  $n = 190$ , Table S4).

120 From the male perspective, 188 individuals sired 3,067 eggs during the time they were socially  
121 paired, 851 of which (27.7%) with females other than their social mate. The corresponding

122 average levels of extra-pair paternity (% of all young sired with extra-pair females) ranged from  
123 32.2% in line 2 'control' to 16.7% in line 2 'low' (Figure 2). Here, selection regime showed a non-  
124 significant trend in the expected direction ( $\beta = 0.278$ ,  $z = 1.7$ ,  $p = 0.09$ ,  $n = 188$ , Table S5).

## 125 **Repeatability of female promiscuity**

126 After one round of breeding and a break of two weeks (housing in unisex groups), we  
127 rearranged all individuals for a second breeding round. Each individual was then allowed to  
128 breed again for seven weeks with a different social mate and a different set of potential extra-  
129 pair mates. Estimates of repeatability for the female's responsiveness to courtship by extra-pair  
130 males ('female extra-pair response') and for the level of extra-pair paternity are relatively low  
131 (Figure 3A, B). This is confirmed by animal models showing that the random effect of social pair  
132 ('Pair ID') explained considerably more variance in measures of female promiscuity than the  
133 random effects that represent female identity (Figure 3C; animal models of 'Genetic' +  
134 'Permanent environment': Tables S6, S7, S10 to S15). In other words, a female's level of  
135 promiscuity is more consistent within a given context (social pair bond, set of extra-pair males)  
136 than between contexts (Figure 3C).

## 137 **Testing the 'intersexual pleiotropy' hypothesis**

138 Figure 4 shows the relationship between measures of female extra-pair behavior and the female  
139 breeding values for male courtship rate. All slopes are positive, but slopes based on data from  
140 the initial study (Figure 4A, B) are steeper than those based on data from the selection lines  
141 (Figure 4C, D), whereby the latter are the more powerful tests. The genetic correlations  
142 between measures of male and female promiscuity are presented in Figure 5; estimates of



143 between-sex genetic correlations from 5-trait animal models based on the initial data (Figure 5A  
144 Table S10, S11) are contrasted with estimates from models based on data from the lines  
145 artificially selected for high and low male courtship rate (Figure 5B; Tables S12, S13). The latter  
146 show between-sex genetic correlations close to zero for male courtship rate (median of four  
147 estimates:  $r_A = 0.04$ , Figure 5B; Table S18), and negative values (i.e. opposite to expectations)  
148 for male extra-pair siring success (median  $r_A = -0.34$ , Figure 5B; Table S18). These estimates  
149 stand in strong contrast to the positive estimates derived from the initial data (Figure 5A). An  
150 updated matrix of genetic correlations estimated from the joint data (initial plus selection lines)  
151 shows weakly positive genetic correlations that are not significantly different from zero (Figure  
152 5C; summary of Tables S6 to S9 showing medians of estimates from four types of models).

### 153 **Testing the ‘intrasexual pleiotropy’ hypothesis**

154 We found a moderately strong positive genetic correlation between female responsiveness to  
155 extra-pair male courtship (‘female extra-pair response’) and female fecundity (Figure 5D), yet its  
156 estimated strength varied considerably across different models (between 0.05 and 0.59, Table  
157 S14 to S17). Estimated genetic correlations between female extra-pair and within-pair response  
158 were weakly positive (Figure 5D), but also not robust (see Tables S14 to S17). Note that genetic  
159 correlations involving ‘female within-pair response’ are particularly difficult to estimate because  
160 the trait shows low repeatability across pair bonds (see Methods: Data Analysis: Female extra-  
161 pair and within-pair response).

### 162 **Discussion**

163 Overall, our data show high context-dependence of female promiscuity and more support for  
164 the ‘intrasexual pleiotropy’ hypothesis than for the hypothesis of ‘intersexual pleiotropy’. This  
165 study thus suggests that female promiscuity is an ‘independent trait’ of females rather than a  
166 ‘corollary’ of male promiscuity [31, 32].

167 The breeding of selection lines for male courtship rate was effective in maximizing the statistical  
168 power for testing whether measures of female promiscuity are genetically correlated with male  
169 courtship rate as a proxy of male promiscuity (see the increased data range in Figure 1 and  
170 Figure 4). Based on the most decisive test for such a genetic correlation (see ‘new data’ in Figure  
171 5B; Table S18), we reject the ‘intersexual pleiotropy’ hypothesis, despite weak supportive trends  
172 in the phenotypic data (Figure 2 and Figure 4D) and weak, positive correlations in the analysis of  
173 the joint data (Figure 5C). Statistical testing suggested a significant effect of the selection regime  
174 on female levels of extra-pair paternity, mostly stemming from reduced levels of extra-pair  
175 paternity in females from the two lines for low male courtship rate (Figure 2, Table S4).  
176 However, we base our conclusions on animal models that control for non-independence of  
177 individuals in the different selection lines via genetic relatedness (Figure 5).

178 We found a significant, positive genetic covariance between female responsiveness to extra-pair  
179 males (‘female extra-pair response’) and female fecundity, and a somewhat lower positive  
180 genetic covariance between female extra-pair responsiveness and her responsiveness to her  
181 social mate (‘female within-pair response’) (Figure 5D). This finding should be interpreted  
182 cautiously (given that the estimates did not seem robust, see also below) and deserves more  
183 study, in particular from populations of different species breeding in the wild.

184 Our study reveals strong context dependence of female extra-pair mating behavior: 'Pair ID'  
185 explained more variation than female identity (Figure 3, Tables S6, S7). This could reflect  
186 variation in the quality of the social pair bond, or in the set of available extra-pair males, which  
187 can be studied further under a social network framework [5].

## 188 **Comparison of the initial study with this study**

189 The conclusions from this study and from our earlier work [29] differ substantially. We discuss  
190 several potential explanations for this difference. First, the initial study was based on a smaller  
191 sample of 150 females. Hence, founder effects [33] may have resulted in some linkage  
192 disequilibrium between alleles for male and female promiscuity by chance alone. Such non-  
193 physical linkage may then have broken up during the breeding of selection lines. Second, the  
194 measures of female extra-pair behavior (mean phenotypes; y-axes in Figure 4) are noisier in the  
195 initial study than in this study, because in the latter they are based on two rounds of breeding  
196 with different social mates and a different set of potential extra-pair partners. Third, estimates  
197 of female breeding values for male courtship rate (x-axes in Figure 4) were based on half the  
198 number of male relatives in the initial study compared to this study, leading to higher error  
199 along the x-axis in the initial study. Updating the breeding value estimates of the females  
200 involved in the initial study with the new information on courtship rate of their sons, grandsons  
201 and great-grandsons, already leads to considerably shallower regression slopes in Figure 4A ( $\beta =$   
202 0.14) and 4B ( $\beta = 0.10$ ).

203 In conclusion, we suggest that the significant finding in our initial study [29] is a type I error  
204 resulting from relatively noisy data. There is no evidence that inadequate modelling caused the

205 difference, because updating the earlier models by including clutch and pair identity ('Clutch ID',  
206 'Pair ID') as additional random effects, did not alter the conclusions (see Figure 5A and Tables  
207 S10 and S11). Note that estimates from Bayesian models in MCMCgImm were smaller, had  
208 larger standard errors and were closer to estimates from the follow-up study than those from  
209 REML models in VCE (Table S18). This confirms the notion that the estimation of genetic  
210 correlations can be problematic when heritabilities are relatively low [34, 35] and sample sizes  
211 are limited.

## 212 **Future directions and conclusions**

213 Female fecundity was positively genetically correlated with measures of female promiscuity  
214 (Figure 5D), but Bayesian models in MCMCgImm again yielded more conservative estimates  
215 (median  $r_A = 0.14 \pm 0.21$ ) than REML models in VCE (median  $r_A = 0.59 \pm 0.20$ ). To assess whether  
216 genetic covariance with fecundity is a more general explanation for the persistence of female  
217 extra-pair mating, follow-up studies in the wild will be needed. Reid et al. [27] reported positive  
218 genetic covariance between female levels of extra-pair paternity and female annual  
219 reproductive success, but it is unclear whether this was due to variation in fecundity or variation  
220 in rearing success. If quantitative genetic analyses are not feasible because detailed pedigree  
221 information is not available, one could still examine whether there is a positive phenotypic  
222 correlation between clutch size and levels of extra-pair paternity. Such analyses, however,  
223 would need to take into account the mechanisms behind extra-pair paternity. For example, the  
224 probability of detecting extra-pair paternity (i.e. that an extra-pair copulation leads to a  
225 fertilization) might increase with clutch size. In field studies, it may also be important to control

226 for breeding density, because the latter may influence both the availability of extra-pair males  
227 and clutch size.

228 Our analyses of female extra-pair behavior across two social environments (Figure 3) revealed a  
229 substantial amount of context-dependence of this behavior. When considering levels of extra-  
230 pair paternity, the most influential factor was the identity of the social pair ('Pair ID', Figure 3C),  
231 indicating consistency across multiple clutches with the same partner and flexibility in behavior  
232 when breeding with different partners (Figure 3B). Such consistency at the level of the social  
233 pair rather than at the level of the female ('Female ID', Figure 3C) is consistent with findings in  
234 coal tits [36] and suggests that extra-pair paternity levels may vary with the strength of the  
235 social pair bond (e.g. behavioral compatibility of mates, as suggested by [37]). Similarly, a  
236 female's responsiveness to courtship of extra-pair males strongly depended on the combination  
237 of male and female identities, i.e. on who courted whom (coded as 'Pair ID' in Figure 3C; Tables  
238 S6, S7). Hence, the occurrence of promiscuous behavior may depend more strongly on aspects  
239 of compatibility between individuals. The dependence on the social context might reflect the  
240 quality of the social pair bond or the availability of specific extra-pair males, or both. The  
241 relative importance of these factors could be addressed by targeted experiments or by social  
242 network analyses [5].

243 Contrary to our previous claim, the artificial selection experiment showed that levels of female  
244 promiscuity cannot be altered by artificially selecting on the courtship rate of unpaired males (a  
245 correlate of extra-pair siring success that can be measured prior to pairing), at least not within  
246 the range covered by our selection lines. Nevertheless, this study suggests that models of  
247 genetic constraint remain in general a viable explanation for the persistence of female extra-

248 pair mating. All examined genetic correlations in Figure 5 (A and B) were positive (instead of 50%  
249 as expected from randomness). Examining these constraints in other study systems appears  
250 both promising and feasible.

## 251 **Methods**

### 252 **Subjects**

253 All study subjects come from a population of zebra finches that has been maintained at the Max  
254 Planck Institute for Ornithology in Seewiesen, Germany since 2004 (population # 18 in [38]).  
255 Housing conditions, diet and aviary specifications for breeding have been described in detail in  
256 the supplementary file to [39]. For this study, the pedigree of this population comprises eight  
257 generations: Parental, F1 to F4, and four generations of selection lines (S1 to S3, see below).

### 258 **Behavioral Observations**

259 We measured behavioral traits related to extra-pair mating under two experimental set-ups: (1)  
260 in cages, where behavior could be measured under standardized conditions, leading to high  
261 individual repeatability; (2) in aviaries, where individuals bred repeatedly and were exposed to  
262 different sets of potential extra-pair partners.

#### 263 **a) Cage Experiments on Unpaired Birds**

264 Before the formation of social pair bonds, we measured for each male in the population ‘male  
265 courtship rate’ (the trait subjected to artificial selection) towards an unpaired female introduced  
266 into his cage. We set up encounters between an unpaired male and an unpaired female that  
267 were unfamiliar to each other. Each encounter (‘trial’) lasted five minutes during which we  
268 recorded the total duration (in seconds) of male courtship, that is, song directed towards the

269 female. For each female, we scored her responsiveness to the male ('female unpaired response')  
270 during each encounter on a five-point scale following [11], where -1 represents a clear rejection  
271 (involving aggression, threat, or fleeing) and +1 a clear acceptance (involving copulation  
272 solicitation, beak wiping, and ritualized hopping) with intermediate scores (-0.5, 0, +0.5) given  
273 for weaker or mixed responses [11, 29]. For this study, we combined 3,776 trials from the initial  
274 study [29] and 3,014 trials on individuals from the selection lines (see below). In total, we  
275 obtained 6,786 measures of 'male courtship rate' (four encounters with missing data were  
276 excluded) and 5,039 measures of 'female unpaired response' (74% of all trials; responsiveness  
277 could not be scored in 1,751 trials, typically when there was no male display). The trials involved  
278 1,556 males and 1,441 females and were carried out between July 2002 and December 2013.  
279 Males encountered on average  $4.4 \pm 1.3$  SD (range 2-8) different females, and females  
280 encountered on average  $4.5 \pm 2.2$  SD (range 1-14) different males (Table S1).

### 281 ***Selection on Male Courtship Rate***

282 We established lines selected for divergent breeding values for male courtship rate, starting in  
283 2009 [some details see 40, 41].

### 284 ***Founder generation 'S0'***

285 Before initiating the breeding of selection lines, we measured the courtship rate of 585 males  
286 from four consecutive generations (P to F3, not including F4 birds) [29] in 2,922 trials. Using  
287 these measurements, we estimated breeding values for male courtship rate with a pedigree-  
288 based animal model. Breeding values of all individuals in the pedigree ( $n = 1219$  from P to F3,  
289 including females) were calculated using VCE 6.0.2 [42]. The single-trait permanent-  
290 environment animal-model was set up as follows. (1) 'Male courtship rate' was squared-root

291 transformed to approach normality and used as the response variable (Table S1). (2) Fixed  
292 effects were male test day (four levels, from day one to day four), time of day of the trial start  
293 (continuous, range: 8:51-18:19), male inbreeding coefficient F (continuous, range: 0-0.25) and  
294 rearing environment of the male (two levels, mixed-sex or unisex). (3) As random effects we  
295 included 'Animal' (additive genetic effect), 'Male ID' (permanent environment effect, 585 levels),  
296 'Female ID' (maternal effect, 203 levels), 'Test batch ID' (period of testing, 8 levels), and 'Cohort  
297 ID' (periods of breeding, 6 levels).

298 We started six breeding lines (two control, two high and two low lines) by choosing founder  
299 individuals with the estimated breeding values for courtship rate (see above) from the pool that  
300 were still alive in May 2009 (n = 773; see Table S19). For each line, we let 15 pairs breed in one  
301 of 90 randomly assigned cages (60×40×45cm) distributed over two breeding rooms (45 cages  
302 each). First, we randomly selected birds from the entire pool for the two control lines. Then, we  
303 selected 30 birds of each sex with the highest breeding values for courtship rate for the two  
304 'high' lines, and randomly allocated half of them to each replicate line. Thereafter, we also  
305 selected six 'replacement' individuals of each sex (in case a high line bird would die during  
306 breeding) with the next highest breeding values and distributed them randomly among the two  
307 lines. The two low lines were selected in the same manner, but using the birds with the lowest  
308 breeding values.

309 Within each line, the 15 breeding pairs were chosen in such a way as to minimize the level of  
310 inbreeding (see Table S19). Each pair was allowed to breed in two 'rounds' over a total period of  
311 about 14 months (from pair formation to independence of the last offspring). In each round, we  
312 allowed pairs to breed until we obtained about 50 juveniles from each line. After round one, we



313 redistributed the birds within each line such that they obtained a new partner (breeding cages  
314 again randomly assigned). In this way, we created maternal and paternal half sibs, which  
315 facilitated the separation of maternal effects from additive genetic effects. We placed juveniles  
316 (age: 35 to about 120 days) of each breeding round in one of two large, mixed-sex groups. Thus,  
317 across both rounds of breeding of each generation (S0, S1 and S2, details see below), roughly  
318 600 offspring were raised in four mixed-sex groups comprising roughly 75 males and 75 females  
319 from all lines.

### 320 ***Breeding generations 'S1' to 'S3'***

321 Birds of the S0 generation produced 568 offspring of which 546 survived until we started  
322 breeding the next generation (see Table S19). 'Male courtship rate' and 'female unpaired  
323 response' of these offspring were measured four times per individual (age of testing is given in  
324 Table S19). These new measurements were added to update the animal model (with the same  
325 fixed and random effects) for the calculation of predicted breeding values for all individuals (n =  
326 1,929). The new model included 4,362 measurements of courtship rate from 947 males.

327 We selected the S1 breeders (15 pairs plus five replacement birds of each sex in each line) as  
328 described above (random selection for control lines and based on breeding values for high and  
329 low lines; Table S19). Again, we assigned breeding pairs in such a way as to minimize and  
330 standardize the average inbreeding coefficient. Specifically, in the most inbred line (high 2), we  
331 minimized inbreeding, while in the other five lines we chose pairs to match the mean value for  
332 this line. The mean inbreeding coefficients of the resulting offspring for each line are given in  
333 Table S19. The following generations S2 and S3 were bred following the same principles (see  
334 Table S19 for summary statistics).

## 335 **b) Aviary Experiments of 'S3' Birds**

336 The S3 generation of the six selection lines consisted of 343 female and 338 male offspring,  
337 most of which had been phenotyped for 'male courtship rate' and 'female unpaired response' in  
338 the cage experiments (see Table S19). For a subset of 219 females and 217 males (about equally  
339 representing the six lines), we also measured other phenotypes directly linked to extra-pair  
340 mating.

341 Between January 2014 and May 2015, we set up 9 breeding aviaries equipped with cameras as  
342 described in [29] and let birds breed, as follows. We created four consecutive testing cohorts,  
343 each comprising 54 males and 54 females randomly drawn from the available pool of birds in  
344 each line (9 males and 9 females from each line per cohort, 216 of each sex in total, plus a few  
345 replacements, see below). Each group was distributed over the nine aviaries such that (1) all  
346 birds within an aviary were unfamiliar with each other and (2) each aviary contained one male  
347 and one female from each selection line. Due to a shortage of line 1 'low' and later also line 2  
348 'high' birds, we used individuals from line 2 'low' and line 1 'high' , respectively, in 11 out of 36  
349 rounds of breeding in aviaries. In all cases, aviaries contained 2 males and 2 females from each  
350 line type, but overall the number of tested birds per line and sex varied from 25 to 47 (Table  
351 S19).

352 With this setup, each individual had a choice of 6 potential mates. Social pairing appeared  
353 random with regard to line (details not shown). Each set of birds spent seven weeks in the  
354 aviary, during which most females laid three clutches; nest boxes were provided from day 1 to  
355 day 45. We collected all laid eggs for parentage assignment as soon as we found them and  
356 replaced them by plastic eggs. Clutches (of plastic eggs) were removed after 10 days of

357 incubation to encourage the female to lay the next clutch. On day 49, all individuals were  
358 separated by sex and placed into different rooms for a two-week period, after which we  
359 initiated an identical, second round of breeding with a different set of potential social and extra-  
360 pair partners (by swapping the six males of one aviary to the next). This allowed us (a) to  
361 quantify the repeatability of the measured traits with different partners, and (b) to disentangle  
362 effects of 'Female ID' from those of 'Male ID' and 'Pair ID'. In the second round, on average 25%  
363 of individuals were familiar to each other due to the joint rearing in one of four large natal  
364 groups. Overall, one male and three females died during the first breeding round and they were  
365 replaced by an individual from the same line in the second round, leading to a total of 217 males  
366 and 219 females participating in the experiments.

367 We fitted all breeding birds with randomly assigned colored leg bands for individual recognition  
368 and observed their behavior. Observations lasted about 30 min (for the 9 aviaries combined)  
369 and were carried out about 120 times per breeding round. We recorded all instances of  
370 "bonding behavior": allopreening, sitting in body contact or close to each other, and visiting a  
371 nest-box together. The start of a pair bond was defined as the day on which >50% of bonding  
372 behaviors were directed to a single male (with a minimum of eight observations on this female-  
373 male combination; see [43] for details).

374 Following the initial study [29], we used video cameras to monitor the birds' courtship behavior  
375 continuously in each aviary. Because courtship was most frequently observed in the early  
376 morning, we analyzed the first hour of recording on every day during the breeding period, plus  
377 another two randomly selected hours per day. In total, we screened 10,656 hours of video (3h x  
378 49.33 days x 9 aviaries x 2 breeding rounds x 4 testing cohorts) at 8-fold speed (equal numbers

379 of hours randomly allocated to two observers D.W. and K.M.), and detected a total of 33,003  
380 courtships. Of those, we scored ‘female extra-pair response’ based on 9,121 courtships of  
381 paired females by potential extra-pair males (involving 206 females) and ‘female within-pair  
382 response’ based on 13,268 courtships by the social partner (involving 200 females). For each  
383 courtship, a single person (K.M.) scored female responsiveness as in the initial study [29]: threat  
384 or aggression toward the male (-1), flying away (-0.5), mixed or ambiguous signs (0), courtship  
385 hopping and beak wiping (+0.5), and copulation solicitation (+1).

386 Data from the initial study consisted of 3,958 scores of ‘female extra-pair response’ (from 141  
387 females) and 4,601 scores of ‘female within-pair response’ (from 143 females; Table S1) [29].

### 388 **Paternity Analysis**

389 In total, we collected 4,041 eggs and placed them in an incubator for 4 days to obtain embryonic  
390 tissue for parentage analysis. We failed to analyze parentage for 685 eggs (14 eggs without yolk,  
391 24 broken eggs, 632 apparently infertile eggs and 15 lost samples or samples with too low DNA  
392 concentration). The remaining 3,356 eggs were unambiguously assigned to parents using 15  
393 microsatellite markers [39], but four eggs were only assigned to their mother (due to  
394 parthenogenesis, mosaicism, or siring by sperm from the previous experimental round).

395 We quantified the proportion of extra-pair young for each female (‘female EPP’) based on a  
396 subset of 2,951 eggs laid by paired females (726 eggs were sired by an extra-pair male, 24.6%).  
397 Similarly, we quantified male extra-pair siring success (‘male EPP’) as the number of eggs a male  
398 sired with a female other than its social mate (3,067 eggs, of which 851 were extra-pair sired,

399 27.7%; the total number of eggs is higher because it includes paired males siring extra-pair  
400 offspring with unpaired females).

401 Data from the initial study included 'female EPP' from 2,253 eggs laid by 149 females and  
402 measures of 'male EPP' from 152 males (Table S1) [29].

### 403 **Female Fecundity**

404 We quantified 'female fecundity' as described in [39]. In brief, 'female fecundity' is the total  
405 number of eggs laid by a female within one breeding round (45 days, see above), determined  
406 based on a combination of genetic assignment of maternity (3,356 eggs) and social assignment  
407 of eggs that could not be genotyped based on observations of nest attendance (610 eggs). For  
408 genotyped eggs, "social assignment" was correct in 93.1% of cases (false assignments resulted  
409 from egg dumping or nest take-over; [44]). Thus, assignment errors appear negligible compared  
410 to the error when omitting all non-genotyped eggs. In total, we obtained 432 estimates of  
411 female fecundity (216 females x 2 breeding rounds, involving 219 individuals) based on 3,966  
412 assigned eggs (mean  $\pm$  SD =  $9.2 \pm 5.1$ , range 0-22). To increase statistical power for quantifying  
413 genetic covariance between female fecundity and measures of promiscuity, we included data on  
414 female fecundity from seven other aviary breeding experiments with genetic parentage  
415 assignment (carried out between 2005 and 2017 and involving 6 generations, the same genetic  
416 population as the selection lines). This includes data from the first four breeding experiments  
417 used in the initial study [29]. Thus, we used a total of 854 fecundity estimates from 461  
418 individual females based on the assignment of 9,127 eggs (mean  $\pm$  SD =  $10.7 \pm 6.8$ , range 0-38).

419 We statistically accounted for potential differences between the eight breeding experiments  
420 (see below).

## 421 **Data Analysis**

422 Sample sizes and descriptive statistics of the data used for quantitative genetic analyses are  
423 given in Table S1 (including the data from the initial study, [29]). We used similar models as in  
424 the initial study, except that we included additional random effects (e.g. 'Pair ID' and 'Clutch ID')  
425 and modelled an effect as random instead of fixed (e.g. 'Test Batch ID'), where appropriate. To  
426 examine whether conclusions of the initial study depended on these decisions about model  
427 structure, we repeated the initial analyses with the updated model structure.

### 428 **a) Mixed-effect Models Testing Extra-pair Paternity Levels of the Selection Lines**

429 We tested whether individuals from the high lines had higher levels of extra-pair paternity than  
430 those from the low lines after three generations of selection on male courtship rate. We used  
431 mixed-effect models in the lme4 package in R 3.4.0 [45, 46] to test for differences in EPP levels  
432 across the six selection lines. For each sex, the number of extra-pair eggs of an individual within  
433 each round was the dependent variable (binomial model of counts of extra-pair young versus  
434 within-pair young using the 'cbind' function in R). As the fixed effect of interest, we fitted  
435 'selection regime' as a covariate with one degree of freedom (low lines = -1, control lines = 0,  
436 and high lines = 1). As random effects, we included either 'Female ID' (for female EPP, n = 190)  
437 or 'Male ID' (for male EPP, n = 188), 'Selection Line ID' (six levels), and 'Individual within  
438 breeding round ID' (each line in the data sheet, n = 325 in females and n = 319 in males as an

439 'observation-level random effect' [47] to control for overdispersion of counts arising from the  
440 non-independence of eggs within an individual's breeding round).

#### 441 **b) Statistical Approach for Quantitative Genetic Models**

442 First, we used generalized linear mixed-effect models [45, 46] to investigate how each of the  
443 traits measured in this study depended on a range of fixed effects. Details of fixed and random  
444 effects given below refer to the joint data set (Table S2: initial study plus data from selection  
445 lines).

##### 446 ***Male courtship rate***

447 'Male courtship rate' was square-root transformed to approach normality (Table S1). 'Male  
448 courtship rate' declined significantly over consecutive test days, declined with time of day,  
449 declined with male inbreeding coefficient, and was higher for males from a mixed-sex rearing  
450 environment compared with the unisex (Table S2). After accounting for these fixed effects, the  
451 random effects 'Male ID' and 'Test Batch ID' (19 levels) explained 46% and 13% of the variance,  
452 respectively.

##### 453 ***Male EPP***

454 The number of extra-pair eggs males sired within each breeding round ('Male EPP') was square-  
455 root transformed to approach normality, and was modelled as the dependent variable (Table  
456 S1). 'Male EPP' increased strongly with the number of days the male was paired. This fixed  
457 effect controls for variation in the duration of the breeding period and in the duration of the  
458 period a male was unpaired. 'Male EPP' also declined with male inbreeding coefficient (Table

459 S2). The random effects 'Male ID' and 'breeding year' (six levels) explained 21% and 8% of the  
460 variance, respectively.

#### 461 ***Female unpaired response***

462 The responsiveness of unpaired females to male courtship ('female unpaired response' in cages)  
463 differed significantly among consecutive test days (4 levels) and was higher for females reared  
464 in mixed-sex as opposed to unisex groups (Table S2). The random effects 'Female ID' and 'Test  
465 Batch ID' (19 levels) explained 37% and 13% of the variance, respectively.

#### 466 ***Female extra-pair and within-pair response***

467 Females interacted with an average of  $5.5 \pm 2.4$  different extra-pair males (range 1-12; 97% of  
468 346 females with two or more). 'Female extra-pair response' declined strongly with time after  
469 sunrise and with the duration of the pair bond (days paired). Based on the initial study [29], we  
470 assumed that 'female extra-pair response' varied over the fertile cycle with highest  
471 responsiveness 3 days before the start of egg laying (day 0) and with a continuous decline over  
472 the laying sequence. Hence, the fertile cycle was modeled as the number of days from day -3 (6  
473 levels: from 0 to 5, > 5 also coded as 5). Since 2007, all courtships had been scored by the same  
474 observer (K.M.). However, we also used data from two additional observers in 2006, so we  
475 included observer ID as a fixed effect. Scores of female extra-pair response varied slightly  
476 among the three observers (Table S2). The random effects 'Female ID', 'Pair ID' (i.e. the  
477 combination of identities of the courted female and the courting extra-pair male) and 'Year'  
478 explained 5%, 23% and 1% of the variance, respectively.



479 The 'female within-pair response' declined strongly with time after sunrise, and increased  
480 strongly with the duration of the pair bond (days paired). Within-pair responsiveness varied  
481 similarly over the fertile cycle as extra-pair responsiveness (Table S2). The random effects  
482 'Female ID', 'Pair ID', and 'Year' accounted for 1%, 15% and 5% of the variance, respectively.

### 483 ***Female EPP***

484 The dependent variable 'Female EPP' was modeled for each egg laid by a paired female, as 0 =  
485 within-pair and 1 = extra-pair (5,194 eggs in total). This model used a Gaussian error structure,  
486 because models with binomial error structure did not converge. 'Female EPP' decreased with  
487 the duration of the pair bond (measured until the start of laying), was higher when the sex-ratio  
488 was female-biased (only relevant for data from 2005 and 2006), and was not influenced by the  
489 inbreeding coefficient of the social partner (Table S2). The random effects 'Female ID', 'Pair ID'  
490 and 'clutch ID' (a clutch was defined as having no laying gaps longer than 4 days) explained 9%,  
491 26% and 37% of the variance, respectively.

### 492 ***Female fecundity***

493 'Female fecundity' (number of eggs laid per breeding round) was square-root transformed to  
494 approach normality. Female fecundity increased with the number of days a female spent in the  
495 aviary (mean  $\pm$  SD = 60  $\pm$  23 days, range 1–112), and decreased with female age (mean  $\pm$  SD =  
496 735  $\pm$  285 days, range 265–1511 days; Table S2). The random effects 'Female ID' and  
497 'Experiment ID' (18 levels after differentiating testing cohorts and breeding rounds) explained  
498 45% and 10% of the variance, respectively.

### 499 **c) Quantitative Genetic Analyses**

500 We used animal models to carry out quantitative genetic analyses, closely following the initial  
501 study [29]. To calculate the parameters, we implemented both a restricted maximum likelihood  
502 (REML) method using VCE 6.0.2 [42], and a Bayesian approach using a Monte Carlo-Markov  
503 Chain (MCMC) with the package MCMCglmm in R 3.4.0 [48]. Within each type of model (VCE or  
504 MCMCglmm), we used two units of analysis: raw data representing single observations and  
505 individual mean trait estimates based on the best linear unbiased predictions (BLUPs).

506 To test the ‘intersexual pleiotropy’ hypothesis, we used four versions of animal models (as in  
507 [29]) to estimate the heritability and genetic correlations between aspects of male and female  
508 extra-pair mating behavior (five traits: ‘male courtship rate’, ‘male EPP’, ‘female unpaired  
509 response’, ‘female extra-pair response’ and ‘female EPP’): a permanent-environment model  
510 with repeated measures on individuals in VCE (model 1) and in MCMCglmm (model 2); a model  
511 on individual estimates in VCE (model 3) and in MCMCglmm (model 4). For models 3 and 4,  
512 individual estimates were BLUPs extracted from the mixed-effect models shown in Table S2. All  
513 models are based on the joint data from the initial study [29] and the selection lines.

514 For comparison between earlier and new findings, we also ran models 1 and 2 on the respective  
515 subsets of data (initial data: models 5 and 6 which are updated for model structure compared to  
516 the ones published previously; new data: models 7 and 8).

517 To test the ‘intrasexual pleiotropy’ hypothesis, we used four versions of animal models (similar  
518 to models 1 to 4 above) to estimate the heritability and genetic correlations within females (five  
519 traits: ‘female fecundity’, ‘female unpaired response’, ‘female extra-pair response’, ‘female  
520 within-pair response’ and ‘female EPP’): a permanent-environment model in VCE (model 9) and

521 in MCMCglmm (model 10); a model on individual estimates in VCE (model 11) and in  
522 MCMCglmm (model 12). All models are based on the joint data.

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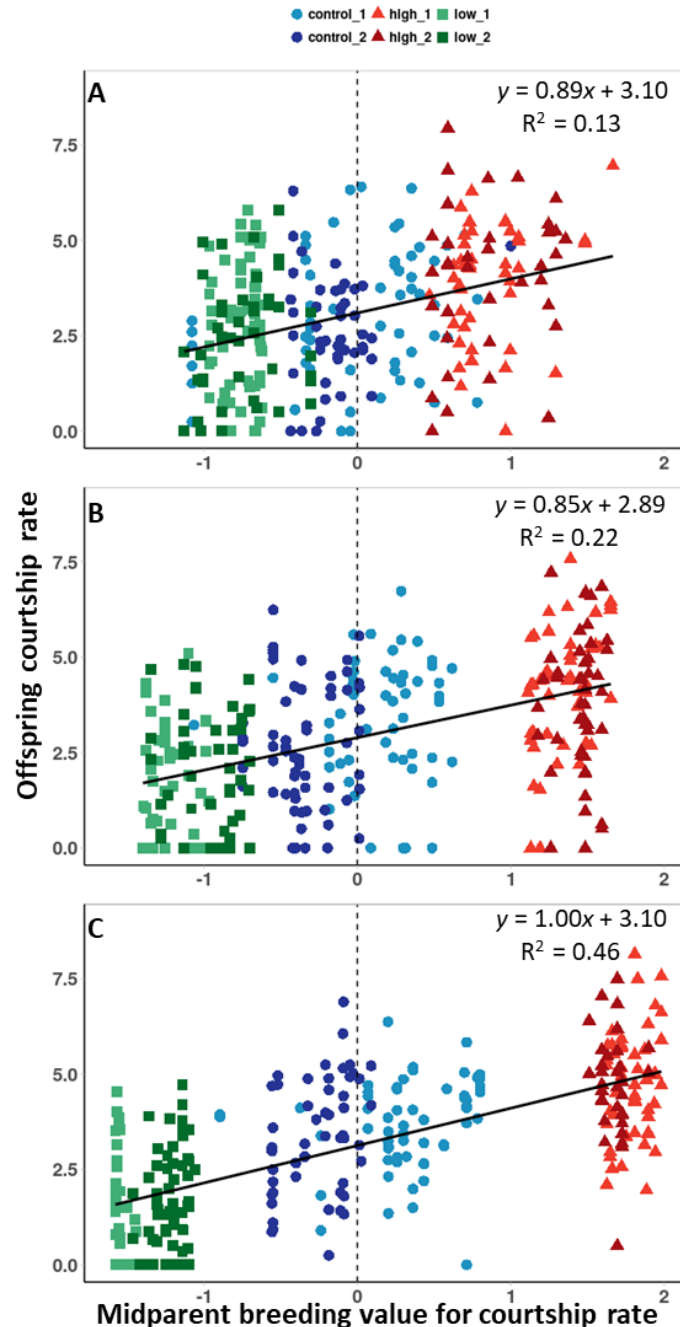
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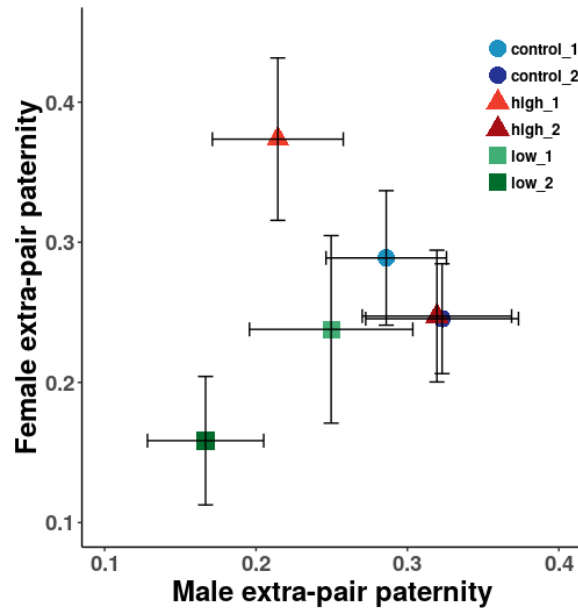
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638

639 Figure 1. Male observed and predicted courtship rate in individuals from six selection lines over three  
640 successive generations (S1-S3, A to C). The y-axis shows the measured courtship rate of male offspring  
641 (seconds in a 5-min trial, averaged across 4 trials per male, square-root transformed). The x-axis shows  
642 the predicted courtship rate, that is, the parents' breeding value for male courtship rate. These values  
643 were estimated prior to breeding (generations S0-S2, without information on offspring phenotypes) from  
644 a single-trait permanent-environment animal model in VCE. Symbol color and shape indicate the three  
645 types of selection lines (high, control, and low). Within each type, light and dark colors indicate the two  
646 replicate lines. Ordinary least-square regression lines and their equations are shown.



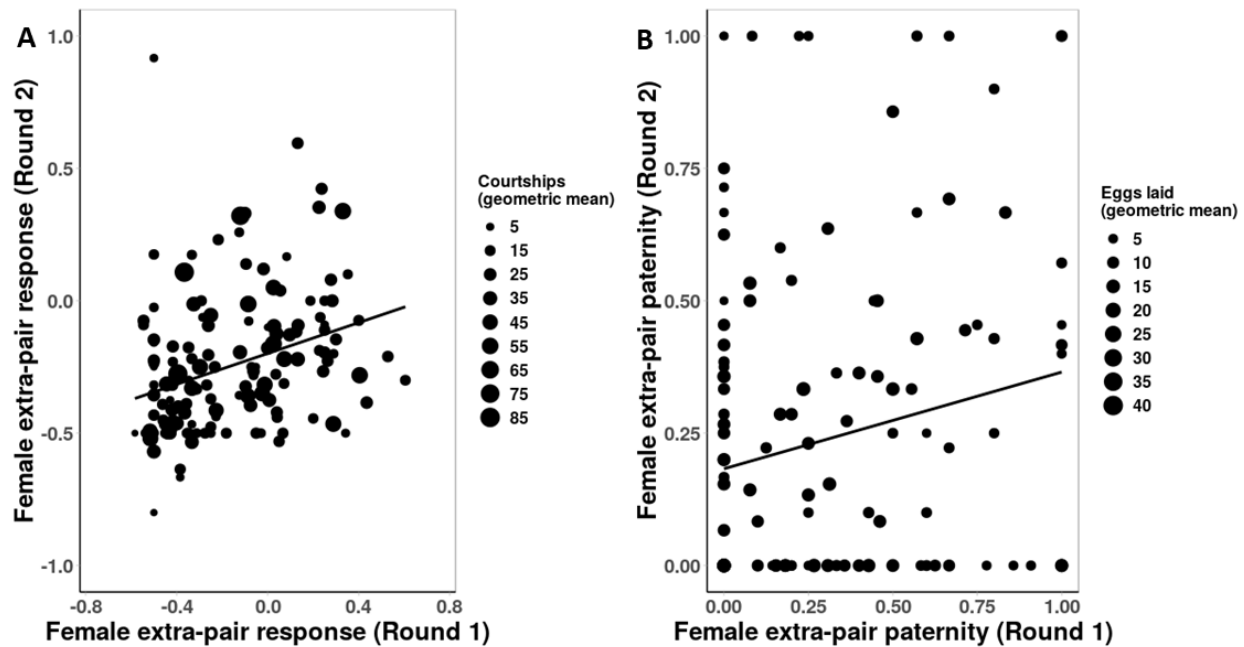
648 Figure 2. Weighted averages ( $\pm$  SE) of levels of male and female extra-pair paternity for each of the six  
649 selection lines in aviary breeding experiments (data on ca. 3,000 eggs from the 'S3' generation, see  
650 Results). Male extra-pair paternity (x-axis) refers to the total proportion of eggs sired by socially paired  
651 males outside their pair bond. Female extra-pair paternity (y-axis) refers to the proportion of eggs laid by  
652 socially paired females that are sired by males other than the social partner.



653



654 Figure 3. Estimates of repeatability of ‘female extra-pair response’ (A, the responsiveness of females to  
 655 courtship by extra-pair males) and levels of extra-pair paternity (B, proportion of eggs sired by extra-pair  
 656 males) across two breeding rounds differing in social pair bonds and in the identity of potential extra-pair  
 657 males. Shown are ordinary least square regression lines weighted by the geometric mean of the two  
 658 rounds ((A) slope  $\beta = 0.37 \pm 0.09$ ,  $N = 151$  females; (B)  $\beta = 0.24 \pm 0.09$ ,  $n = 135$  females). Dot size refers to  
 659 the geometric mean of the relevant sample sizes in the two breeding rounds (number of extra-pair  
 660 courtships and number of eggs laid, respectively). (C) Variance components estimation of the random  
 661 effects based on mixed-effect models with ‘female extra-pair response’ and ‘female EPP’ (each egg  
 662 modeled as 0 = within-pair and 1 = extra-pair) as the dependent variable, respectively.



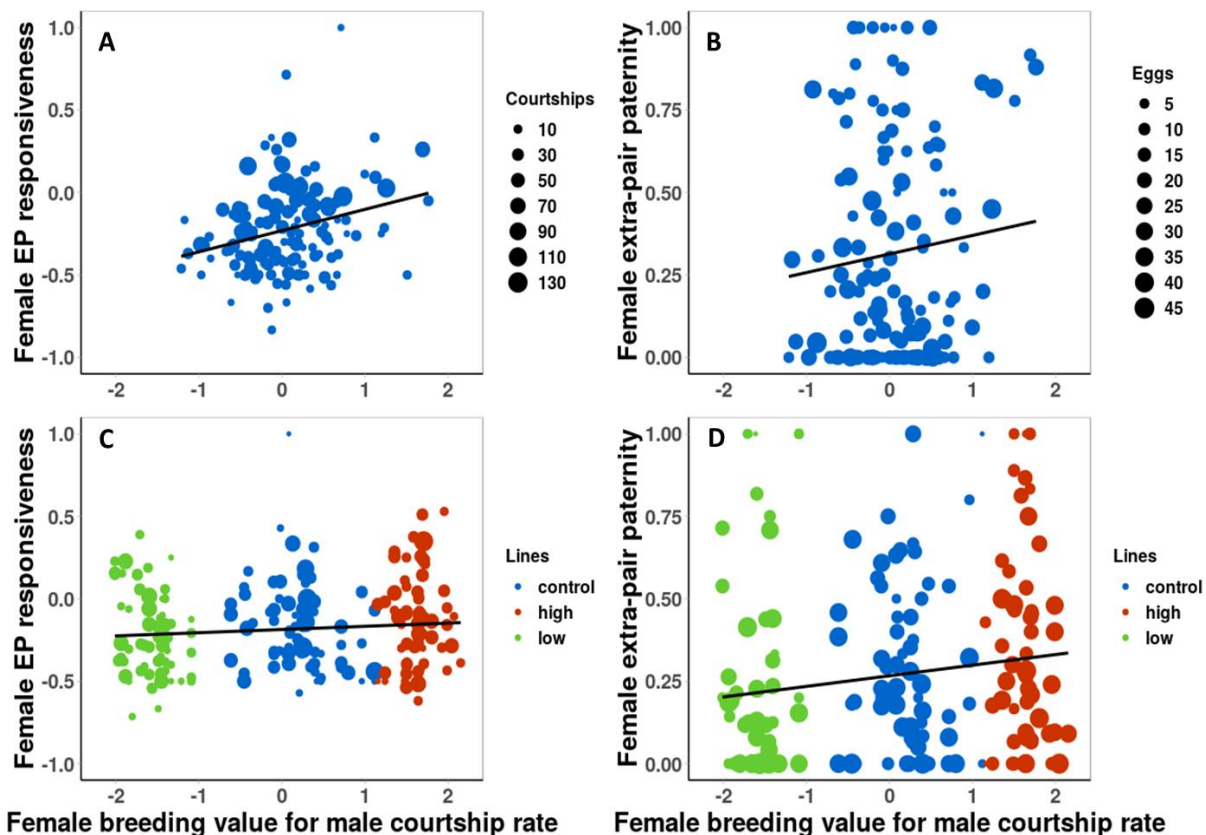
**C**

	Female extra-pair response (n = 9,117 extra-pair courtships)	Female extra-pair paternity (n = 2950 extra-pair eggs)
Female ID	6.2%	6.1%
Male ID	2.8%	9.9%
Pair ID	20.4%	16.1%
Clutch ID	-	39.4%
Residual	70.6%	28.5%

663

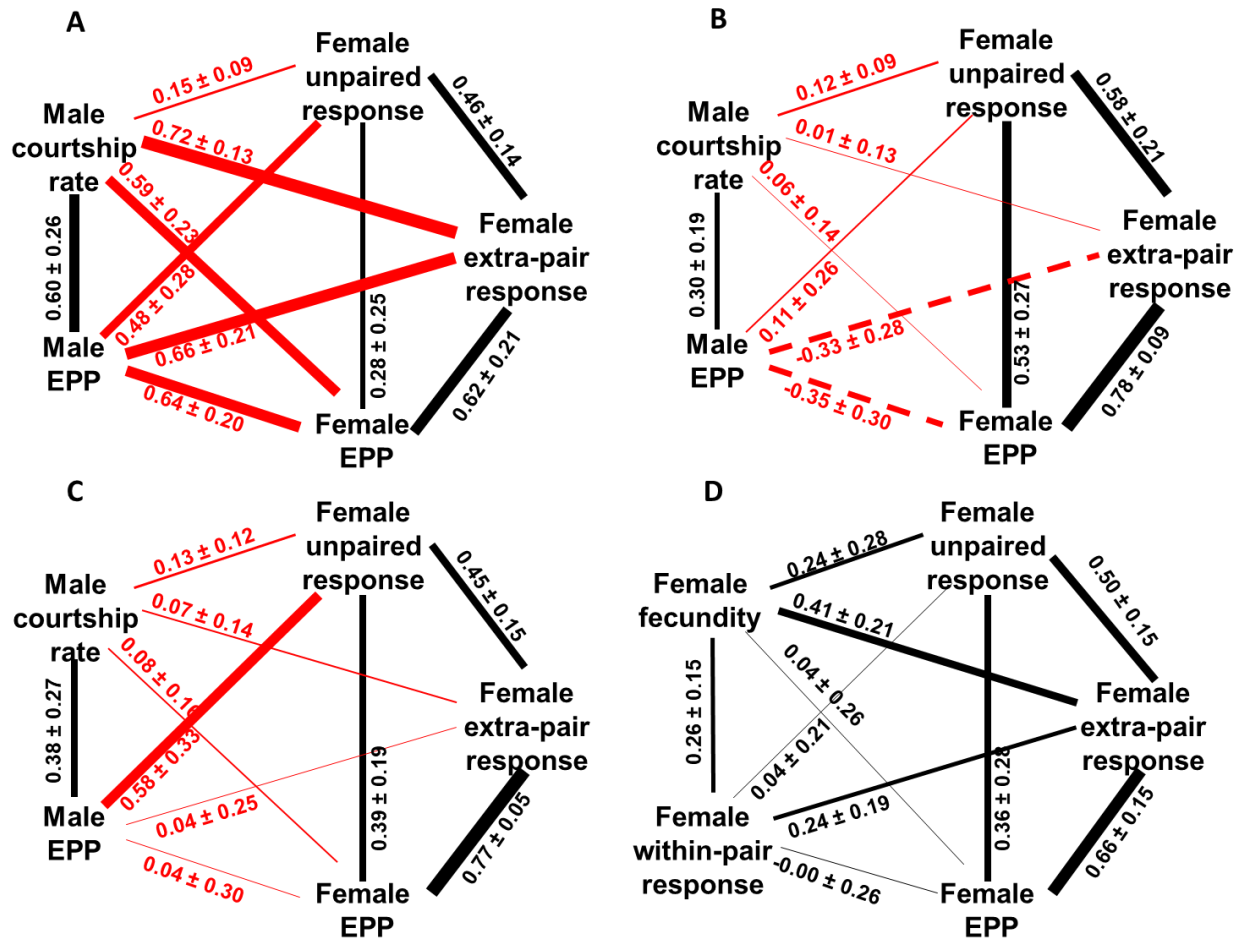
664

665 Figure 4. Relationships between measures of female extra-pair behavior and their estimated breeding  
666 value for male courtship rate. (A) and (B) Data from Forstmeier et al. (2011) (generations F1-F3)[29]; (C)  
667 and (D) data from the selection lines (generation S3). (A) Average female responsiveness to courtship by  
668 extra-pair males ('Female EP responsiveness',  $n = 141$  females, 3,958 courtships) in relation to their  
669 estimated breeding value for male courtship rate. Dot size refers to the number of extra-pair courtships  
670 observed for each female (range: 1–138, median: 19). Shown is the regression line weighted by the  
671 number of courtships (slope  $\beta = 0.14 \pm 0.03$ ). (B) Average level of female extra-pair paternity (the  
672 proportion of eggs sired by extra-pair males;  $n = 149$  females, 2,253 eggs) in relation to their estimated  
673 breeding value for male courtship rate. Dot size refers to the number of eggs laid by each female (range:  
674 1–45, median: 14). Shown is regression line weighted by the number of eggs ( $\beta = 0.10 \pm 0.04$ ). (C)  
675 Average female responsiveness to extra-pair male courtship ( $n = 205$  females, 9,117 courtships) in  
676 relation to their estimated breeding value for male courtship rate. Colors refer to the type of selection  
677 line (control, high, low). Dot size refers to the number of extra-pair courtships observed for each female  
678 (range: 1–219, median: 33). Shown is the weighted regression line ( $\beta = 0.02 \pm 0.01$ ). (D) Average level of  
679 female extra-pair paternity ( $n = 190$  females, 2,951 eggs) in relation to their estimated breeding value for  
680 male courtship rate. Dot size refers to the number of eggs laid by each female (range: 1–32, median: 15).  
681 Shown is the weighted regression line ( $\beta = 0.03 \pm 0.01$ ). Female breeding values for male courtship rate  
682 come from a single-trait permanent environment model conducted in VCE based on courtship rates from  
683 800 (A,B) and 1,651 (C,D) male relatives. Note that the regression lines are for illustration only, because  
684 other influential fixed effects are not taken into account.



685

686 Figure 5. Estimates of genetic correlations between measures of male and female extra-pair mating  
 687 behavior. A, initial data from the previous study (29). Shown are median estimates ( $\pm$  median SE) from  
 688 two versions of animal models (Table S10 and S11; see description of models 5 and 6 in Methods). B,  
 689 selection lines. Shown are median estimates ( $\pm$  median SE) from two versions of animal models in this  
 690 study (Table S12 and S13; see description of models 7 and 8 in Methods). C, joint data from the initial  
 691 study and the selection lines. Shown are median estimates ( $\pm$  median SE) from four versions of animal  
 692 models (Table S6 to S9; models 1 to 4 in Methods). D, genetic correlations among female traits. Shown  
 693 are median estimates ( $\pm$  median SE) from four versions of animal models (Table S14 to S17; models 9 to  
 694 12 in Methods). Between-sex genetic correlations are shown in red, within-sex genetic correlations in  
 695 black. Line thickness reflects the strength of the correlation. ‘Female EPP’: paternity of each egg laid by a  
 696 paired female, scored as 0 = within-pair and 1 = extra-pair; ‘Male EPP’: the number of extra-pair eggs  
 697 males sired within each breeding round. Note that all traits are measured during breeding in communal  
 698 aviaries except for “male courtship rate” and “female unpaired response” which reflect the behavior of  
 699 unpaired birds in standardized cage trials (see Methods).



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