

Postcopulatory sexual selection favors fertilization success of restocking hybrid quails over native Common quails (*Coturnix coturnix*)

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Abstract:	<p>Postcopulatory sexual selection plays an important role in the reproductive success of males in many species. Differences in fertilization success could affect rates of admixture and genetic introgression between divergent lineages. We investigated sperm precedence in matings in captivity involving Common quails (<i>Coturnix coturnix</i>) and farm quails of hybrid origin (<i>C. coturnix</i> x domestic Japanese quail, <i>C. japonica</i>), the last used in restocking practices to increase hunting bags. These interspecific matings in natural conditions are claimed to represent an important threat for the conservation of native Common quail populations. Results showed that fertilization success of each male depended on 1) the time it spent with the female, 2) the presence of sperm from a previous male in the female oviduct, 3) the time that the previous partner had been copulating with the female, and, most importantly, 4) the genetic origin of the male (wild or farm). Farm hybrid males showed higher fertilization success than wild Common males, they needed less time with the female to fertilize the same proportion of eggs. The presence of sperm from another male in the female oviduct reduced the percentage of fertilized eggs by a male. However, this reduction was higher for wild males when the precedent mate was a farm male. In summary, the sperm of farm hybrid males may outcompete the sperm of native males and this could be favoring the introgression of domestic Japanese alleles into the Common quail population, thus constituting a severe conservation threat to wild Common quail populations.</p>

1 **Postcopulatory sexual selection favors fertilization success of restocking hybrid**
2 **quails over native Common quails (*Coturnix coturnix*)**

3

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19

20 **ABSTRACT**

21

22 Postcopulatory sexual selection plays an important role in the reproductive success of males in
23 many species. Differences in fertilization success could affect rates of admixture and genetic
24 introgression between divergent lineages. We investigated sperm precedence in matings in
25 captivity involving Common quails (*Coturnix coturnix*) and farm quails of hybrid origin (*C.*
26 *coturnix* x domestic Japanese quail, *C. japonica*), the last used in restocking practices to
27 increase hunting bags. These interspecific matings in natural conditions are claimed to
28 represent an important threat for the conservation of native Common quail populations. Results
29 showed that fertilization success of each male depended on 1) the time it spent with the female,
30 2) the presence of sperm from a previous male in the female oviduct, 3) the time that the
31 previous partner had been copulating with the female, and, most importantly, 4) the genetic
32 origin of the male (wild or farm). Farm hybrid males showed higher fertilization success than
33 wild Common males, they needed less time with the female to fertilize the same proportion of
34 eggs. The presence of sperm from another male in the female oviduct reduced the percentage
35 of fertilized eggs by a male. However, this reduction was higher for wild males when the
36 precedent mate was a farm male. In summary, the sperm of farm hybrid males may outcompete
37 the sperm of native males and this could be favoring the introgression of domestic Japanese
38 alleles into the Common quail population, thus constituting a severe conservation threat to wild
39 Common quail populations.

40

41 **Keywords:** *Coturnix*, cryptic female choice, introgression, postcopulatory selection, quail,
42 restocking, sperm competition.

43

44

45 **INTRODUCTION**

46

47 Female promiscuity is widespread in nature (Birkhead and Møller 1998), creating a favorable
48 scenario for sexual selection both before and after copulation (Shuster et al. 2013).
49 Postcopulatory sexual selection plays an important role in sexual evolution (e.g. Birkhead and
50 Møller 1998; Birkhead and Pizzari 2002; Birkhead et al. 2009) and can occur through different
51 mechanisms. One of them is sperm competition, where gametes of different males compete to
52 fertilize the same set of ova (Parker 1970; Pizzari and Parker 2009). Another mechanism is
53 cryptic female choice, by which females are able to bias paternity in favor of a particular male
54 after having copulated with several males (Eberhard 1996; Kempnaers 1999; Pitnick and

55 Brown 2000; Birkhead and Pizzari 2002; Dean et al. 2011). Both mechanisms are usually
56 closely related and, thus, difficult to disentangle (Birkhead 1998), resulting in sperm precedence
57 patterns by which some gametes take precedence over others when fertilizing ova.

58 Sperm competition and cryptic female choice are facilitated by the capability of females
59 to store viable sperm in their reproductive tract. Sperm storage permits that sperm from several
60 males compete to fertilize the ovum and allows females to impose some kind of postcopulatory
61 selection, playing an important role in mating strategies (Smith 1984; Birkhead and Parker
62 1997). Sperm-Storage Tubules (SST) are the structures for sperm storage found in the oviduct
63 of birds and some other taxa (Birkhead and Møller 1993; Pearse and Avise 2001). The
64 persistence of viable sperm within the female varies among bird species, from days to weeks
65 (Birkhead and Møller 1992a), allowing the fertilization of ova long after copulation (Cohen 1977;
66 Shugart 1988; Birkhead and Møller 1993).

67 The Common quail (*Coturnix coturnix*) is a migratory galliform species that inhabits
68 grasslands and croplands in Europe, North Africa and West Asia during the breeding season
69 and African grasslands during winter (Guyomarc'h et al. 1998; Rodríguez-Teijeiro et al. 2012). It
70 is a game species, with economic importance in several European countries, where thousands
71 of birds are hunted every year (Guyomarc'h 2003). Although the census size is estimated to be
72 globally large, their populations are annually reinforced in many hunting grounds with the
73 release of thousands of quails bred in farms to boost the hunting population. The number of
74 individuals released can be up to 5 to 20 times larger than the breeding population in the same
75 areas (Sanchez-Donoso et al. 2012). A recent genetic analysis has shown that farm individuals
76 are not Common quails but descendants of crosses with domestic Japanese quail (*C. japonica*,
77 Sanchez-Donoso et al. 2012). Released farm quails are competing with native quails for mating
78 opportunities and their admixture in the wild has been identified as the main conservation
79 problem for Common quail populations (Guyomarc'h 2003; Perennou 2009). Domestic
80 Japanese quails have been artificially selected to show, among other traits, a reduced migratory
81 drive. It has been shown that this character can be inherited by hybrids (Derégnaucourt 2000 in
82 Barilani et al. 2005; Derégnaucourt et al. 2005a, b), as farm quails intended for restocking, so it
83 could impact natural populations if they interbreed. This admixture could entail a loss of
84 migratory impulse and a sedentariness of native populations, which would thus winter in
85 unfavourable latitudes. Moreover, growing in captivity makes farm quails poorly fitted to the wild
86 conditions when released. These individuals suffer a higher predation (Puigcerver et al. 2014)
87 and could also be subject to farm diseases or parasites due to lower immune system response
88 as a result of relaxed selection in captivity (as in other related species, Villanúa et al. 2008).
89 Recent studies have confirmed that farm and wild quails interbreed and produced offspring in
90 the wild (Puigcerver et al. 2014; Sanchez-Donoso et al. 2014).

91 One of the factors that could determine the success of the admixture between wild and
92 farm quails is postcopulatory sexual selection. Since domestic Japanese quails may have been

93 artificially selected to be highly fertile (Pérez y Pérez 1966), their male farm descendants could
94 be outcompeting wild males, which would be promoting the introgression of invasive alleles.
95 Several characteristics of both Common and domestic Japanese quails lead to think that they
96 can be exposed to postcopulatory sexual selection (either by sperm competition and/or by
97 cryptic female choice), which could be influencing the success of hybridization in the wild.

98 (i) Females are polyandrous in both species. Domestic Japanese quail females are receptive to
99 multiple males in laboratory settings and males can force copulations if females are not
100 receptive (Adkins-Regan 1995). Common quail females usually mate with several males in a
101 single reproductive attempt (Rodríguez-Teijeiro et al. 2003). Although male and female create a
102 bond that lasts several days while the female is sexually active, opportunistic extra-pair
103 copulations or mate-switching often occur during that time (Rodrigo-Rueda et al. 1997).

104 (ii) Females of both species present SST which would allow ejaculates from different males to
105 accumulate, even if matings occur on different days. Domestic Japanese quail females can
106 store viable sperm for a median of 8 days (Sittmann and Abplanalp 1965; Birkhead and Fletcher
107 1994).

108 (iii) Males of both species have large testes relative to their body mass (Puigcerver 1990;
109 Rodríguez-Teijeiro et al. 1992) which is a common feature in bird species with high frequency of
110 extrapair paternity (Møller 1991; Møller and Briskie 1995) and in species with intense sperm
111 competition (Møller 1988; Jennions and Passmore 1993; Gage 1994), because the investment
112 in sperm production is increased (Clulow and Jones 1982; Møller 1989; Cook 1997).

113 (iv) Quail males have a penis (Puigcerver et al. 1994), which could facilitate forced copulations
114 (as in waterfowl, Coker et al. 2002) and some degree of postcopulatory female selection could
115 have developed to manage the output of these copulations (Briskie and Montgomerie 1997).

116 In this paper we investigated whether postcopulatory sexual selection exists between the
117 sperm of Common quail males and hybrid males intended for restocking. We assessed if
118 fertilization success for both kinds of males was the same and also how fertilization success
119 was modified when other sperm was found in the female's oviduct. To study these questions,
120 we carried out an experiment in captivity with females mating with wild Common males or with
121 hybrid farm males. Some of those females were secondarily exposed to another male of the
122 same or different genetic origin. We studied the sperm precedence pattern from these crosses
123 by genetically analyzing the resulting embryos and, thus, we assessed the fertilization success
124 for each kind of male. All in all, we investigated whether postcopulatory sexual selection
125 between farm-reared hybrids and Common quails could contribute to the admixture between
126 these two lineages in the wild, increasing the threat imposed by the massive restocking
127 campaigns.

129 **METHODS**

130

131 ***Experimental design***

132 Experimental crosses were initially attempted using wild Common females captured from the
133 field. However, they did not succeeded in breeding in captive conditions, since it is very difficult
134 for pure Common quail females to lay eggs in captivity (Caballero de la Calle and Peña
135 Montañés 1997). Therefore, experiments were conducted with 16 females from an experimental
136 farm of the University of Castilla-La Mancha (Spain), which were descendants of admixed
137 females backcrossed with wild Common quail males for about 20 generations, so they had
138 domestic Japanese mitochondrial DNA (Barilani et al. 2005) but nuclear DNA indistinguishable
139 from Common quail (Sanchez-Donoso et al. 2012).

140 Twenty-seven males were used in the experimental crosses. Ten of them were pure
141 Common quails (without any evidence of admixed ancestry) captured from the wild in three
142 cereal crop areas in Spain (four in Barcelona, four in Ciudad Real and two in Seville provinces;
143 see Puigcerver et al. 2007 for capture methods); the remaining 17 males were hybrid individuals
144 with variable degree of admixture, hatched and reared in three different Spanish game farms
145 intended for restocking. Species identification for all individuals was done following Sanchez-
146 Donoso et al. (2012) (data not shown). All individuals were about one year old to avoid any
147 possible effect of age on their fertility (Santos et al. 2013).

148 Quails were kept in aviaries during at least eight months before the start of the
149 experiments, in order to get them habituated to captive conditions. They were moved to
150 individual 20x20x20 cm cages fourteen days before the beginning of the experiments to ensure
151 that no viable sperm was stored in females' SST (Birkhead and Fletcher 1994; Miranda et al.
152 2013) and to avoid sperm depletion in males (Preston et al. 2001). All experimental crosses
153 were done during the natural breeding season of the Common quail (from the beginning of June
154 to the end of July of 2010).

155 Females remained in their cage during the experiments to avoid disturbances and males
156 were the ones moved in and out the cages as needed (see below). Individuals were used just
157 once in the experiment to avoid any effect of their copulatory history over subsequent pre- and
158 postcopulatory traits or behavior. All individuals were sexually active during the experiments.
159 Female sexual maturity was confirmed by their capability of laying eggs while for male its sexual
160 activity status was assessed by their swollen cloaca (measured in length; Sachs 1969; Siopes
161 and Wilson 1975) and/or the presence of foam in the cloacal gland (Adkins-Regan 1995).
162 Physical condition and health were assessed by the weight and physical appearance and only
163 individuals in good condition were used in the experiments.

164 Statistical analyses were conducted in R 2.15.2 (R CoreTeam 2012). Generalized Linear
165 Models (GLMs) were fitted using the `glm()` function and constructed assuming a Binomial
166 error distribution and a Logit link function. GLMs were tested for the presence of outliers,
167 homoscedasticity, normality and overdispersion. Overdispersed models were recalculated
168 assuming a Quasibinomial error distribution. The significance of the variables studied was
169 evaluated by an analysis of deviance with the function `Anova()` available in the package `car`
170 (Fox and Weisberg 2011). In models that were not overdispersed, the analysis of deviance was
171 evaluated by a likelihood-ratio test (Chi-square test); an F-test was used in overdispersed
172 models.

173

174 **Experiment 1: Simple crosses - Male fertilization potential**

175 A set of simple crosses (one male and one female) was designed to study the fertility of farm
176 and wild Common quail males without other sperm in the female's oviducts. Six wild Common
177 males and 10 farm males were caged with a female each. After the female laid an average of
178 six eggs (from 3 to 8), the male was removed from the cage and Experiment 1 finished. The
179 number of days that female and male were caged together was registered, as well as daily
180 laying events (absence/presence of egg laid). Eggs were collected every day, individually
181 labeled and incubated in the laboratory during a week. After that time, they were opened to
182 determine whether they were fertilized or not by the presence or absence of an embryo.

183 We analyzed with a GLM whether genetic origin of males could affect their egg
184 fertilization capability. The proportion of fertilized eggs during the time male and female were
185 caged together was the response variable and male origin was the explanatory factor tested.
186 The number of days the male spent with the female was added as a covariate, since we
187 suspected that it could have some effect on the proportion of fertilized eggs.

188

189 **Experiment 2: Double crosses - Sperm competition**

190 To study the possible sperm competition between males of the same or different origin, we
191 carried out a second experiment with 11 females after concluding Experiment 1 (four females
192 that had been with wild males and seven with farm males). At the same time that the first male
193 (M1) was taken out of the cage, it was replaced by a second male (M2). M2 was caged with the
194 female until she laid an average of 7 (from 3 to 11) more eggs. After that, M2 was removed from
195 the cage, and Experiment 2 finished. Three different types of crosses were done regarding the
196 origin of the males and the order in which they encountered the female: 1) wild M1 - farm M2
197 (w-f); 2) farm M1 - wild M2 (f-w); 3) farm M1 - farm M2 (f-f). Four crosses were performed for the
198 first and second type of cross, and three for the last type.

199 Eggs were handled as in Experiment 1. Number of eggs laid and eggs fertilized were
200 registered. We genotyped the embryos and assessed their paternity. Since sperm stored in the
201 female oviduct can fertilize ova with the same probability during six days after mating (Birkhead
202 and Fletcher 1994), we registered the number of eggs fertilized by each male and the number of
203 non-fertilized eggs laid during the first six days after the male exchange. During that period,
204 fertilization by M1 or M2 would only depend on the possible postcopulatory selection over the
205 sperm of the two males.

206 Firstly, we analyzed whether the presence of sperm from a previous male, stored in the
207 female's oviduct, had an effect on the proportion of eggs fertilized by a second male. The
208 proportion of eggs sired by a male over the total number of eggs laid was the response variable
209 of the GLM. Presence or absence of sperm from a previous male was the factor of interest. Wild
210 and farm males were analyzed independently to observe the stored sperm effect over each kind
211 of male. Secondly, we tested whether the proportion of non fertilized eggs changed before and
212 after male exchange, to assess if it was dependent on the presence of sperm of one or two
213 males in the oviduct. Finally, we studied whether fertilization capability of M2 was determined by
214 its own origin and the origin of the previous male. We tested differences in the proportion of
215 embryos sired by M2 among the three male arrangements: w-f, f-w and f-f. The number of days
216 M1 had been with the female was included as a covariate.

217

218 **Experiment 3: Time alone – Sperm longevity in SST**

219 After removing M2, the female was left in the cage during 14 days. Eggs laid were collected,
220 incubated and checked for embryos as before, and paternity analyses were done for all
221 embryos. We registered the number of eggs and length of time during which these were
222 fertilized in the absence of males.

223

224 ***Paternity analyses***

225 Paternity of embryos was assessed by genetic analyses using a panel of autosomal
226 microsatellite loci. A blood sample (100 µl) was obtained for each adult and a tissue sample for
227 each embryo. Samples were stored at -20 °C in 95% ethanol until DNA was extracted using
228 DNeasy Blood & Tissue Kit (Qiagen), following manufacturer's protocols. Individuals were
229 genotyped for 11 unlinked autosomal microsatellite loci originally developed for Japanese quail
230 by Kayang et al. (2002; 2004). These markers were highly polymorphic and had previously been
231 used for hybrid identification (Sanchez-Donoso et al. 2012; Sanchez-Donoso et al. 2014). Some
232 loci were amplified by polymerase chain reaction (PCR) in a multiplex (some of them with
233 primers modified to facilitate multiplexing, as in Sanchez-Donoso et al. 2014), while others were
234 amplified separately and subsequently pooled before electrophoresis (detailed protocols are

235 available upon request). All PCR products were electrophoresed on an ABI 3730 sequencer
236 (Applied Biosystems), following manufacturer's protocols. Alleles were sized and scored using
237 the software GeneMapper v3.5 (Applied Biosystems).

238 Paternity of embryos was assigned by using the maximum likelihood method
239 implemented in CERVUS 3.0.3 (Kalinowski et al. 2007). This software assigns parentage based
240 on a pair-wise likelihood comparison. Simulations, based on allele frequency analyses, were run
241 in advance to estimate the significance of these differences between likelihoods for a defined
242 confidence level (see Marshall et al. 1998; Kalinowski et al. 2007; for details). Each mating
243 experiment was analyzed separately. Simulations of parentage were run for 100,000 offspring.
244 For all embryos analyzed, paternity was unambiguously assigned to one of the putative parents
245 with a probability over 99%. In all cases, genetic analyses confirmed as mother the female in
246 the cage, showing the reliability of the markers used in the study.

247

248

249 **RESULTS**

250

251 Fertile eggs did not necessarily occur on successive days, but could be separated by days with
252 non-fertilized eggs or without egg. This laying pattern was concordant with previous findings
253 (Adkins-Regan 1995). In all cases, no more than one egg was laid per day.

254

255 **Experiment 1: Simple crosses – Male fertilization potential**

256 The duration of the experiment was variable among cases, lasting on average 18.6 days
257 (standard deviation, SD=12.1). During this time, females laid 6.0 eggs on average (SD=1.2).

258 Both male origin and number of days the male spent with the female had a significant
259 effect on the proportion of eggs fertilized. The longer the male spent caged with the female, the
260 larger the proportion of fertilized eggs ($\chi^2_1=5.93$, $p=0.015$, Fig. 1). Although this pattern was
261 observed for both types of males, there were clear differences between them ($\chi^2_1=7.19$,
262 $p=0.007$): for the same amount of time, farm males fertilized more eggs than wild males (Fig. 1).

263

264 **Experiment 2: Double crosses - Sperm competition**

265 Experiment 2 lasted 10.4 days on average (SD=2.5) during which each of the 11 females laid
266 7.0 eggs on average (SD=2.8).

267 The first egg sired by M2 was one of the first three eggs laid during this experiment and
268 during the four first days after changing males. After that, a switch in paternity occurred in the
269 egg series and none of the following eggs was sired by M1. In eight cases, eggs were fertilized
270 by sperm from M1 stored in the female's oviduct after the change in males had taken place. In
271 two of these eight cases, M1 sired only the first egg after the change of males, in four M1 sired
272 the two first eggs, and in two M1 was the father of the three first eggs.

273 The presence of sperm from a previous male stored in the female's oviduct led to a
274 reduction in the proportion of eggs fertilized by a male (Fig. 2). For wild males, the reduction
275 was 74% (proportion of eggs fertilized by a wild male as M1 vs. proportion of eggs fertilized as
276 M2 -with a farm male as M1-: $F_{(1,8)}=12.88$, $p=0.007$, Fig. 2). For farm males, the reduction was
277 31%, less accentuated but still significant (farm male as M1 vs. farm male as M2: $F_{(1,15)}=5.01$,
278 $p=0.041$, Fig. 2). In this case, the origin (wild or farm) of the previous male (M1) did not have a
279 significant effect ($F_{(1,5)}=0.01$, $p=0.923$).

280 The proportion of non-fertilized eggs did not differ significantly before and after the male
281 exchange ($F_{(1,25)}=0.26$, $p=0.617$). This indicated that the reduction in the proportion of fertilized
282 eggs by a male when another male (M1) had been with the female before was due to the
283 presence of the eggs fertilized by M1, and not due to non fertilized eggs after the male
284 exchange.

285 The proportion of embryos sired by M2 (thus, excluding non-fertilized eggs) was
286 dependent on the combination of males with regard to their origin while controlling for the time
287 M1 had been before with the female ($\chi^2_2=10.66$, $p=0.005$). The longer the stay of M1 with the
288 female, the lower proportion of eggs fertilized by M2 (Fig. 3). Farm males that copulated after
289 another male (independently of its origin) fertilized a higher proportion of eggs than wild males
290 that copulated after a farm male ($\chi^2_1=10.54$, $p=0.001$). Even though the proportion of eggs
291 fertilized by M2 males was reduced, the proportion of eggs fertilized by farm males as M2 was
292 not significantly different from M1 wild males ($F_{(1,11)}=0.25$, $p=0.625$).

293

294 **Experiment 3: Time alone – Sperm longevity in SST**

295 Out of the 11 females in Experiment 2, one did not lay eggs during the time she was left alone,
296 so it was not taken into account for Experiment 3. The ten remaining females were monitored in
297 Experiment 3. Among them, three laid exclusively non fertilized eggs while six laid fertilized and
298 non fertilized eggs. These females retained viable sperm during a maximum of 10 days
299 (average: 6.2; SD=2.5). During this time, a maximum of 7 eggs could be fertilized by the sperm
300 stored in the SST (average: 3.5; SD=2.2). Eggs were sired in all cases by the last male that
301 stayed with the female.

302

303

304

305 **DISCUSSION**

306 Several factors can modulate a male's ejaculate competitiveness (Cook et al. 1997; Pitnick et al.
307 2009; Pizzari and Parker 2009), such as ejaculate size (volume and absolute number of
308 spermatozoa; Martin et al. 1974; Gage and Morrow 2003), spermatozoan traits (length,
309 swimming speed or longevity), seminal plasma biochemistry, and the interactions between the
310 ejaculates of competing males and also with the female's oviduct (Cook et al. 1997; Pitnick et al.
311 2009; Pizzari and Parker 2009). In the case of the quail, also the production of foam by the
312 proctodeal gland has been found to be of importance in male fertility (Cheng et al. 1989; Adkins-
313 Regan 1999; Finseth et al. 2013). All these traits, together with cryptic female choice, can
314 contribute to determine the relative fertilization success of the different males mating with the
315 same female. However, in this paper we have not focused on the mechanisms underlying the
316 sperm precedence pattern, but on the output of this process.

317 Results showed that the time a male spent caged with a female had a positive effect on
318 his probability of fertilising a female's egg. The longer the male stayed with the female, the
319 higher the proportion of eggs from the clutch fertilized by his sperm. This period also had an
320 effect on the fertilization success for any male subsequently copulating with that same female.
321 This time dependency of the fertilization success would explain the natural mating behavior of
322 the Common quail. Male and female establish a pair-bond for a period of time during which the
323 male guards his partner against possible extra-pair copulations (EPC). Since mate-switching
324 has also been observed (Rodrigo-Rueda et al. 1997), the longer a male guards its mate, the
325 higher the chances will be to fertilize the clutch even if the female copulates later with another
326 male. In addition, our results also show that quail sperm could be viably maintained in the
327 female's oviduct during an average of 6 days, with an average of 3.5 eggs fertilized during those
328 days (what is not very different from previous findings, Birkhead and Fletcher 1994). Taking into
329 account that Common quail clutches usually total about 10 eggs (Gallego et al. 1993), males
330 would be unlikely to fertilize entire clutches by mating just once or a few times over a short time
331 period with the same female, so the guarding behavior is likely to increase their siring potential.

332 Previous studies have already reported that males that copulate more times or for longer
333 have higher fertilization success (Dickinson 1986; Parker and Simmons 1991). A higher number
334 of copulation events increases the volume of sperm introduced into the female, what increases
335 the siring probability of that male. A male's fertilization success will generally increase with the
336 quantity of his sperm relative to that of other males (Parker et al. 1990). In our study, after the
337 first egg sired by the second male (M2) was laid, a change in the paternity in the egg series
338 occurred, and none of the following eggs was sired by the first male (M1), neither while being
339 with M2 nor when she was alone. Our results did not support the last-male precedence pattern
340 observed in other bird species (Warren and Kilpatrick 1929; Cheng et al. 1983; Briskie 1996;

341 Birkhead and Pizzari 2002) - by which the last male that copulates with the female is the one
342 siring the majority of the clutch –, since in our case not only the order of the males would have
343 been affecting the sperm precedence pattern, but this pattern would be under the effect of the
344 time male and female would had been copulating. On the contrary, our results did not
345 completely reject the “last in, first out” pattern (Birkhead and Møller 1992b; Stai and Searcy
346 2010), by which some kind of sperm stratification occurs in the SST: the sperm of the first male
347 is stored at the bottom of the tubules and the sperm of subsequent mates is deposited in top of
348 it, this sperm being those that would fertilize eggs first. When this has been used up, the sperm
349 beneath it gets exposed, so the last eggs are fertilized by the first male sperm. We did observe
350 a complete replacement of the male siring the embryos after a given point. However, since in
351 our case embryo paternity was determined by the order of males and the length of time spent
352 with the female, we do not know if a shorter stay of M2 would allow the reappearance of eggs
353 sired by M1. In any case, some eggs were fertilized by M1 after M2 had been introduced in the
354 cage, which implies that it is not just the last sperm inseminated that will fertilize eggs. When a
355 male stops copulating with a female, the amount of sperm in the female’s oviduct gets reduced
356 through time (Birkhead and Fletcher 1994) because it is used up fertilizing ova and because
357 there is a passive sperm loss that makes its viability decline (Birkhead et al.1995; Birkhead and
358 Pizzari 2002). The reduction of viable sperm stored also reduces the probability of fertilization.
359 Our results supported this observation, since fertilization of eggs stopped after some time being
360 without a male. When a second male inseminated sperm into the female’s oviduct, the first
361 male’s fertilization success would be reduced faster, by passive sperm loss plus by competition
362 with an increasing quantity of sperm from the second male.

363 Results from this study show that farm males had higher fertilization success than wild
364 males. Farm males needed to spend less time paired with the female than Common males to
365 sire the same proportion of embryos in a clutch. This implies that farm males during the
366 breeding season are potentially able to fertilize a larger number of eggs, even in just a few days,
367 thus gaining a competitive advantage.

368 Previous matings by the female reduced male’s fertilization success. This reduction in
369 the proportion of eggs fertilized by the second partner was because some of them were sired by
370 the previous mate, since the overall fertilization success was not affected by the presence of
371 sperm from more than one male. This reduction depended again on the amount of time the
372 previous partner was with the female: it was less accentuated when the time available for
373 copulation with the first male had been shorter. However, this reduction in the fertilization
374 success was also affected by the origin of the second male: farm males suffered a less
375 accentuated reduction than wild males. In fact, the proportion of eggs that farm males could
376 fertilize in the presence of competitor’s sperm in the female’s oviduct was not different from the
377 proportion of fertilized eggs by Common quail males when no other sperm was present. This
378 could also give farm males a great advantage to fertilize eggs over Common quail males.

379 Differences in sperm competitiveness have been observed between poultry breeds
380 (Prieto et al. 2011; Santiago-Moreno et al. 2014), that implies that a heritable component may
381 be playing a role. The domestication process and selection for increased productivity in captivity
382 could have increased fertility in many domestic species (Darwin 1868). This could also be the
383 case for domestic Japanese quails that contributed to the origin of the farm quails analyzed
384 here. The results of this study suggest that restocked farm quails may have higher fertility than
385 wild Common quails and may dominate sperm competition. Similar results could have been
386 obtained if the wild birds used in the mating experiments performed worse due to the lack of
387 habituation to life in captivity. However, we tried to prevent this possibility by keeping in captivity
388 all the birds used in these experiments for at least eight months before the beginning of the
389 study. The higher fertility potential of domestic Japanese males over Common quail males
390 would also be supported by their relative testes size: domestic Japanese males show a higher
391 gonadal index (weight of both testes x 100) / body weight) (2.26 g, Clulow and Jones 1982) than
392 Common quail males (1.75 g, Puigcerver 1990). Since relative testes size has been described
393 as a good indicator of sperm competitiveness potential (Møller 1988; Jennions and Passmore
394 1993; Gage 1994), it would be explaining the observed pattern of fertilization success in favor of
395 domestic Japanese males.

396 Our results indicate that restocking practices have the potential for a higher impact on
397 the native population than initially suspected by just considering the number of crosses between
398 wild and farm quails. Puigcerver et al. (2014) have shown that, despite a higher mortality for
399 restocked quails and the preference for intra-specific matings, interspecific matings in natural
400 conditions can occur. In fact, Sanchez-Donoso et al. (2014) have detected introgression of
401 alleles of farm origin into wild Common quail populations. In the present study, we have
402 detected an advantageous postcopulatory selection in favor of males of farm origin. These
403 results indicate that a relatively small number of successful interspecific matings could have an
404 important contribution to the introgression of farm alleles into Common quail populations. Given
405 the superior reproductive potential of the quails of farm origin, we advice against restocking
406 practices.

407

408

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410

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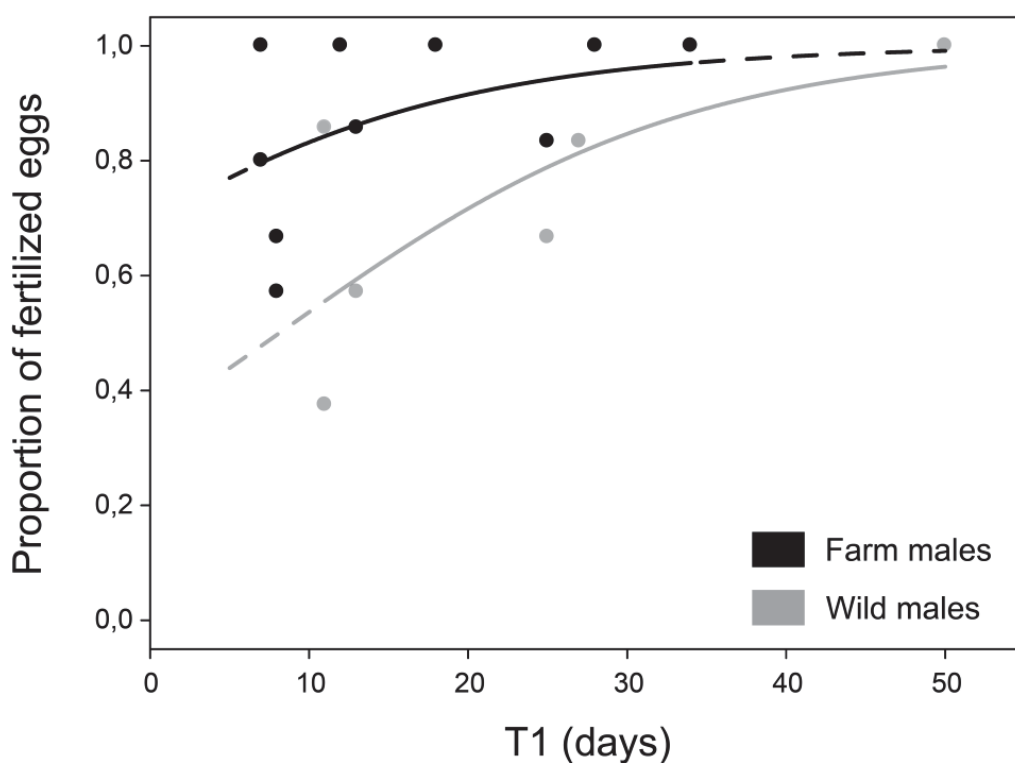
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615 **FIGURES**

616

617 **Fig. 1. Proportion of eggs fertilized by hybrid farm males (black dots) and wild males**
618 **(grey dots) depending on the days spent with the female (T1).** Curves show the generalized
619 linear model that best fitted the data. Dashed curves show the inferred fitting of the model
620 beyond observations. Probability of egg fertilization increases over time for both male types, but
621 the probability of fertilization was higher for farm males.

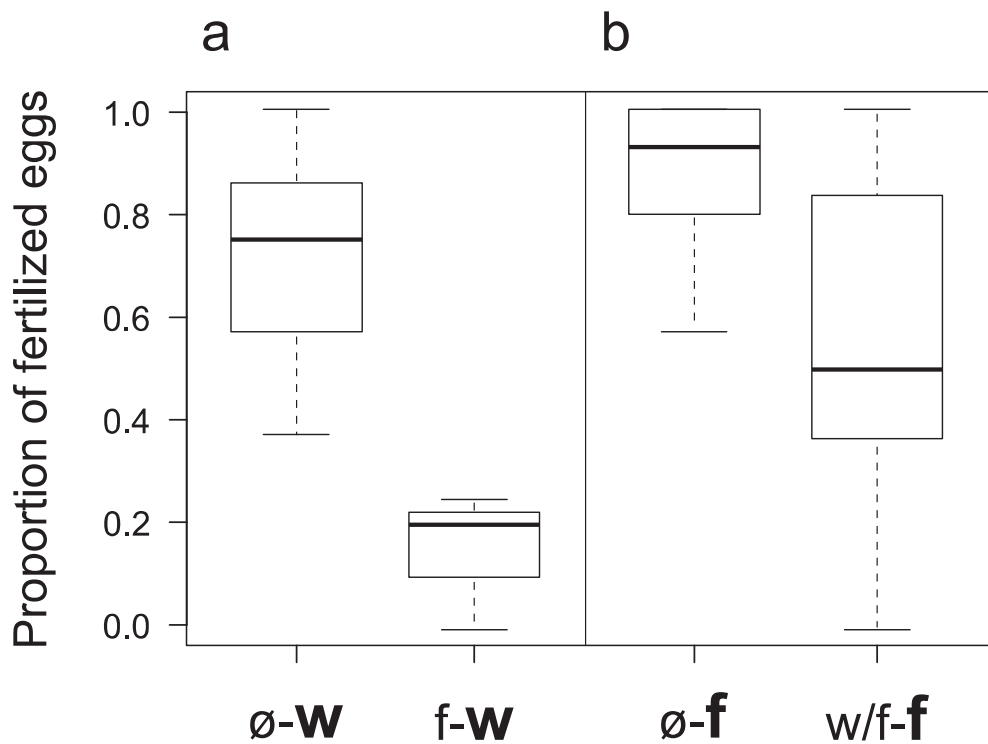
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623

624 **Fig. 2. Proportion of eggs fertilized by the second male quail (marked in bold) mating**
625 **with a female in different kinds of crosses.** The bottom and top of each box are the first
626 and third quartiles, the band inside the box is the median and the range of the data is
627 marked by the whiskers. "Ø": no male before; "w": wild male; "f": farm male. The proportion
628 of eggs fertilized by a wild (a) or a farm (b) male when the female had mated before with
629 another male was lower than when the female had not. In the case of farm males, there
630 were no differences regarding the origin of the first male

631

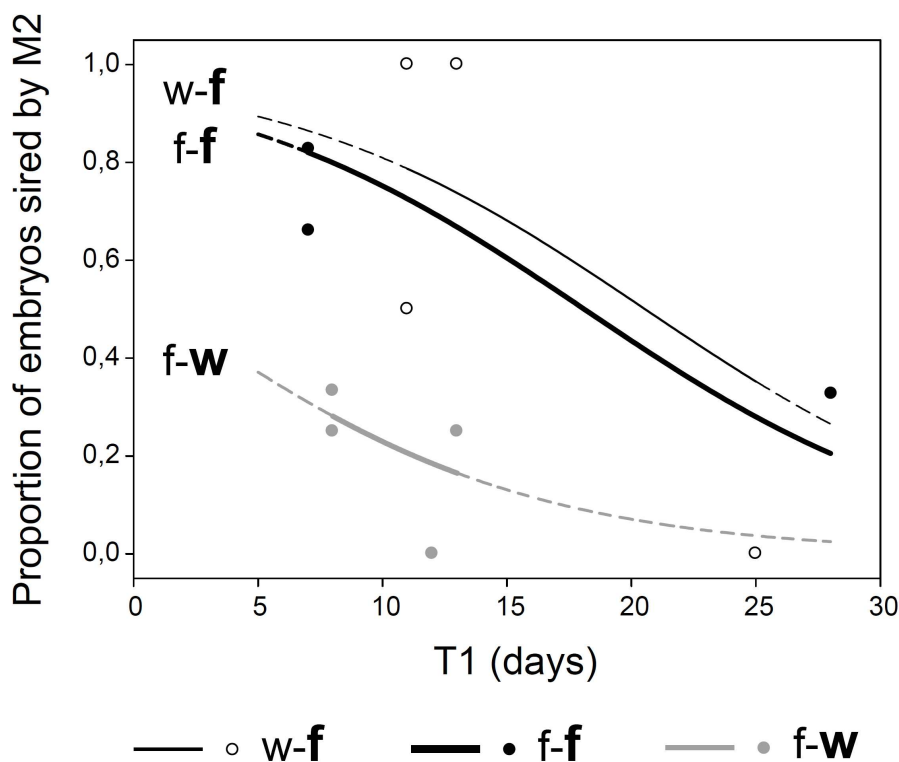


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633

634 **Fig. 3. Proportion of embryos sired by the second male (M2, in bold) depending on**
635 **the time the first male (M1) had been with the female (T1, in days), for each male's**
636 **origin combination ("w": wild male, "f": farm male).** Curves show the generalized linear
637 model that best fitted the data. Dashed curves show the inferred fitting of the model beyond
638 observations. The longer the previous male had been with the female, the lower proportion
639 of embryos was sired by the following male. Wild males acting as second partner sired
640 significantly less embryos than farm males did. Although plotted separately to facilitate
641 understanding, levels "w-f" and "f-f" were not significantly different.

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