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1 **Post-copulatory sexual selection allows females to alleviate the fitness costs**
2 **incurred when mating with senescing males**

3

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21

22 **Abstract**

23 Male senescence has detrimental effects on reproductive success and offspring fitness. When
24 females mate with multiple males during the same reproductive bout, post-copulatory sexual
25 selection that operates either through sperm competition or cryptic female choice might allow
26 females to skew fertilization success towards young males and as such limit the fitness costs
27 incurred when eggs are fertilized by senescing males. Here, we experimentally tested this
28 hypothesis. We artificially inseminated female North African houbara bustards with sperm
29 from dyads of males of different (young and old) or similar ages (either young or old). Then,
30 we assessed whether siring success was biased towards young males and we measured several
31 life history traits of the progeny to evaluate the fitness costs due to advanced paternal age. In
32 agreement with the prediction, we found that siring success was biased towards young males,
33 and offspring sired by old males had impaired hatching success, growth and post-release
34 survival (in females). Overall, our results support the hypothesis that post-copulatory sexual
35 selection might represent an effective mechanism allowing females to avoid the fitness costs of
36 fertilization by senescing partners.

37

38 **Keywords:** offspring growth, offspring survival, paternal age, reproductive senescence,
39 siring success, sperm selection

40

41 **Introduction**

42 Reproductive senescence refers to the age-dependent decline in breeding success,
43 involving physiological and behavioural changes [1,2]. In males, reproductive senescence is
44 often associated with poor ejaculate quality [3–5], potentially impairing male fitness through
45 reduced fertilization success [6]. However, the effect of paternal age extends well beyond the
46 probability to fertilize eggs, since offspring sired by old fathers have been shown to suffer from
47 reduced fitness, both under laboratory and natural conditions [7,8]. Negative effects of paternal
48 age on offspring fitness can arise because of the accumulation of *de novo* mutations in the highly
49 replicative male germline or because of epigenetic modifications [9–12]. Therefore, while
50 paternal age has a direct effect on male fitness, it can also affect female fitness, due to poor
51 offspring performance [1].

52 Given the potentially deleterious effects of male age on offspring fitness, it has been
53 suggested that females should avoid mating with senescing males [13,14]. Other authors have,
54 however, suggested that age might also be considered as a reliable signal of male quality, and
55 females mating with old males might gather direct (i.e., experienced partners providing better
56 parental care) or indirect (i.e., genetic) benefits for the progeny [15,16]. The idea that females
57 might gather genetic benefits from mating with old males has, nevertheless, been criticized on
58 several grounds. Indeed, longevity does not necessarily reflect fitness if there are negative
59 correlations between fitness-linked traits expressed at early and late life [16,17], and inherited
60 genes have all the same age, irrespective of individual age [18]. A handful of studies have
61 experimentally investigated whether females show any preference for young or old partners and
62 both types of response (as well as no response at all) have been reported [19–22]. This is perhaps
63 not so surprising given that different processes might be at play in different species. For
64 instance, the rate of senescence might differ between pre- and post-copulatory traits; with some
65 species having faster decline of pre-copulatory traits [23], others having faster decline of post-

66 copulatory traits [5]. Therefore, female preference for young or old males might be modulated
67 by male age-specific investment in maintenance of traits associated with mating and
68 fertilization success. Predicting any adaptive mate choice based on age should explicitly take
69 into account the net effect of paternal age on fitness of the progeny [24].

70 Preference for or avoidance of old males can be achieved during pre-copulatory mate
71 choice. However, this requires that females can reliably use phenotypic attributes of potential
72 mates to assess their age [21,22]. Post-copulatory sexual selection [25] might also allow females
73 to discriminate between males of different ages. When females copulate with more than one
74 male, a widespread mating system in animals, different ejaculates compete for the fertilisation
75 of the ova (sperm competition) [26], and females might actively bias fertilisation success
76 towards sperm of preferred males (cryptic female choice) [27]. Therefore, if young males
77 produce sperm with superior competitiveness ([20,28]; but also see [29]) or females can actively
78 select sperm from young males, post-copulatory sexual selection might be an effective
79 mechanism allowing females to avoid fertilization by old males [13].

80 Here, we aimed to test this hypothesis, and to this purpose, we conducted an artificial
81 insemination experiment in a bird species, the North African houbara bustard (*Chlamydotis*
82 *undulata undulata*), kept in a conservation breeding program. Artificial insemination offers the
83 possibility to remove the effects of pre-copulatory sexual selection and to solely focus on post-
84 copulatory sexual selection. In the North African houbara bustard, the combination of
85 polyandry [30] and the storage of sperm for several weeks [31] offers the potential for post-
86 copulatory sexual selection to operate. In this species ejaculate quality (e.g., number of sperm
87 in the ejaculate, sperm motility) reaches peak value at the age of 4-6 years and then declines
88 with advancing age [5]. When eggs are fertilized by senescing males they have a lower
89 probability to hatch and, when they do, chicks have an impaired growth [32]. We artificially
90 inseminated females with sperm from dyads of males of different ages (one at prime age, one

91 in the senescence stage), while two other groups of females were inseminated with sperm from
92 dyads of males of similar ages (either young or old). In addition to the probability to fertilize
93 eggs when sperm of young and old males compete in the female reproductive tract, we also
94 assessed the fitness consequences for the offspring sired by a young or an old father, in terms
95 of hatching success, growth rate, early survival under captive conditions and long-term post-
96 release survival in the wild.

97

98 **Materials and methods**

99 *Breeding programme management*

100 All birds used in this study are part of the Emirates Center for Wildlife Propagation
101 (ECWP), a conservation breeding programme located in eastern Morocco, aiming at reinforcing
102 natural populations of the North African houbara bustard (see ESM for a description of the
103 ecology of the species). This programme relies entirely on artificial inseminations [31] and
104 follows strict rules where breeding is designed to maintain genetic diversity within the captive
105 flock [33]. Ejaculates are routinely collected and immediately assessed for quality (see ESM
106 for details). Females are regularly checked for reproductive status and inseminated with freshly
107 collected semen when ready to lay.

108 *Experimental groups and artificial inseminations*

109 The study was conducted during the 2016 breeding season. We used 92 females [mean
110 age (\pm sd) = 4.5 ± 1.7 years, min-max = 3-8 years, corresponding to the peak of breeding
111 performance in females], split into three groups (female age did not differ between groups:
112 Kruskal-Wallis test: $\chi^2_2 = 0.63$, $p = 0.732$): 30 females were inseminated with a mix of
113 semen from a dyad of young [mean age (\pm sd) = 3.5 ± 1.0 years, min-max = 3-6 years] and old
114 [mean age (\pm sd) = 13.0 ± 1.0 years, min-max = 12-16 years] males (hereafter called young-old
115 group); 33 females were inseminated with a mix of semen from a dyad of young males (young

116 group, same mean age and range as above); 29 females were inseminated with a mix of semen
117 from a dyad of old males (old group, same mean age and range as above). In total, 71 males (31
118 young, 40 old) were used in the experiment, and the semen of each male was used 2.6 ± 2.7
119 times (mean \pm sd, min-max = 1-19).

120 Male age categories were chosen based on the pattern of senescence that has been
121 previously reported in the North African houbara bustard [5]. Nevertheless, to make sure that
122 the same pattern of age-associated decline in ejaculate quality was observed among males that
123 were available in the captive flock in 2016, we ran a linear mixed effect model confirming that
124 ejaculate quality (i.e. proportion of motile sperm and number of sperm in the ejaculate) reached
125 peak values at the age of 4-6 years and then declined (Table S1; Fig. S1A,B in ESM).

126 One obvious consequence of this age-associated decline in ejaculate quality is that,
127 when mixing ejaculates of young and old males, each age category might potentially contribute
128 with different number of sperm of different quality. Previous work has already shown that when
129 females are inseminated with mixes of sperm, paternity is systematically skewed towards males
130 that contribute the most to the mix [34]. Therefore, we wished to go beyond the simple “the
131 more (or better) sperm, the higher the chance to fertilize the eggs” paradigm. To do this, we
132 equalised the number of sperm that each male contributed to the mixes and made sure that
133 ejaculates from young and old males had similar motility (t value = 1.488, $p = 0.140$; Table S2
134 in ESM). As such, the sample of old males used for the study was not randomly drawn from
135 the total population of old males present in the captive breeding (Tables S3 and S4 in ESM).

136 Females were inseminated once with $32.9 \times 10^6 \pm 8.3$ sperm (mean \pm sd), which is close
137 to the mean number of sperm per ejaculate produced by the 71 males used in the study ($29.8 \times$
138 $10^6 \pm 20.9$). Inseminations took place between February 8th and April 18th and there was no
139 difference in insemination date among experimental groups (one-way ANOVA: $F_{2,89} = 0.132$,
140 $p = 0.876$).

141 We collected up to the first five eggs laid. However, note that not all 92 females laid
142 five eggs (see ESM for details), and therefore a total of 401 eggs were collected. Number of
143 eggs laid did not differ among experimental groups (generalized linear model with a Poisson
144 distribution of errors: $F_{2,89} = 0.17$, $p = 0.844$). Eggs were laid between February 13th and June
145 3rd and laying date did not differ among experimental groups (linear mixed effects model: $F_{2,86.4}$
146 $= 1.30$, $p = 0.279$). Three hundred seventy-four eggs out of the 401 laid eggs were incubated
147 following a standardized protocol (see ESM for details, including fertility assessment).

148 *Offspring traits*

149 Chick body mass (± 1 g) and size (head-skull length ± 0.5 mm), were measured at day
150 1, 9, 18, 26, 40, 60 and 90 post-hatching (see ESM for details on rearing conditions). Survival
151 in captivity was monitored daily up to October 26th. Survival in the wild was monitored for 142
152 birds. To this purpose, birds were fitted with a GPS satellite transmitter (GPS - PTT), two weeks
153 prior to release. At that time, birds were at least 4-month-old [mean age (\pm sd) = 190 ± 18 days,
154 min-max = 145-233 days]. They had become sexually dimorphic and could therefore be sexed
155 (77 males and 65 females). All birds were released on October 27th 2016. Age, body mass and
156 body size at release did not differ between birds sired by young and old fathers (Table S5 in
157 ESM). Survival was monitored until March 2019 (i.e. 28 months post-release). For each bird,
158 we also computed the maximal distance travelled as the distance (in kilometres) between the
159 release location and the furthest location from release. This measure served as a proxy of
160 movement behaviour (dispersal and/or exploration).

161 *Paternity analyses*

162 We genotyped 13 microsatellite loci designed for the species. Paternity was assigned
163 using CERVUS 3.0. Individuals were included in parentage analyses whenever they were
164 genotyped at a minimum of 11 loci, and only paternities assigned with a confidence level of
165 95% were considered (see ESM for further details).

166 *Statistical analyses*

167 Analyses were performed with R 3.1.3 [35].

168 Siring success within the young-old group was estimated as the probability for each egg
169 to be fertilised by a young or an old male. Siring success was therefore analysed using a
170 generalized linear mixed effects model ('glmer' function of the 'lme4' package) with a binomial
171 distribution of errors. We tested whether the intercept significantly deviated from 0.5, which
172 represents the null-hypothesis of equal siring probability between young and old males.
173 Maternal identity and males' identities within dyads were included as random effects (to take
174 into account the non-independence of offspring produced by the same mother and the repeated
175 use of the sperm of certain males among dyads, respectively).

176 Hatching success was analysed using a generalized linear mixed effects model with a
177 binomial distribution of errors ('glmer' function of the 'lme4' package), while hatchling body
178 mass and size, and offspring growth, were analysed using linear mixed effects models ('lmer'
179 function of the 'lme4' package) with a normal distribution of errors. In all models, paternal age
180 was included as a fixed effect, whereas maternal identity and males' identities within dyads
181 were included as random effects.

182 When analysing offspring growth, we also included offspring age and the interaction
183 with paternal age as fixed effects. We tested for linear and quadratic effects of offspring age on
184 growth by comparing two models including age, or age and squared age, respectively, based on
185 their AICc. A linear effect of age received the best statistical support for body mass, while for
186 body size, the best model included the squared age term. Offspring identity nested within
187 maternal identity was also included as a random effect to take into account the repeated
188 measurements on the same chicks over time and the non-independence of chicks produced by
189 the same mother.

190 An information theoretic model averaging approach was used to test for the significance
191 of fixed effects as predictors of the response variables using the function ‘dredge’ of the
192 ‘MuMIn’ package. Significance of each effect was based on the p -value of the corresponding
193 conditional model-averaged coefficient. For generalized linear mixed effects models with a
194 binomial distribution of errors, we also systematically checked whether data were
195 overdispersed. For all models the ratio between residual deviance and residual degrees of
196 freedom was comprised between 1.17 and 1.3, and the test proposed by Bolker [36] showed no
197 evidence for overdispersion.

198 Offspring survival under captive conditions and after release in the wild was analysed
199 using a mixed effects Cox model (function ‘coxme’ of the ‘coxme’ package). The model on
200 survival in captivity included paternal age as a fixed effect, and maternal identity and males’
201 identities as random effects. For the model on post-release survival, we could also include
202 offspring sex and the interaction between paternal age and offspring sex as fixed effects,
203 because released birds were old enough to be sexed based on sexual dimorphism. Among the
204 142 birds that were released in the field with PTTs, 18 were lost and were censored in the
205 survival analysis. Offspring movement (as log-transformed maximal distance travelled from
206 the release site) was analysed using a linear mixed effects model. Offspring age, sex, paternal
207 age and the two-way interactions were included as fixed effects, while maternal identity and
208 males’ identities as random effects.

209

210 **Results**

211 *Siring success when females are inseminated with sperm of young and old males*

212 Females that were inseminated with a mix of sperm from young and old males laid 118
213 fertile eggs, and paternal identity could be assigned for 86 of them. Based on these 86 offspring,

214 we found that old males had significantly lower within-dyad siring success compared to young
215 males (33.7% *versus* 66.3%; z value = 2.220, p = 0.026; Fig. 1).

216 Given that the paternity of 27% of laid eggs could not be assigned, one possibility could
217 be that the differential siring success reflects differential embryo mortality (all eggs for which
218 paternity could not be assigned had early embryo mortality). To address this issue, we ran
219 another model that was restricted to the first egg laid by each female, for which the rate of
220 paternity assignment was high (28 out of 29). This restricted model confirmed that siring
221 success was significantly lower for old compared to young males (25% *versus* 75%; z value =
222 2.517, p = 0.012; Fig. 1).

223 *Fitness consequences for offspring sired by young or old males*

224 Eggs fertilized by old males had a lower probability to hatch compared to eggs fertilized
225 by young individuals (58% *versus* 70%, n = 331 eggs from the three experimental groups; z
226 value = 2.146, p = 0.033; Table S6 in ESM).

227 Hatchling body size was slightly lower for chicks sired by young fathers, while
228 hatchling body mass and size-corrected body mass were not affected by paternal age (Tables
229 S7 and S8 in ESM).

230 We assessed growth rate of chicks during their first 3 months of life, in terms of body
231 size, body mass and size-corrected body mass (Table S7 in ESM). Growth rate of body size did
232 not differ between chicks sired by young or old fathers (Table S9 in ESM). However, offspring
233 sired by young fathers had higher body mass growth and size-corrected body mass growth
234 (Table 1). The slope relating body mass to offspring age was steeper for chicks sired by young
235 fathers (offspring age \times paternal age interaction; Table 1). The model that also included body
236 size (providing therefore a measure of size-corrected body mass) showed a significant three-
237 way interaction between offspring age, paternal age and body size (Table 1). To visualize the
238 effect of paternal age on size-corrected body mass growth, we computed the residuals of body

239 mass on body size and plotted them against chick age for the two paternal age groups. The
240 figure shows that chicks sired by young fathers initially gained more mass for their size
241 compared to chicks sired by old fathers (Fig. 2).

242 Offspring survival was high in the captive breeding (86% of chicks were alive prior to
243 release) and did not differ between individuals sired by young and old fathers (mixed effects
244 Cox model: $z = -0.240$, $p = 0.810$).

245 Among the 142 birds that were released in the field with PTTs in October 2016, 79%
246 had died 28 months later (March 2019). A mixed effects Cox model showed that survival was
247 affected by the interaction between paternal age and offspring sex ($z = -2.280$, $p = 0.022$; Table
248 S10 in ESM), with female offspring sired by young fathers having the highest survival rate (Fig.
249 3). To investigate whether the effect of paternal age on female survival was due to a differential
250 propensity to move further away from the release site, we ran a linear mixed effects model on
251 the log-transformed maximal distance covered by each individual (mean \pm sd = 37.7 ± 62.9 km,
252 min-max: 2.3-483.8 km, $n = 142$). This model showed that paternal age did not affect offspring
253 movement (Table S11 in ESM), suggesting that the effect on survival was not mediated by
254 differences in exploratory/dispersal behaviour.

255

256 **Discussion**

257 We found that when females were concomitantly inseminated with sperm from young
258 and old males, egg fertilization success was biased towards young males. We also found
259 evidence showing that when eggs are fertilized by old males, hatching rate was reduced, chick's
260 growth rate was decreased, and long-term survival in the wild was lower for female offspring.
261 Therefore, this work supports the hypothesis that post-copulatory sexual selection, that operates
262 when females mate with multiple males, may allow females to limit the fitness cost incurred
263 when eggs are fertilized by the sperm of senescing males [13].

264 The North African houbara bustard is an ideal candidate to test the hypothesis that post-
265 copulatory sexual selection might allow females to avoid the cost of egg fertilization by old
266 mates, because females are polyandrous (and the decision to mate with several males is under
267 female control), old males produce ejaculates with fewer sperm of lower motility, and paternal
268 age is associated with fitness costs for the progeny. Here, we used a very stringent experimental
269 approach where young and old males used to prepare the mixes of semen had similar ejaculate
270 quality and similarly contributed to the mixes in terms of sperm motility and number. Our
271 decision to use a non-random sample of old males has pros and cons. Among the cons, we might
272 speculate about the possible consequences if there are trade-offs among ejaculate attributes
273 and/or between ejaculate attributes and other phenotypic traits [6,37,38]. An intriguing
274 possibility would be that, if there is a trade-off between sperm motility and longevity, sperm of
275 old birds with high motility might also have the shortest longevity, possibly due to their higher
276 mutation load, which might account for their poorer fertilization success. Further work is
277 definitely needed to explore how age affects the strength of the trade-offs among different
278 ejaculate attributes, as well as between traits involved in pre- and post-copulatory sexual
279 selection. Additional ejaculate attributes (e.g. sperm velocity) measured using computer-
280 assisted sperm analysis might contribute to investigate these trade-offs.

281 This last point brings us also to the difficulties to properly identify and fully tease apart
282 any male and female effects as drivers of the observed fertilization bias in favour of young
283 males. We controlled for two obvious traits that are known to contribute to the probability of
284 egg fertilization when sperm from different ejaculates compete, but there are many more
285 phenotypic attributes that we could not control for (for instance, how long sperm from young
286 and old males live once in the female reproductive tract, sperm morphology, etc.). In addition
287 to the phenotypic attributes, when studying the effect of male age, one should also control for
288 any possible mutation load and/or epigenetic changes that are known to occur in the highly

289 replicative male germ line. Therefore, in line with the conclusions of a recent review article
290 [27], we think that it is extremely challenging to tease apart male (traits leading to higher sperm
291 competitiveness) from female effects (active female sperm selection). The difficulties to
292 identify the mechanism underlying the biased fertilization success do not, however, preclude to
293 conclude that females, by mating with multiple males may open the way for post-copulatory
294 selection to operate and, as such, limit the risk of being fertilized by senescing mates.

295 In addition to the possible mechanisms that might allow the sperm of young males to
296 outcompete sperm from senescing individuals (i.e., higher longevity in the female reproductive
297 tract), there might also be direct mechanisms of female sperm selection [27]. For instance, it
298 has been shown that females might control male fertilization success by ejecting sperm of non-
299 preferred males (as in the case of sub-dominant males in the feral fowl, [39]). Obviously, such
300 a mechanism cannot operate in the present study, because females were artificially inseminated
301 with a mix of semen, making impossible to selectively eject the sperm of one of the two males.
302 Other possible mechanisms might involve the selective action of the female immune system
303 towards sperm of old males. If senescing males produce sperm carrying higher mutation load,
304 they might be recognized by female immune cells and destroyed. Finally, the difficulty to
305 identify and tease apart any male and female effect as drivers of the outcome of post-copulatory
306 selection is further illustrated by the finding that the two might not be mutually exclusive and
307 might actually interact, as in the case of the guppy (*Poecilia reticulata*) where female ovarian
308 fluids mediate male sperm velocity [40].

309 Estimating siring success when females are inseminated with ejaculates of different
310 males implies that paternity can be assigned, and this is usually achieved with genetic tools.
311 However, when embryos have an interrupted development at very early stages, it might be
312 impossible to retrieve enough genetic material to assign paternity. Therefore, when comparing
313 two groups of fathers, differential siring success might arise because of differential embryo

314 mortality. In a companion study, we investigated whether post-copulatory sexual selection
315 might allow female North African houbara bustards to avoid insemination with kin. We found
316 evidence that paternity was skewed towards unrelated mates, but this skew was entirely due to
317 the low hatching success of eggs that were fertilized by males that were genetically related to
318 the female [41]. Here, we found that eggs sired by old fathers also had reduced hatching success,
319 potentially raising the same concern about differential embryo mortality as the driver of the
320 observed differential siring success between young and old males. However, when restricting
321 the analysis to first laid eggs, where paternity assignment was very successful (only one egg
322 had a missing paternity), we still found a highly skewed paternity towards young males.
323 Therefore, we are confident that our finding of differential siring success between young and
324 old males is not an artefact due to differential embryo mortality and biased paternity
325 assignment.

326 The idea that females might engage in multiple matings and let post-copulatory selection
327 to operate as to limit fertilization by senescing males is corroborated by our finding that eggs
328 fertilized by old fathers had lower hatching success and chicks sired by old fathers had reduced
329 growth rate. These findings add evidence for deleterious effects of paternal age on offspring
330 fitness, a topic that has attracted considerable attention in the last years [8,42]. In addition to
331 these short-term effects of paternal age, we also investigated whether offspring survival was
332 affected both during the period that offspring spent in the captive breeding and more notably
333 during almost 2 years and a half after their release in the wild. While early survival in the captive
334 breeding did not differ as a function of paternal age (likely the consequence of the very benign
335 environmental conditions encountered in captivity), we found a sex-dependent effect of
336 parental age on long-term survival in the wild. In particular, female offspring sired by young
337 males had better survival compared to female offspring sired by old fathers, whereas there was
338 no effect on male offspring survival. Although this is not the first study to report offspring sex-

339 dependent effect of parental age [8,43,44], it is difficult to precisely identify the underlying
340 mechanisms. For instance, females generally start breeding younger than males, i.e. during their
341 first year of life. Hence, released females might have been more exposed to environmental
342 hazards (predation, hunting, inability to find appropriate food items) when prospecting for nest
343 sites or during incubation, and females sired by old fathers might have performed less well than
344 females sired by young fathers. Alternatively, although we did not find any evidence suggesting
345 a propensity to cover longer post-release distances as a function of paternal age, differences in
346 habitat use or other behavioural and/or physiological traits might explain the observed sex-
347 dependent effect of paternal age on long-term survival.

348 To conclude, we provided experimental evidence showing that post-copulatory sexual
349 selection can be an effective mechanism allowing females to avoid egg fertilization by
350 senescing males and limit the associated fitness costs for the progeny. Although very
351 challenging, further work might try to attempt identifying the possible drivers (better sperm
352 competitiveness of young males, active female sperm preference, or both), and disentangle male
353 and female effects of age-dependent fertilization skew.

354

355 **Ethics**

356 All birds used in the present study were bred in captivity and released into the wild in
357 agreement with Moroccan authorities: Ministère de l'Agriculture, Développement Rural et des
358 Pêches Maritimes, Direction Provinciale de l'Agriculture de Boulemane, and Service
359 Vétérinaire (Nu DPA/48/285/SV) under permit number 01-16/VV; OAC/2007/E; Ac/Ou/Rn.
360 All releases were recorded by the Haut Commissariat aux Eaux et Forêts et à la Lutte Contre la
361 Désertification. Bird handling and sampling were performed by trained bird keepers or
362 experimenters of the ECWP.

363

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375 analyses. The authors declare no conflict of interest.

376

377 **Authors' Contributions**

378 PV, LL, GL, TC, MSJ, FL, YH and GS designed the experiment; PV and AB conducted
379 the experiment; PV performed statistical analyses; PV drafted out the manuscript, which was
380 revised by GS and YH. All authors gave final approval for publication.

381

382 **Data, code and materials**

383 The datasets supporting the results are available from the Dryad Digital Repository:
384 <https://datadryad.org/review?doi=doi:10.5061/dryad.47fd240>.

385

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- 503

504 **Table**

505

506 Table 1. Linear mixed effects models exploring the effect of paternal age on chick growth rate
 507 during the first three months of life (n = 215 chicks). Growth rate was assessed as age-dependent
 508 change in body mass and size-corrected body mass. The model on body mass included offspring
 509 age, paternal age and the two-way interaction as fixed effects and maternal identity, offspring
 510 identity nested within the maternal identity, and the identities of the two males forming the dyad
 511 as random effects. The model on size-corrected body mass also included body size (head
 512 length), as well as all the two- and three-way interactions. The old paternal age category was
 513 included in the intercept. Significant *p* values are in bold.

<i>Body mass</i>				
Fixed effects	Estimate	SE	t value	<i>p</i>
Intercept	3.510	7.062		
Offspring age	11.404	0.113	101.180	<0.001
Paternal age	-4.757	9.123	-0.520	0.930
Paternal age x Offspring age	0.333	0.145	2.300	0.022
Random effects	Variance	SD		
Offspring ID:Maternal ID	1573.300	39.653		
Maternal ID	244.800	15.647		
Male ID 1	0.006	0.079		
Male ID 2	1.5 x 10 ⁻⁴	0.012		
<i>Size-corrected body mass</i>				
Fixed effects	Estimate	SE	t value	<i>p</i>
Intercept	-383.624	23.235		
Offspring age	-17.802	1.289	-13.813	<0.001
Paternal age	-87.145	29.945	-2.910	0.376
Body size	11.093	0.579	19.157	<0.001
Paternal age x Offspring age	-3.931	1.634	-2.407	0.459
Offspring age x Body size	0.233	0.010	22.282	<0.001
Paternal age x Body size	2.392	0.749	3.196	0.151
Offspring age x Paternal age x Body size	0.027	0.013	2.071	0.039

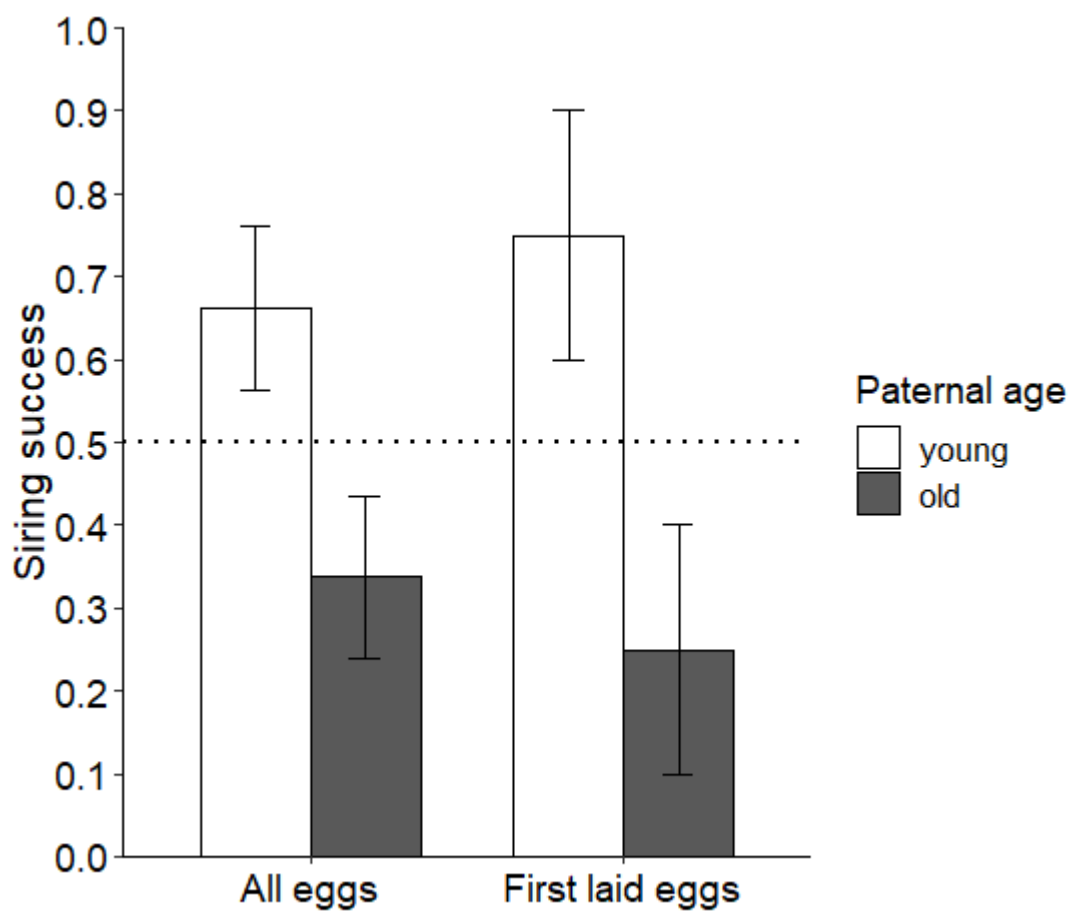
Random effects	Variance	SD
Offspring ID:Maternal ID	434.100	20.840
Maternal ID	188.800	13.740
Male ID 1	4.2×10^{-7}	6.5×10^{-4}
Male ID 2	0.000	0.000

514

515 **Figures**

516

517 Figure 1. Within-dyad siring success (with 95% confidence intervals), considering all eggs (n
518 = 86) and first laid eggs only (n = 28), for the two paternal age categories (young and old). The
519 dotted line represents the random (0.5) expectation.

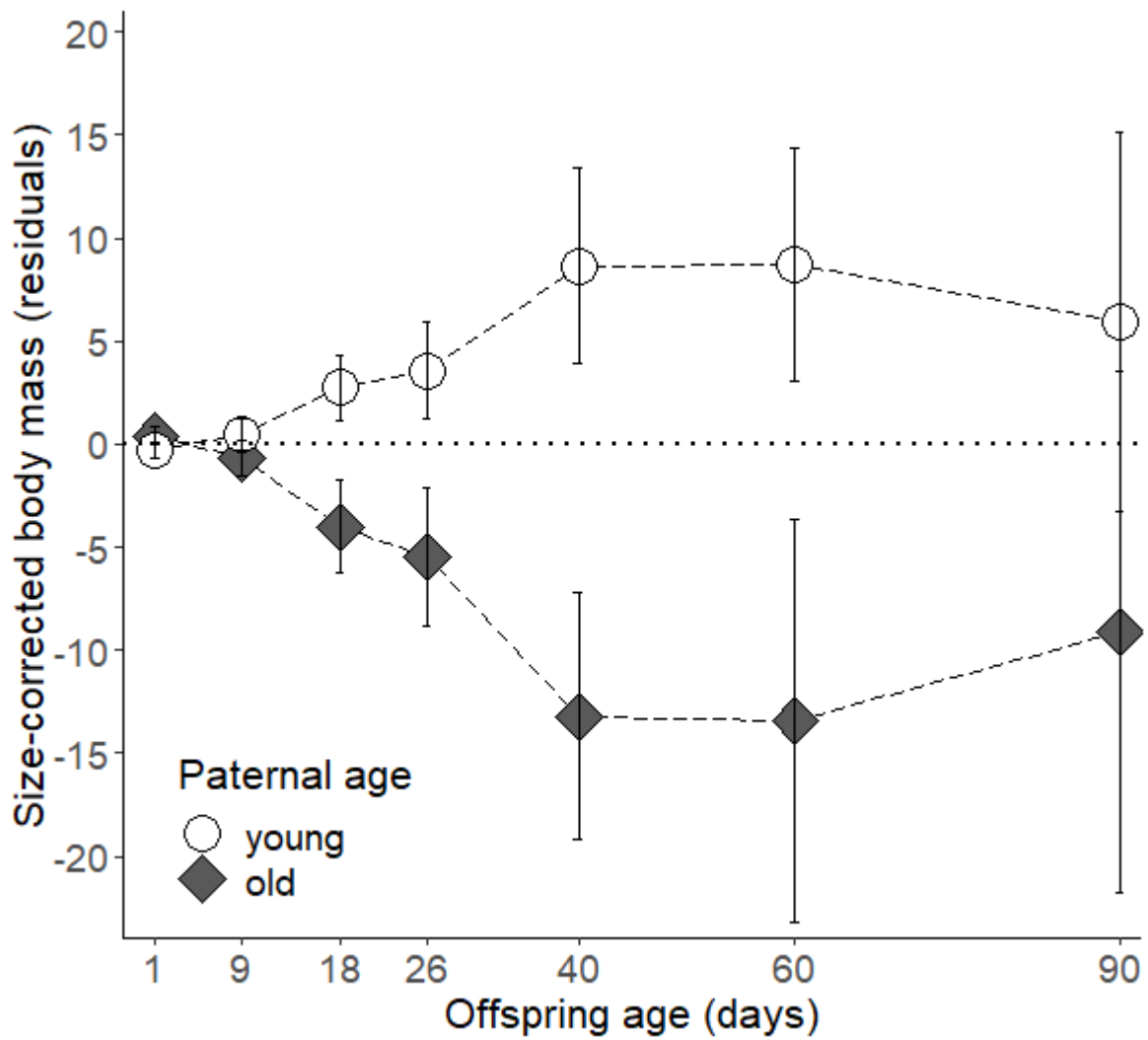


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523 Figure 2. Age-dependent changes, during the first three months of life, in size-corrected body
524 mass of chicks sired by young or old fathers in the North African houbara bustard. Size-
525 corrected body mass refers to the residuals of a regression of body mass on body size (head
526 length). Values are means \pm se.

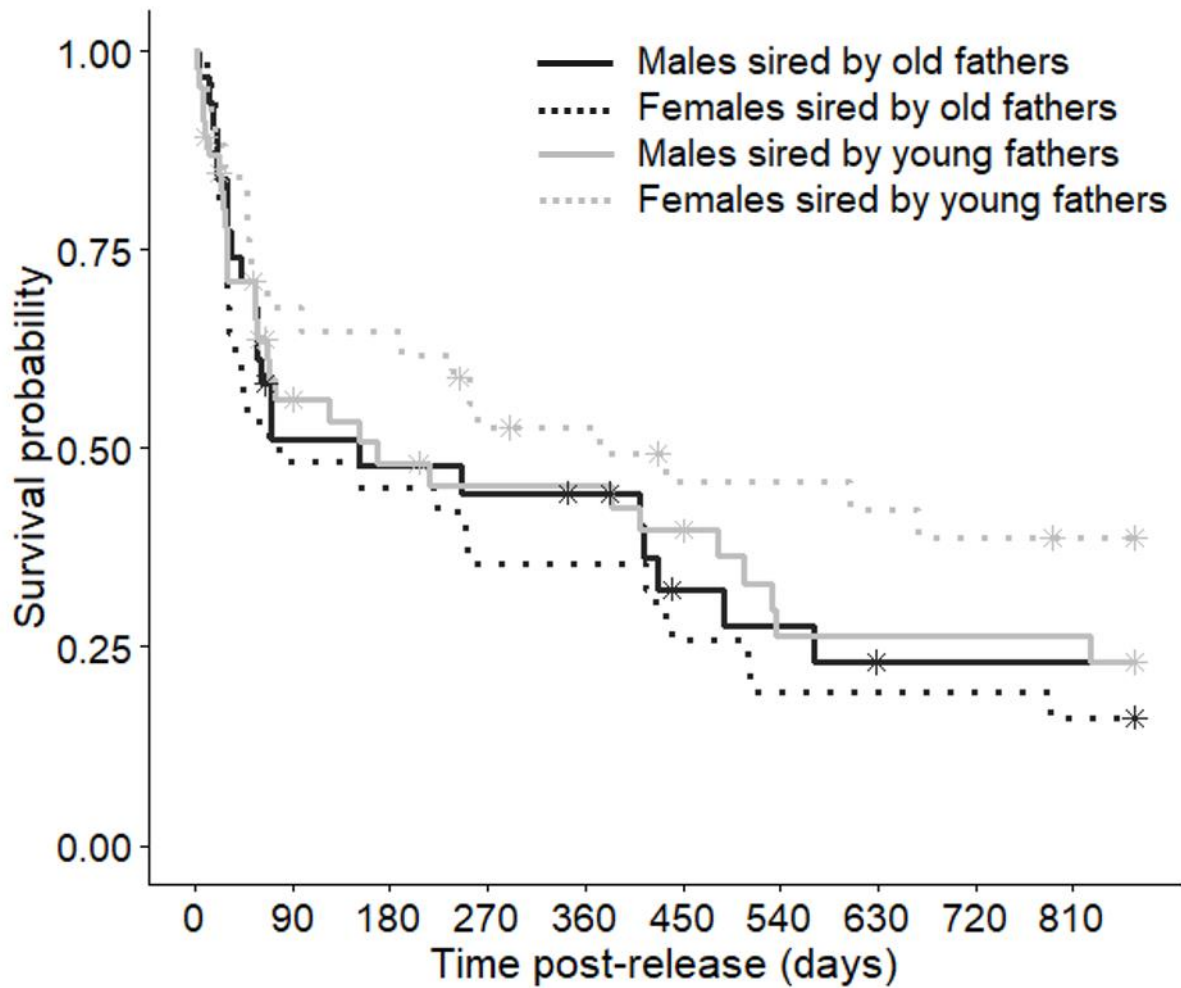


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530 Figure 3. Survival of North African houbara bustard as a function of sex and paternal age, up
531 to 28 months post-release in the wild (n = 142). Solid lines refer to male offspring and dashed
532 lines to female offspring. Stars represent censored observations (i.e., lost individuals).



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