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4 **Experimentally flight-impaired females show**
5 **higher levels of extra-pair paternity in the pied**
6 **flycatcher *Ficedula hypoleuca***

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22 **ABSTRACT**

23 There is no consensus yet on the reasons why females engage in extra-pair copulations
24 (EPC). In some species, females have been shown to accrue some indirect benefits, but
25 these effects are not consistent across species and studies. The sexual conflict
26 hypothesis posits that extra-pair paternity (EPP) is the result of strong selection for male
27 pursuit of EPC without real benefits for females. In order to test this hypothesis, we
28 experimentally reduced wing area (reversibly tying together some primary feathers), in
29 a group of pied flycatcher females (*Ficedula hypoleuca*). The manipulation increases
30 wing loading (body mass/wing area), which is negatively associated with flying ability,
31 and thus with the capacity to escape from unwanted copulations. We compared the
32 levels of EPP in this experimental group with those of a group of un-manipulated
33 females. Experimental females almost doubled the proportion of extra-pair young
34 (EPY) with respect to control females. In addition, more males sired EPY in
35 experimental than in control broods containing EPY. These results suggest that in our
36 study population, EPP could be partially a product of female capacity to avoid EPCs.
37 We also discuss the alternative hypothesis that results might be due to an eventual
38 reduction of female attractiveness.

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47 **INTRODUCTION**

48 Most socially monogamous birds show extra-pair paternity (EPP) [1, 2] and, although
49 90% of them display bisexual parental care [3], at least 70% also present cases of
50 offspring sired by a male outside the pair bond [4]. However, even though EPP may
51 constitute a strong driver of sexual selection, its incidence is poorly understood [5, 6].

52 The main adaptive explanations for female involvement in extra-pair copulations
53 (EPC) propose that females may obtain indirect benefits [4, 7] mainly by gaining “good
54 genes”, enhancing heterozygosity or ensuring fertilization. Great effort has been made to
55 test these hypotheses [7-10], but results are not clear even within the same or closely
56 related species [2, 4, 7]. Contrary to this, the sexual conflict hypothesis argues that
57 males may drive the incidence of EPP towards their benefit overriding female choice [2,
58 9, 11]. Costs for females derived from EPC have been mentioned in the literature [11-
59 14] while benefits have not always been found [15-17]. For an extra-pair fertilization to
60 be successful, it is required that an extra-pair male encounters a female and achieves
61 copulation. In some species, females seek out such encounters, but in others males
62 initiate them [18-20]. There is little information on the proportion of EPP obtained
63 through female solicitation of EPCs, and male-initiated EPCs constitute the most
64 commonly observed events in birds [2]. In some birds females never initiate or solicit
65 EPCs but stay passive or try to escape from extra-pair males [13, 21].

66 Under a female mate choice scenario, it is expected that older, more experienced
67 or larger females should be more able to escape from their mate and more capable of
68 selecting high-quality extra-pair sires [22-24]. But if EPP is the consequence of sexual
69 conflict [2, 11], we expect it to depend on the social mate’s mate-guarding capacity
70 [16], and on the female’s ability to avoid unwanted extra-pair male encounters [13],
71 which may vary with their size, age, social dominance [25] or flight ability. A recent
72 study [26] found that older pied flycatcher (*Ficedula hypoleuca*) females, with longer

73 wings, showed a lower occurrence of EPP, suggesting that traits related to capacity of
74 avoiding unwanted males can decrease the incidence of EPP. Wing loading (body
75 mass/wing area [27]) has been theoretically and empirically negatively related to flight
76 capacity at short distances [28, 29] through the modification of the centre of gravity
77 [27], and in a sexual conflict scenario we should expect a positive relationship between
78 female wing loading and EPP [2].

79 In the present study we manipulated wing loading by reversibly reducing wing
80 area in an experimental group of females, to investigate the effect of this manipulation
81 on EPP in pied flycatchers, a model species in this context as it shows genetic
82 polyandry [13, 21, 30-33]. We had previous evidence of the effects of experimental
83 modification of flying ability by reducing wing area in closely related species [34]
84 which induces gaps in the wing and impairs flight performance [35-37]. Our prediction
85 is that, if EPP is explained by an adaptive mate choice hypothesis, experimental females
86 with a higher wing loading would suffer a reduced capacity to both evade their guarding
87 mates and locate extra-pair sires, thus showing reduced levels of EPP. However, if EPP
88 is driven mainly by sexual conflict, we would expect the opposite pattern [21].

89

90 **MATERIAL AND METHODS**

91 **(a) Field methods**

92 The study was conducted in 2016 in an oak *Quercus pyrenaica* forest in central Spain,
93 where 300 nest-boxes have been installed [38].

94 Nests were randomly assigned to either control or experimental treatments on
95 the first day of construction [39]. On that day, females were captured by using a
96 conventional nest-box trap [40]. The trap was active for a maximum of 1 h to minimize
97 disturbance. All females were identified by their rings or ringed if necessary and mass

98 was recorded with a Pesola spring balance (accuracy 0.25 g). After that, experimental
99 females were handicapped by taping primary remiges five to seven as described in
100 Senar et al. [34]. A rectangular notch was cut on each side of the 3 rachises and they
101 were placed side by side. These remiges were tied all three together with a strip of tape
102 within the notched area, thus creating two wing gaps (Fig.1). We also cut the notched
103 area from the same feathers in control birds, but no strip was added. We took a digital
104 photograph of the wing before and after applying the treatment in 8 experimental
105 females. Pictures were analyzed to estimate surfaces [25]. Reduction in wing area was
106 of $5.75 \pm 0.73\%$, which falls within the normal range for birds when molting [41]. On the
107 2nd day of incubation females were again captured in the nest-box during daytime.
108 They were weighed and the tape in the experimental group was removed. Two females
109 from the experimental treatment and two controls changed nest-box to restart breeding
110 and they were removed from the experiment, so 24 control and 25 experimental nests
111 were included in analyses.

112 There is previous evidence that only inseminations (and also extra-pair
113 inseminations) occurring from day -2 (laying date=0) until the day the penultimate egg
114 is laid [13, 42, 43] result in fertilizations [32, 42].

115 All adults were captured during daytime while feeding nestlings of 7–8 days
116 (nestlings fledge 16–19 days after hatching (hatching day=day 1). They were identified
117 by their rings or ringed if necessary and again weighed. A sample of blood from the
118 brachial vein (10–20 μ l) was taken and stored on Flinders Technology Associates
119 reagent loaded cards (Whatman Bioscience, Florham Park, NJ, USA) until needed for
120 paternity analyses. The following traits associated with EPP in a previous descriptive
121 study of the same population [26] were recorded: female age estimated from ring data,

122 female wing length measured with a ruler and male dorsal blackness as percentage of
123 black plumage on the mantle.

124 When nestlings were 13 days old they were ringed, and a small blood sample
125 from the brachial vein was taken for paternity analyses. Carcasses and abandoned eggs
126 found inside the nest-boxes were collected and frozen for paternity analyses through
127 tissue extraction. Hatching failure affected 42 of 297 eggs in 24% of the nests. Of these,
128 15 did not show any trace of embryonic development suggesting that they were
129 infertile.

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131 **(b) Genetic analyses**

132 We collected samples from 49 families, all of them including the two social mates and
133 their brood at 12 d of age (98 adults, 270 nestlings). DNA was obtained from blood
134 samples using a standard extraction protocol that digests the cards where the blood is
135 fixed and animal tissues from the carcasses and eggs. BioSprint Blood kits (QiaGen,
136 Duren, Germany) were used to extract and purify genomic DNA and Type-it kits
137 (QiaGen, Duren, Germany) to amplify it in the PCR.

138 For genotyping the samples we used 10 pied flycatcher microsatellite loci [44]
139 and the PCR standard protocol described [26]. By running a paternity analysis in
140 CERVUS 3.0.7 (Kalinowski et al. 2007) we determined parentage using the same
141 published criteria [26, 45] (see supplementary material).

142

143 **(f) Statistical analyses**

144 We explored possible differences between treatments in breeding variables by using *t*-
145 tests for hatching date (normally distributed) and Mann–Whitney *U*-test for clutch size
146 (not normal). We then compared changes in female body mass during the treatment

147 period between groups to detect if differences in female flight capacity were caused by
148 changes in wing area (unpaired *t*-test).

149 We analyzed two indicators of the intensity of extra-pair mating interactions,
150 namely the incidence of EPP (presence/absence of EPY in the nest) and the proportion
151 of EPY (number of extra-pair young divided by brood size) as dependent variables in
152 two sets of Generalized Linear Models in R (v 3.5.3; R Foundation for Statistical
153 Computing, Vienna, Austria) with binomial and quasi-binomial distributions to test the
154 effects of our treatment on the dependent variables. We included as covariates the three
155 variables that were significantly associated with EPP in a previous descriptive study in
156 the same population [26]: female wing length, female age and male dorsal blackness. In
157 the case of EPY proportion, we calculated the over-dispersion parameter (\hat{c}) in the full
158 model and used this value to adjust the Akaike information criterion values (AICc),
159 yielding quasi-AICc values corrected for over-dispersion (QAICc). We conducted the
160 *dredge* automated model selection function (MuMIn package Barton, 2019) on all
161 possible combinations of the 3 covariates. We report the conditional average models
162 taking into account all models that differed in less than 4 units from the model with
163 lowest QAIC.

164 We also compared the number of extra-pair mates in nests with EPP in the two
165 treatments with a Mann-Whitney U-test. All values are presented with SE.

166

167 **RESULTS**

168 EPP was found in 22 out of 49 broods (44%) and affected 65 of 270 nestlings (24.1%).
169 Nests with EPP contained an average of 2.95 ± 0.41 EPY (range 1-6) and mostly
170 involved one extra-pair male (66%). In six nests we found extra-pair sires that could not
171 be identified.

172 No significant differences were found with respect to hatching date and clutch
173 size between treatments (Table 1). Mass and mass changes between captures were not
174 substantially different between groups (Table 1).

175 Our treatment caused a strong effect on the proportion of EPY, which was
176 significantly higher in the experimental group (Table 2, Fig.2 and Table S4) and
177 doubled the proportion of EPY with respect to the control group (effect size=58%).
178 Treatment was included in 6 of the 8 most plausible models. The frequency of nests
179 with EPP was 0.33 ± 0.10 in the control group and 0.56 ± 0.09 in the experimental group
180 (effect size=41%). Treatment was conserved in 4 of the 8 most plausible models (delta
181 AIC<4), with a near-significant effect in the average model including male dorsal
182 blackness and female age and wing length (Table 2 and Table S3) although the null
183 model had the lowest AIC.

184 Within nests with EPP, there were more extra-pair fathers per brood in the
185 experimental (1.93 ± 0.16) than in the control group (1.30 ± 0.22) (Mann-Whitney U-test:
186 $Z=2.12$, $P=0.034$).

187

188 **DISCUSSION**

189 We found that females with an impaired flight capacity caused by experimental
190 reduction of wing area showed a large and significant increase in the proportion of EPY
191 and a near-significant increase in the incidence of EPP, controlling for certain traits of
192 the social mate or of the female involved. Experimental females effectively doubled the
193 proportion of EPY in their broods with respect to controls. Moreover, a higher number
194 of extra-pair mates fathered young in experimental than in control broods containing
195 EPY. Brood EPP occurrence and percentage of nestlings affected are slightly higher

196 than in other studies in the same population (28.8 and 13.1% in 2010, [46]; 38.3 and
197 17.6% in 2011 [26]).

198 The majority of the females from both groups increased their body mass during
199 the experiment, although this increase was not significantly different between groups
200 and it probably responds to a parental strategy to carry energetic reserves to lay high
201 quality eggs. The absence of differences between treatments in mass variation suggests
202 that any modification in female flight capacity was exclusively due to changes in wing
203 area in the experimental group.

204 Our main results suggest that handicapped females were less able to escape
205 unwanted copulations with extra-pair males. This increase in EPP frequency is
206 compatible with a scenario in which the levels of EPP are influenced by male pursuit
207 instead of female choice, and is backed up by previously published descriptive data
208 from the same population [26]. This effect was robust after controlling for the possible
209 influence of additional factors in EPP [47, 48]. However, alternative explanations
210 cannot be ruled out if we assume that the manipulation could lead to reduced female
211 attractiveness. In this case, males paired to experimental females may experience: (1)
212 reduced mate-guarding, thus allowing them to pursue EPCs; (2) reduced copulation rate
213 or sperm transfer, leading to sperm-depletion [49]. Both possibilities would lead
214 females to show increased levels of EPP. However, we have no evidence that
215 experimental females were less attractive to their males [50]. Similarly, we could argue
216 that handicapped females may seek EPC to secure feedings or protection from extra-pair
217 partners, however there is no evidence that this happens in this species [51].

218 Our results provide evidence in agreement with the idea that EPP may not be
219 adaptive for females in some species being the result of strong selection in males [11,
220 52]. The occurrence of EPP is likely the result of behavioural and ecological issues [2,

221 11, 26] in which both males and females interact. Since each of the players has its own
222 reproductive interests depending on their own costs and benefits [2, 11], our results
223 suggest that in our study population, EPP is at least partially driven by extra-pair male
224 pursuit and not female benefit.

225

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237

238 **FIGURE CAPTIONS**

239

240 **Figure 1.** Female pied flycatcher female showing the wing manipulation of the
241 experimental group (primary remiges five to seven tied together).

242

243 **Figure 2.** Proportion of EPY in “Experimental” (mean±SE, 0.33±0.06) and “Control”
244 (0.14±0.06) treatments (Central points represent means, boxes SE and whiskers
245 95% CI).

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247 **REFERENCES**

248 [1] Petrie, M. & Kempenaers, B. 1998 Extra-pair paternity in birds: explaining variation
249 between species and populations. *Trends in Ecology & Evolution* **13**, 52-58.
250 (doi:[https://doi.org/10.1016/S0169-5347\(97\)01232-9](https://doi.org/10.1016/S0169-5347(97)01232-9)).

251 [2] Westneat, D.F. & Stewart, I.R.K. 2003 Extra-Pair Paternity in Birds: Causes,
252 Correlates, and Conflict. *Annual Review of Ecology, Evolution, and Systematics* **34**,
253 365-396. (doi:10.1146/annurev.ecolsys.34.011802.132439).

254 [3] Lack, D. 1968 *Ecological adaptations for breeding in birds.*, Methuen & Co. Ltd.,
255 London; 7-12 p.

256 [4] Griffith, S.C., Owens, I.P.F. & Thuman, K.A. 2002 Extra pair paternity in birds: a
257 review of interspecific variation and adaptive function. *Molecular Ecology* **11**, 2195-
258 2212. (doi:doi:10.1046/j.1365-294X.2002.01613.x).

259 [5] Møller, A.P. & Birkhead, T.R. 1994 The Evolution of Plumage Brightness in Birds
260 is Related to Extrapair Paternity. *Evolution* **48**, 1089-1100. (doi:10.2307/2410369).

261 [6] Brouwer, L., van de Pol, M., Aranzamendi, N.H., Bain, G., Baldassarre, D.T.,
262 Brooker, L.C., Brooker, M.G., Colombelli-Négrel, D., Enbody, E., Gielow, K., et al.
263 2017 Multiple hypotheses explain variation in extra-pair paternity at different levels in a
264 single bird family. *Molecular Ecology* **26**, 6717-6729. (doi:10.1111/mec.14385).

265 [7] Forstmeier, W., Nakagawa, S., Griffith, S.C. & Kempenaers, B. 2014 Female extra-
266 pair mating: adaptation or genetic constraint? *Trends in Ecology & Evolution* **29**, 456-
267 464. (doi:<https://doi.org/10.1016/j.tree.2014.05.005>).

268 [8] Griffith, S.C., Blomqvist, D., Andersson, M., Küpper, C., Cuthill, I.C., Kis, J.,
269 Lanctot, R.B., Sandercock, B.K., Székely, T., Wallander, J., et al. 2003 Why do birds
270 engage in extra-pair copulation? *Nature* **422**, 833. (doi:10.1038/422833b).

271 [9] Boulton, R.A., Zuk, M. & Shuker, D.M. 2018 An Inconvenient Truth: The
272 Unconsidered Benefits of Convenience Polyandry. *Trends in Ecology & Evolution* **33**,
273 904-915. (doi:<https://doi.org/10.1016/j.tree.2018.10.002>).

274 [10] Krist, M. & Munclinger, P. 2011 Superiority of extra-pair offspring: maternal but
275 not genetic effects as revealed by a mixed cross-fostering design. *Molecular Ecology*
276 **20**, 5074-5091. (doi:10.1111/j.1365-294X.2011.05337.x).

- 277 [11] Arnqvist, G. & Kirkpatrick, M. 2005 The Evolution of Infidelity in Socially
 278 Monogamous Passerines: The Strength of Direct and Indirect Selection on Extrapair
 279 Copulation Behavior in Females. *The American Naturalist* **165**, S26-S37.
 280 (doi:10.1086/429350).
- 281 [12] Poiani, A. & Colin, W. 2000 Sexually Transmitted Diseases: A Possible Cost of
 282 Promiscuity in Birds? *The Auk* **117**, 1061-1065. (doi:10.2307/4089652).
- 283 [13] Alatalo, R., Gottlander, K. & Lundberg, A. 1987 Extra-Pair Copulations and Mate
 284 Guarding in the Polyterritorial Pied Flycatcher, *Ficedula Hypoleuca*. *Behaviour* **101(1)**,
 285 139-154.
- 286 [14] Birkhead, T.R., Hatchwell, B.J., Lindner, R., Blomqvist, D., Pellatt, E.J., Griffiths,
 287 R. & Lifjeld, J.T. 2001 Extra-Pair Paternity in the Common Murre. *The Condor* **103**,
 288 158-162.
- 289 [15] Blomqvist, D., Andersson, M., Küpper, C., Cuthill, I.C., Kis, J., Lanctotk, R.B.,
 290 Sandercock, B.K. & B., W.J.K. 2002 Genetic similarity between mates and extra-pair
 291 parentage in three species of shorebirds. *Letters of Nature* **419(6907)**, 613-615.
 292 (doi:10.1038/nature01104).
- 293 [16] Moreno, J., Martínez, J.G., Morales, J., Lobato, E., Merino, S., Tomás, G.,
 294 Vásquez, R.A., Möstl, E. & Osorno, J.L. 2010 Paternity Loss in Relation to Male Age,
 295 Territorial Behaviour and Stress in the Pied Flycatcher. *Ethology* **116**, 76-84.
 296 (doi:doi:10.1111/j.1439-0310.2009.01716.x).
- 297 [17] Lifjeld, S.D., S; Ellegren, H; Jan T; Slagsvold, Tore; Dale, Svein; Ellegren, Hans.
 298 1997 A Sexually Selected Paradox in the Pied Flycatcher: Attractive Males Are
 299 Cuckolded. *The American Ornithologist Union* **114(1)**, 112-115.
- 300 [18] Westneat, D.F. 1992 Do female red-winged blackbirds engage in a mixed mating
 301 strategy. *Ethology* **92(1)**, 7-28.
- 302 [19] Dickinson, J.L. 1997 Male detention affects extra-pair copulation frequency and
 303 pair behaviour in western bluebirds. *Animal Behaviour* **53**, 561-571.
 304 (doi:<https://doi.org/10.1006/anbe.1996.0331>).
- 305 [20] Kleven, O.M., G; Lifjeld, JT; Marthinsen, Gunnhild; Lifjeld, Jan T. 2006 Male
 306 extraterritorial forays, age and paternity in the socially monogamous reed bunting
 307 (*Emberiza schoeniclus*). *Journal für Ornithologie* **147(3)**, 468-473.
- 308 [21] Björklund, M. & Westman, B. 1983 Extra-pair copulations in the Pied Flycatcher
 309 (*Ficedula hypoleuca*). *Behavioral Ecology and Sociobiology* **13**, 271-275.
 310 (doi:10.1007/bf00299674).
- 311 [22] Bouwman, K.K., Jan. 2005 Old female reed buntings (*Emberiza schoeniclus*)
 312 increase extra-pair paternity in their broods when mated to young males. *Behaviour*
 313 **142(11)**, 1449-1463.
- 314 [23] Ramos, A.G., Nunziata, S.O., Lance, S.L., Rodríguez, C., Faircloth, B.C., Gowaty,
 315 P.A. & Drummond, H. 2014 Interactive effects of male and female age on extra-pair
 316 paternity in a socially monogamous seabird. *Behavioral Ecology and Sociobiology* **68**,
 317 1603-1609. (doi:10.1007/s00265-014-1769-9).
- 318 [24] Whittingham, L.A. & Dunn, P.O. 2010 Fitness benefits of polyandry for
 319 experienced females. *Molecular Ecology* **19**, 2328-2335. (doi:doi:10.1111/j.1365-
 320 294X.2010.04640.x).
- 321 [25] Plaza, M., Cantarero, A., Cuervo, J.J. & Moreno, J. 2018 Female incubation
 322 attendance and nest vigilance reflect social signaling capacity: a field experiment.
 323 *Behav. Ecol. Sociobiol.* **72**, 24. (doi:10.1007/s00265-017-2423-0).
- 324 [26] Moreno, J., Martínez, J.G., González-Braojos, S., Cantarero, A., Ruiz-de-
 325 Castañeda, R., Precioso, M. & López-Arrabé, J. 2015 Extra-pair paternity declines with
 326 female age and wing length in the Pied Flycatcher. *Ethology* **121**, 501-512.

327 [27] Videler, J.J. 2005 *Avian flight*, Oxford University Press.

328 [28] Kullberg, C., Metcalfe, N.B. & Houston, D.C. 2002 Impaired flight ability during
329 incubation in the pied flycatcher. *Journal of Avian Biology* **33**, 179-183.
330 (doi:10.1034/j.1600-048X.2002.330209.x).

331 [29] Pennycuik, C.J. 1982 The Flight of Petrels and Albatrosses (Procellariiformes),
332 Observed in South Georgia and its Vicinity. *Philosophical Transactions of the Royal*
333 *Society of London Series B-Biological Sciences* **300**, 75-106.
334 (doi:10.1098/rstb.1982.0158).

335 [30] Ellegren, H., Lifjeld, J.T., Slagsvold, T. & Primmer, C.R. 1995 Handicapped males
336 and extrapair paternity in pied flycatchers: A study using microsatellite markers.
337 *Molecular Ecology* **4**, 739-744. (doi:10.1111/j.1365-294X.1995.tb00274.x).

338 [31] Rätti, O., Hovi, M., Lundberg, A., Tegelström, H. & Alatalo, R.V. 1995 Extra-pair
339 paternity and male characteristics in the pied flycatcher. *Behavioral Ecology and*
340 *Sociobiology* **37**, 419-425. (doi:10.1007/bf00170590).

341 [32] Lifjeld, J.T., Slagsvold, T. & Ellegren, H. 1997 Experimental mate switching in
342 pied flycatchers: male copulatory access and fertilization success. *Animal Behaviour* **53**,
343 1225-1232. (doi:<https://doi.org/10.1006/anbe.1996.0430>).

344 [33] Gelter, H.P. & Tegelström, H. 1992 High frequency of extra-pair paternity in
345 Swedish pied flycatchers revealed by allozyme electrophoresis and DNA fingerprinting.
346 *Behavioral Ecology and Sociobiology* **31**, 1-7. (doi:10.1007/bf00167810).

347 [34] Senar, J.C., Domènech, J. & Uribe, F. 2002 Great tits (*Parus major*) reduce body
348 mass in response to wing area reduction: a field experiment. *Behavioral Ecology* **13**,
349 725-727. (doi:10.1093/beheco/13.6.725).

350 [35] Tomotani, B.M., Muijres, F.T., Koelman, J., Casagrande, S. & Visser, M.E. 2018
351 Simulated moult reduces flight performance but overlap with breeding does not affect
352 breeding success in a long-distance migrant. *Functional Ecology* **32**, 389-401.
353 (doi:doi:10.1111/1365-2435.12974).

354 [36] Kiat, Y., Izhaki, I. & Sapir, N. 2016 Determinants of wing-feather moult speed in
355 songbirds. *Evolutionary Ecology* **30**, 783-795. (doi:10.1007/s10682-016-9838-3).

356 [37] Kiat, Y. 2017 Divergent primary moult-A rare moult sequence among Western
357 Palaearctic passerines. *PloS one* **12**, e0187282-e0187282.
358 (doi:10.1371/journal.pone.0187282).

359 [38] Lambrechts, M., Adriaensen, F., Ardia, D.R., Artemyev, A.V., Atiénzar, F.,
360 Bánbura, J., Barba, E., Bouvier, J.-C., Camprodon, J., Cooper, C.B., et al. 2010 The
361 design of artificial nestboxes for the study of secondary hole-nesting birds: a review of
362 methodological inconsistencies and potential biases. *Acta Ornithologica* **45**, 1-26.

363 [39] Moreno, J., Merino, S., Lobato, E., Ruiz-De-Castañeda, R., Martínez-De La
364 Puente, J., Del Cerro, S. & Rivero-De Aguilar, J. 2009 Nest-dwelling ectoparasites of
365 two sympatric hole-nesting passerines in relation to nest composition: An experimental
366 study. *Écoscience* **16**, 418-427. (doi:10.2980/16-3-3233).

367 [40] Cantarero, A., López-Arrabé, J., Plaza, M., Saavedra-Garcés, I. & Moreno, J. 2016
368 Males feed their mates more and take more risks for nestlings with larger female-built
369 nests: an experimental study in the Nuthatch *Sitta europaea*. *Behav. Ecol. Sociobiol.* **70**,
370 1141-1150. (doi:10.1007/s00265-016-2122-2).

371 [41] Hedenström, A. 1998 The Relationship between Wing Area and Raggedness
372 during Molt in the Willow Warbler and Other Passerines (La Relación Entre el Área del
373 Ala y la Desaliñez Durante la Muda de *Phylloscopus trochilus* y de Otras Paserinas).
374 *Journal of Field Ornithology* **69**, 103-108.

375 [42] von Haartman, L. 1956 Territory in the Pied Flycatcher. *Ibis* **98**, 460-475.
376 (doi:10.1111/j.1474-919X.1956.tb01431.x).

- 377 [43] Chek, A.A.L., Jan T.; Robertson, Raleigh J. 1993 Captive study of copulation in
378 the Pied Flycatcher *Ficedula hypoleuca*. *Fauna Norvegica Series C Cinclus* **16(2)**, 67-
379 73.
- 380 [44] Leder, E.H., Karaiskou, N. & Primmer, C.R. 2008 Seventy new microsatellites for
381 the pied flycatcher, *Ficedula hypoleuca* and amplification in other passerine birds. *Mol.*
382 *Ecol. Resour.* **8**, 874-880. (doi:10.1111/j.1755-0998.2008.02096.x).
- 383 [45] Kalinowski, S., Taper, M. & Marshall, T. 2007 Revising how the computer
384 program CERVUS accommodates genotyping error increases success in paternity
385 assignment. *Molecular Ecology* **16**, 1009-1106.
- 386 [46] Moreno, J., Martínez, J.G., González-Braojos, S., Ruiz-de-Castañeda, R.,
387 Cantarero, A. & Sánchez-Tojar, A. 2013 Extra-pair matings, context-dependence and
388 offspring quality: a brood manipulation experiment in pied flycatchers. *Behaviour* **150**,
389 359-380. (doi:10.1163/1568539x-00003056).
- 390 [47] Sirkiä, P.M. & Laaksonen, T. 2009 Distinguishing between male and territory
391 quality: females choose multiple traits in the pied flycatcher. *Animal Behaviour* **78**,
392 1051-1060. (doi:10.1016/j.anbehav.2009.06.022).
- 393 [48] Moreno, J., Velando, A., Gonzalez-Braojos, S., Ruiz-de-Castañeda, R. &
394 Cantarero, A. 2013 Females Paired with More Attractive Males Show Reduced
395 Oxidative Damage: Possible Direct Benefits of Mate Choice in Pied Flycatchers.
396 *Ethology* **119**, 727-737. (doi:10.1111/eth.12112).
- 397 [49] Pizzari, T., Cornwallis, C.K., Løvlie, H., Jakobsson, S. & Birkhead, T.R. 2003
398 Sophisticated sperm allocation in male fowl. *Nature* **426**, 70-74.
399 (doi:10.1038/nature02004).
- 400 [50] Cantarero, A., López-Arrabé, J., Palma, A., Redondo, A.J. & Moreno, J. 2014
401 Males respond to female begging signals of need: a handicapping experiment in the
402 Pied flycatcher *Ficedula hypoleuca*. *Animal Behaviour* **94**, 167-173.
- 403 [51] Sonia Kleindorfer, H.H., Donald Blomqvist and Birgit Fessl. 2005 High frequency
404 of extra-pair fertilisations in the moustached warbler, a songbird with a variable
405 breeding system. *Behaviour*, 1133-1148. (doi:10.1163/156853905774405272).
- 406 [52] Forstmeier, W., Martin, K., Bolund, E., Schielzeth, H. & Kempenaers, B. 2011
407 Female extrapair mating behavior can evolve via indirect selection on males.
408 *Proceedings of the National Academy of Sciences* **108**, 10608-10613.
409 (doi:10.1073/pnas.1103195108).
- 410 [52] Plaza M, Canatarero A, Gil D, Moreno J, Data from: Experimentally flight-
411 impaired females show higher levels of extra-pair paternity in the pied flycatcher
412 *Ficedula hypoleuca*. Dryad Digital Repository. (doi:10.5061/dryad.5sp0m4t)
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414 **Table 1.** Average values (\pm SE) and results of Unpaired *t*-tests and Mann–Whitney *U*-
 415 test for relevant variables in the “Experimental” and “Control” groups.

	Control	Experimental	Statistic	<i>P</i>
Hatching date	61.87 \pm 0.80	63.44 \pm 0.79	<i>t</i> = 1.38	0.17
Clutch size	6.12 \pm 0.12	5.96 \pm 0.11	<i>U</i> = 256	0.37
Female mass I	13.48 \pm 0.32	12.96 \pm 0.30	<i>t</i> = -1.17	0.24
Female mass II	14.74 \pm 0.17	14.49 \pm 0.16	<i>t</i> = -1.00	0.32
Change in female mass	1.25 \pm 0.34	1.47 \pm 0.31	<i>t</i> = 0.46	0.64

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417 **Table 2.** Average models calculated from the set of most plausible models for EPY
 418 proportion (number of EPY/brood size) (Δ QAIC<4) and EPP
 419 (occurrence/absence) (Δ AIC<4) as dependent variables with binomial
 420 distributions, and experimental treatment, female age, female wing length and
 421 male dorsal blackness as predictors (models in supplementary material).
 422 Estimates for the binomial model were transformed (antilogarithm) to convert
 423 them into odds-ratios.

	EPY				EPP			
	Estimate	Std. Error	Z value	<i>P</i>	Estimate	Std. Error	Z value	<i>P</i>
Treatment	1.49	0.38	3.74	<0.01	3.25	0.69	1.64	0.09
Female age	-0.03	0.17	0.18	0.85	1.36	0.33	0.91	0.36
Female wing	-0.52	0.19	2.61	<0.01	0.98	0.35	0.05	0.95
Male blackness	-0.01	0.01	1.19	0.23	0.98	0.02	0.88	0.37

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