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An experimental increase in female mass

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during the fertile phase leads to higher levels

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of extra-pair paternity in pied flycatchers

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Ficedula hypoleuca

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Mireia Plaza^{1,*}, Alejandro Cantarero² and Juan Moreno¹

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12 MP ORCID: 0000-0001-7847-2081; AC ORCID: 0000-0002-5816-701X

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14 ¹ Museo Nacional de Ciencias Naturales (CSIC). Dpto. Ecología Evolutiva. C/ José
15 Gutiérrez Abascal 2, 28006 Madrid.

16 ² Section of Ecology, University of Turku, Turku 20014, Finland

17

18

19 *Corresponding author: mireia.plaza@gmail.com

20 Telephone number: +34 91 4111328 1218

21 **ABSTRACT**

22 Female mass in most altricial birds reaches its maximum during breeding at egg-laying,
23 which coincides temporally with the fertile phase when extra-pair paternity (EPP) is
24 determined. Higher mass at laying may have two different effects on EPP intensity. On
25 the one hand, it would lead to increased wing loading (body mass/wing area), which
26 may impair flight efficiency and thereby reduce female's capacity to resist unwanted
27 extra-pair male approaches (sexual conflict hypothesis). On the other hand, it would
28 enhance female condition, favouring her capacity to evade mate-guarding and to search
29 for extra-pair mates (female choice hypothesis). In both cases, higher female mass at
30 laying may lead to enhanced EPP. To test this prediction, we reduced nest building
31 effort by adding a completely constructed nest in an experimental group of female pied
32 flycatchers (*Ficedula hypoleuca*). Our treatment caused an increase in mass and thereby
33 wing loading and this was translated into a significantly higher EPP in the manipulated
34 group compared with the control group as expected. There was also a significant
35 negative relationship between EPP and laying date and the extent of the white wing
36 patch, an index of female dominance. More body reserves at laying mean not only a
37 higher potential fecundity but a higher level of EPP as well. This interaction had not
38 previously received due attention but should be considered in future studies of avian
39 breeding strategies.

40 **Significance statement**

41 While most research has been focused on determining possible criteria for extra-pair
42 mate choice by females, less effort has been made on establishing if female traits are
43 related to EPP and its intensity. One such trait is mass at laying which attains its highest
44 level for breeding females of altricial birds. Our study indicates that a higher mass
45 during the fertile phase not only has implications for female fecundity and predation
46 risk but also for EPP in the resulting brood as more mass means a higher EPP.

47

48 **Keywords:** extra-pair paternity, wing loading, flight ability, nest building costs, female
49 traits.

50

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55 **INTRODUCTION**

56 Most female altricial birds show important changes in body mass in the course of the
57 breeding cycle, increasing in mass before egg laying to a maximum just at laying,
58 maintaining partly this high mass during incubation and losing it after hatching when
59 feeding the chicks, thus returning to pre-breeding levels (Moreno 1989). This seasonal
60 variation in female body mass has been interpreted as the result of a parental adaptive
61 strategy and constitutes an important aspect of avian breeding biology. Firstly, a high
62 body mass at laying would allow females to carry enough energetic reserves to lay high
63 quality eggs, and then to keep a good condition when activity is reduced during
64 incubation, when foraging is compromised. Later on, body mass would have to be
65 reduced to enhance flying efficiency during nestling provisioning (Norberg 1981).
66 Those changes in female body mass have been observed even in experiments where
67 parents were supplementary fed (Moreno 1989; Sanz and Moreno 1995; Lothery et al.
68 2014). Changes in mass affect crucially female flight ability during the breeding cycle
69 through the modification of wing loading (body mass/wing area) (Videler 2005), a trait
70 that has been theoretically and empirically negatively related to flight capacity at short
71 distances (Pennycuick 1982; Kullberg et al. 2002).

72 In the last two decades, increasingly accurate molecular tools have revealed that
73 90% of socially monogamous bird species show extra-pair paternity (EPP), resulting
74 from mating outside the social pair-bond (Petrie and Kempenaers 1998; Westneat and
75 Stewart 2003). Given its influence on fitness, EPP must be an important factor in sexual
76 selection (Møller and Birkhead 1994; Griffith et al. 2002; Garamszegi and Møller
77 2004). However, although great effort has been made to test adaptive explanations
78 behind extra-pair copulation (EPC) behaviour within and across species, there is yet no
79 consensus on the key factors that are behind it (Griffith et al. 2003; Forstmeier et al.
80 2014; Boulton et al. 2018).

81 EPP results from the complex interaction between a female, an extra-pair male
82 and the social mate, so the behaviour and traits of each of those parties is of importance
83 for the resulting EPP patterns. Most adaptive explanations propose that females may
84 obtain indirect benefits from EP behaviour (Møller and Birkhead 1994; Forstmeier et al.
85 2014), e.g. through improving offspring viability through the choice of more attractive
86 extra-pair sires. Under this point of view, the outcome of EPP depends on the interplay
87 of two factors. Firstly, on the social male's capacity to guard their mates and fight off
88 male intruders, which is a function of his aggressiveness and dominance (Moreno et al.
89 2010b). And secondly, on the female's ability to evade mate guarding tactics (Alatalo et
90 al. 1987), which may depend on her size, age (Bouwman and Komdeur 2005; Ramos et
91 al. 2014), social dominance expressed through ornaments (Plaza et al. 2018) or flight
92 ability (Stutchbury and Robertson 1987). In this respect, a high female body condition
93 would favor the capacity of females to evade the attention of their mates and fly in
94 search of extra-pair mates, roaming more easily through the breeding area.

95 In contrast with the above explanation, the sexual conflict hypothesis (Westneat
96 and Stewart 2003; Arnqvist and Kirkpatrick 2005) derived from sexual selection,
97 proposes that EPP results from a dynamic interplay in which both sexes strive towards
98 conflicting ends. Under this scenario, strong selection in males to seek copulations
99 independent of female choice would lead to higher incidence of EPP despite female
100 costs to avoid EPCs (Arnqvist and Kirkpatrick 2005; Forstmeier et al. 2014). A
101 consideration of female traits that relate to EPP may help us detect whether variation in
102 female capacity to avoid EPCs explains EPP patterns. For instance, if EPCs are the
103 result of male coercion (Westneat and Stewart 2003; Boulton et al. 2018), an increase in
104 female mass would result in a higher wing loading which is translated into a reduced
105 flight ability and a diminished capacity of the females to evade unwanted suitors.

106 Indeed, such a negative relation between EPP and female flight ability has been found
107 in some recent studies (Moreno et al. 2015; Plaza et al. 2019).

108 Bird nests have traditionally been considered as a simple receptacle for eggs and
109 nestlings (Deeming 2013), while their functional characteristics in relation to avian
110 reproduction have recently been taken into account (Cantarero et al. 2015b; Bailey et al.
111 2016). The costs of nest building have largely been documented (Hansell 2000) in terms
112 of physiological stress for the builders (Morales et al. 2008; Moreno et al. 2008), their
113 health and body condition (Tomás et al. 2006) or survival (Gill and Stutchbury 2005).
114 The effort spent on this task may constrain reproductive behaviour during subsequent
115 breeding phases, particularly so for the sex that is mainly involved in nest building. We
116 have shown in a previous experiment that females whose nest construction costs are
117 experimentally reduced, display improved body condition that results in a higher
118 reproductive success (Moreno et al. 2010a). In many species, nest building precedes or
119 overlaps in time with the fertile period and the time when reserves are accumulated in
120 preparation for egg laying. Thus, we may expect that experimentally reducing or
121 eliminating the cost of nest building may lead to an enhanced accumulation of reserves
122 prior to laying (Moreno 1989), resulting in a higher condition but also in a higher wing
123 loading during the fertile phase.

124 In the present study, we manipulated female body condition and wing loading,
125 by drastically reducing female nest building effort in order to investigate the effect of
126 this manipulation on EPP in pied flycatchers (*Ficedula hypoleuca*), a model species in
127 studies of genetic polyandry e.g. (Ellegren et al. 1995). The manipulation involved
128 adding a completely built nest to an experimental set of nest-boxes. In this species, nest
129 building is conducted mainly (Gelter and Tegelström 1992; Martínez-de la Puente et al.
130 2009) or exclusively (Curio 1959) by the female. Our previous evidence shows that this

131 modification of nest building effort exclusively increases female body condition
132 (Moreno et al. 2010a), whereas a food supplementation experiment would have also
133 affected males (Moreno et al. 1999). We test the hypothesis that increases in female
134 body mass at this sensitive period will lead to increased EPP levels through enhanced
135 condition or reduced flight efficiency. To take into account female quality and
136 dominance we included laying date and the extent of a female social plumage signal as
137 independent variables, as well as a plumage signal of the social mate's dominance.

138

139 **MATERIAL AND METHODS**

140 **General field methods**

141 This study was conducted during the spring of 2016 in a deciduous forest of Pyrenean
142 oak *Quercus pyrenaica*, at 1200 m.a.s.l. near Valsaín, central Spain (40°54'N, 4°01'W).
143 A total of 450 nest-boxes have been installed in this area since 1991, leading to a series
144 of long term studies of pied flycatchers breeding in them (the bottom area of the nest-
145 box was 175 cm² and the distance from the bottom to the entrance hole was 12.5 cm,
146 Lambrechts et al. (2010)). The breeding season of this species lasts from the middle of
147 April when the first birds arrive from migration, to the beginning of July when all
148 chicks have fledged. We clean all nest-boxes every year after breeding is over. Daily
149 checking was done from April 15 to detect the initiation and progress of nest building
150 until the end. Afterwards, all occupied nest-boxes were checked every 2-3 days to
151 record laying date (Julian calendar), clutch size, hatching date and brood size. The
152 modal clutch size in the population is 6, and most females begin incubation on the
153 laying of the penultimate egg (Ruiz-de-Castañeda et al. 2012) so we considered
154 incubation to begin on the laying of the fifth egg (mean incubation period is 14 days).

155

156 **Nest manipulation**

157 The average reported time spent in nest building by flycatchers is 4 to 11 days (Curio
158 1959; Lundberg and Alatalo 1992; Moreno et al. 2008). Although intra-pair copulations
159 have been reported 9 days before the laying of the first egg (Von Haartman 1956),
160 experiments by Lifjeld et al. (1997) showed that only inseminations occurring from day
161 -2 before the laying of the first egg until the day the penultimate egg is laid result in
162 fertilizations. This short fertilisation window coincides in time with most observed
163 copulations, which are confined to this relatively short period immediately before the
164 start of egg laying (Von Haartman 1956; Alatalo et al. 1987; Chek et al. 1993). In the
165 year in which this study was conducted (2016), a cold spell in May at the time of nest
166 building led to delays in laying (the average time between the end of nest construction
167 and laying date was $11 \pm \text{SE } 0.57$ days). This is in contrast with the typical pattern in
168 which only a few days elapse between nest completion and laying (Moreno et al.
169 2010a). Thus, nest building did not overlap the period when females were fertile, so the
170 effects of the experiment in terms of changes in EPP cannot be due to behavioural
171 changes occurring during nest building. There was no association between the length of
172 the interval from finished nest building to start of laying and EPP (Spearman's rank
173 correlation: $r_{57} = 0.15$, $P = 0.23$). This suggests that the degree of overlap between nest
174 building activities and the fertile phase did not affect the results of our experiment.

175 We randomly assigned nests to either control or experimental treatments on the
176 first building day, which was detected by the presence of a few nest material pieces
177 placed in a circle (*Cistus laurifolius* bark strips and oak leaves). We discarded nests if
178 they were more advanced than this early stage. In total 36 control nests and 23
179 experimental nests were included in the experiment. A full description of nest material
180 composition for pied flycatchers in our study area is provided in Moreno et al. (2009).

181 The manipulation consisted in placing a completed flycatcher nest inside the nest-box
182 on the day when the treatment was assigned to the experimental group. Control nests on
183 the contrary were not manipulated until they were naturally completed and simply
184 exchanged for other completed flycatcher nests. In this way, we made sure that all
185 active nests (where eggs were laid) had experienced the same level of human
186 disturbance, with the difference that in the experimental group female building costs
187 were greatly reduced with respect to the control group. Nest completion was determined
188 by the same observer following the standard criteria of the presence of a rounded
189 compact nest cup (Moreno et al. 2010a). All added (experimental) or exchanged
190 (control) nests were obtained from freshly completed Pied flycatcher nests that we had
191 previously found abandoned in the study area before hatching of nestlings in previous
192 reproductive seasons, since when they had been frozen at -20°C until use. We weighed
193 all of them once defrosted and shortly before their usage, as well as all the substituted
194 nests in the control group. No differences in mass between introduced ($21.80 \pm \text{SE}1.63$ g)
195 and substituted ($24.20 \pm \text{SE}1.45$ g) nests were found ($F_{1,57} = 1.27$, $P = 0.48$). As in both
196 groups females added some material after the manipulation, all nests were also weighed
197 after laying so the amount of material collected by females was known for both groups
198 (difference in mass between the supplemented nests and the final ones). Accordingly,
199 the average total amount of material collected by control and experimental females was
200 $24.72 \pm \text{SE}1.50$ and $5.46 \pm \text{SE}1.88$ g respectively, showing that control females provided
201 almost five times as much material as experimental females, with the difference being
202 significant between the two treatments ($F_{1,57} = 63.5$, $P < 0.01$). After manipulation no
203 nest desertion was detected.
204
205

206 **Capture and sampling**

207 All females were captured on day 7 of incubation in order to weigh them after laying
208 (capturing them sooner may lead to desertion), by simply blocking the nest-box
209 entrance and catching them during daytime. Later in the season, all adults were captured
210 in their nest-boxes while feeding nestlings of 7–8 days (nestlings fledge 16–19 days
211 after hatching) by using a conventional nest-box trap set at the entrance of the nest-box
212 (Cantarero et al. 2016b). The trap was active for a maximum of 1 h to minimize
213 disturbance to adult birds and nestlings, and it was removed earlier if both adults were
214 trapped before that time. No individual remained more than 5 minutes inside the nest-
215 box after the trap closed. All birds were identified by their rings or ringed if necessary
216 and mass was recorded with a Pesola spring balance (accuracy 0.25 g). Females were
217 aged by their rings, and for the ones that were not ringed we assigned the age of 2 years
218 (typical age at which females are recruited to the breeding population in our studies).
219 We also measured wing length with a stopped ruler to the nearest mm. As a measure of
220 female plumage ornaments, a digital photograph of the white wing patch was taken
221 from above at a height of 10 cm from the animal by placing the wing in its natural
222 folded position on a flat surface with a ruler besides for reference, and forming a
223 roughly 135° angle with the wing. The same photographic technique has been used in
224 previous studies (Moreno et al. 2014; Cantarero et al. 2016a). All digital photos were
225 later analyzed with Adobe Photoshop CS5 v.11.0. to estimate surfaces with the
226 reference to the ruler. A zoom of 400 % and a paintbrush of 17 pixels, with 100 %
227 hardness and 25 % spacing were used to estimate white wing patch areas estimated in
228 cm² (Sirkiä et al. 2015). The percentage of male dorsal blackness was estimated by
229 scoring black feathers in the head and mantle at 10 point intervals from 5 (0-10%) to 95
230 (90-100%) (Canal et al. 2011). A small sample of blood from the brachial vein (10–20

231 μ l) was taken and stored on Flinders Technology Associates reagent loaded cards
232 (Whatman Bioscience, Florham Park, NJ, USA) until needed for the paternity analyses.
233 All captures were performed between 8 and 10 a.m. in the morning.

234 We ringed all chicks when they were 13 days old (hatching day = day 1), and
235 we similarly collected a small blood sample from the brachial vein for paternity
236 analyses. All carcasses and abandoned eggs found inside the nest-boxes during regular
237 checks were collected and frozen on the same day for later paternity analyses through
238 tissue extraction. Hatching failure affected 20 of 348 eggs in 33% of the nests (N = 20).
239 However, 13 eggs did not show any trace of embryonic development suggesting that
240 they were infertile (this can easily be visually detected by examining the egg in contrast
241 to the light). Moreover, 10 chicks (of two different nests) were predated so we left those
242 nests out of the experiment.

243

244 **Genotyping**

245 We obtained samples from 59 families, including the two social mates and their whole
246 brood at 12 d of age (112 adults, 325 nestlings). DNA was obtained from blood samples
247 using a standard extraction protocol that digests the cards where the blood was fixed
248 and animal tissues from the carcasses and eggs. We used BioSprint Blood kits (QiaGen,
249 Duren, Germany) to extract and purify genomic DNA from the blood samples and
250 Type-it kits (QiaGen, Duren, Germany) to amplify approximately 5 ng of template
251 DNA in the PCR.

252 We used 10 pied flycatcher microsatellite loci for genotyping, following
253 published primer sequences described in Leder et al. (2008). Two multiplex PCR
254 reactions were designed as described before (Moreno et al. 2015), in which we
255 amplified loci Fhy301, Fhy466, Fhy336, Fhy370 and Fhy452 in one reaction (set I) and

256 Fhy328, Fhy223, Fhy236, Fhy304 and Fhy407 in the other (set II). The PCR program
257 consisted in a denaturing step of 94°C during 2 min, then 30 cycles with 30 s at 94°C,
258 30 s at 55°C and 30 s at 72°C, finally an extension step of 2 min at 72°C. Conditions
259 were the same for both multiplex sets. With 13, 14, 18, 17, 15, 25, 17, 29, 10 and 15
260 alleles respectively, all loci were polymorphic and a combined non-exclusion
261 probability of second parent of 0.00000114 as calculated by CERVUS 3.0.7
262 (Kalinowski et al. 2007). Three loci (Fhy336, Fhy236 and Fhy452) significantly
263 deviated from Hardy Weinberg equilibrium after Bonferroni correction, but in only one
264 locus (Fhy452) CERVUS estimated a null allele frequency that was higher than 0.05.

265

266 **Paternity analysis**

267 We determined genetic parentage by comparing the genotypes of chicks with those of
268 female and male nest owners. We considered that chicks were the offspring of the adults
269 if their genotypes were compatible for the loci typed. To confirm this, we ran a paternity
270 analysis using CERVUS (v 3.0.7. Field Genetics), specifying for all chicks the identity
271 of the mother and allowing the software to assign the genetic father from the whole
272 sample of adult males. In the paternity analyses, we used a level of confidence of 95%,
273 we allowed a proportion of 5% mistyped loci and assumed that the proportion of
274 candidate parents sampled was 85%, with a minimum number of 6 loci typed. CERVUS
275 assigned paternity to the male with the highest LOD score (obtained by taking the
276 natural log of the overall likelihood ratio; the likelihood ratio is the probability for the
277 candidate parent to be the true parent divided by the probability for the candidate parent
278 of not being the true parent). We accepted this as the genetic father of a given nestling
279 only when the difference between the LOD scores of the first and the second most
280 probable fathers was statistically significant (Kalinowski et al. 2007). We considered as

281 extra-pair offspring those nestlings (82 in total) with two or more mismatched loci with
282 respect to their social fathers by CERVUS (the mismatch never involving markers that
283 deviated from Hardy–Weinberg equilibrium). From all these nestlings, 46 cases were
284 assigned to a male which was not included in the population male pool (most probably a
285 non-territorial floater). However, when the difference in LOD score between the first
286 and the second most probable father was not significant, we did not assign a genetic
287 father (36 cases in total). We also visually checked if those males assigned by CERVUS
288 as fathers of extra-pair offspring matched the genotypes of the nestlings they were
289 assigned to. We took a conservative rule, and considered as a father–offspring pair in 9
290 out of the 46 cases of extra-pair chicks assigned by the program, since these
291 mismatched the social male in only one locus.

292 We considered that a single locus mismatch between the genotypes of the male
293 and a chick could be due to mutation or genotyping mistakes, and for this reason we
294 overruled the CERVUS decision of considering these as extra-pair offspring. One
295 mismatch between females and offspring occurred in 14 cases (8 cases in the control
296 group and 6 in the experimental group), and in 16 cases with fathers (7 cases in the
297 control group and 9 in the experimental group).

298

299 **Statistical analyses**

300 We first investigated possible differences between groups in breeding variables
301 (hatching date and clutch size) and relevant female and male traits which could
302 influence the effect of our treatment. When they were not normally distributed we
303 performed Mann–Whitney *U*-tests.

304 We then examined the effect of our treatment on female wing loading by
305 performing an unpaired *T*-test as it was normally distributed. Following Moreno et al.

306 (2015) we extracted an index of wing loading (g/dm^2) by dividing female body mass by
307 the square of wing length ($n = 47$). This index was validated with direct measurements
308 of wing areas in the field in a pilot study conducted on birds not included in the
309 experiment. In this study conducted in 2017, wing area was estimated from photographs
310 ($n = 41$) of the contour of flattened wings against a sheet of paper with a ruler for
311 reference as described above. The correlation of the two measures shows that our index
312 was an acceptable proxy for wing loading (Spearman's rank correlation: $r_{71} = 0.77$;
313 $P < 0.001$). As we wanted to examine potential changes in wing loading caused by the
314 effect of our treatment on female body mass, we first checked for differences in female
315 wing length and then also in female mass. To that end, we performed two unpaired *T*-
316 tests as both variables were normally distributed. As these mentioned traits are related
317 to age, we also examined differences between groups in female age by performing a
318 Mann–Whitney *U*-test, due to its lack of normality. All analyses mentioned were done
319 with the STATISTICA package, v 10.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

320 The incidence of EPP was analyzed in two ways. On the one hand, as a binary
321 response (occurrence vs. absence of EPP) within nests by a univariate generalized linear
322 model using the GENMOD procedure in SAS v9.4 (StatSoft, Inc., Tulsa, Oklahoma,
323 USA), with a binomial distribution, to test the effect of our treatment on EPP
324 occurrence. Three additional potentially relevant independent variables were also taken
325 into account, trying not to add unnecessary complexity to the analyses. First, we
326 included laying date since extra-pair behaviour could be influenced by the availability
327 of reproductive individuals, which varies throughout the season as reproductive pairs
328 are established. Second, we took into account the extent of the area of the female white
329 wing patch (since we have previously shown that it is a predictor of individual social
330 signalling capacity (Plaza et al. 2018) and territorial defence behaviour, through

331 testosterone levels (Cantarero et al. 2016a). Finally we also included the social male
332 dorsal blackness as a measure of his dominance which is positively related to the mate
333 guarding effect, and significantly negatively related to EPP in previous published
334 studies (Moreno et al. 2015). We also examined potential differences in those female
335 and male characteristics between groups. On the other hand, we conducted a similar
336 analysis using a different univariate generalized lineal model following the same
337 procedure, but using instead the proportion of EPY (number of extra-pair young divided
338 by brood size with “event/trial” syntax) as a measure of extra-pair paternity. All values
339 are presented with standard error.

340

341 **Data availability**

342 The datasets generated during and/or analyzed during the current study are available
343 from the corresponding author MP on reasonable request.

344

345 **RESULTS**

346 EPP occurred in 21 out of 59 broods (35.59%) and affected 82 of 325 nestlings
347 (25.23%). We found non-identified extra-pair sires in five nests. The number of EPY in
348 nests with EPP ranged from 1 to 7 nestlings, being on average 3.90 ± 0.42 EPY, which
349 represents $67.76 \pm 0.40\%$ of the broods on average.

350 The two experimental groups were similar in hatching date and clutch size
351 (Table 1, both $P > 0.40$). We did not find differences in female wing length and age
352 between experimental and control groups (Table 1). However, we did find significant
353 differences in female mass (Table 1), which was higher in the experimental than in the
354 control group. Also the experiment was successful in inducing differences in female

355 wing loading during incubation between treatments due to higher values in the
356 experimental group with respect to the control one (Table 1).

357 Our manipulation also caused an effect on the occurrence of EPP, which was
358 significantly higher in the experimental group (Table 2, Fig. 1). The final model also
359 included significant negative associations between EPP occurrence and the extent of the
360 female white wing patch (Table 2; mean wing patch area for nests with EPP and without
361 EPP were 1.28 ± 0.07 and 1.42 ± 0.05 cm² respectively; $t = 1.41$; $P = 0.16$), and between
362 EPP and laying date (Table 2; mean laying date for nests with EPP and without EPP
363 were 49.10 ± 0.46 and 49.92 ± 0.33 respectively, day 1=April 1 ; $t = 1.45$; $P = 0.15$).

364 We also found a significant effect of our treatment on the proportion of EPY
365 (Table 2) which was higher in the experimental than in the control group (means for
366 each group were 0.31 ± 0.07 and 0.15 ± 0.05 , effect size was 67% following Nakagawa
367 and Cuthill (2007). We also found significant negative relationships between the
368 proportion of EPY and both the extent of female white wing patch and laying date
369 (Table 2), although the associations on their own were not significant (white wing patch
370 Spearman's rank correlation: $r_{57} = -0.19$, $P > 0.05$; laying date Spearman's rank
371 correlation: $r_{57} = -0.22$, $P > 0.05$) (Table 2). There were no differences between groups
372 in the extent of the female white wing patch and male dorsal blackness (Table 1).

373

374 **DISCUSSION**

375 The experimental reduction of nest building effort resulted in a significant increase in
376 female body mass and wing loading and a subsequent increase in the occurrence of EPP
377 and EPY in the experimental group. We also found that the probability of a nest
378 containing EPP and the proportion of EPY were negatively related to the extent of the
379 female white wing patch and laying date.

380 In the present study, control females took an average of 3 days to build the nest
381 and collected almost 24 g of nest material, which is similar to values reported in other
382 studies of populations breeding in central Spain (Moreno et al. 2008; Moreno et al.
383 2010a). Females constructed their nests at a rate of 6 g/day. These high rates may imply
384 important energy costs as indicated by associations of building rate with female
385 physiological costs in this species (Moreno et al. 2008), causing a significant effect on
386 female body mass and therefore on wing loading, as we detected when comparing this
387 variable between groups. Predation on adult females has been found to be high during
388 nest building and egg laying, caused by vulnerability when collecting nest materials due
389 to the increased female mass during this stage (Slagsvold and Dale 1996). However
390 there was no predation in our study population in either of the experimental groups as
391 deduced from the absence of cases of early nest abandonment.

392 Our experimental results showed that females of the experimental group
393 displayed a higher condition and wing loading as well as higher EPP levels. These
394 results are in accordance with a previous study reported by (Plaza et al. 2019), in which
395 handicapped females with a diminished flying ability caused by an increased wing
396 loading, also displayed higher EPP levels. Wing loading has previously been negatively
397 correlated with flying capacity and a reduced manoeuvring ability (van den Hout et al.
398 2010; Salewski et al. 2014). In our treatment we found a higher body mass (translated
399 into higher wing loading) in the experimental group during the incubation period (soon
400 after our nest manipulation treatment was applied). Assuming that mass at incubation
401 reflects mass during nest building, these results suggest that females that did not have to
402 build a complete nest before laying could dedicate more time to feed themselves and
403 increase their reserves to better provision their eggs with resources. Moreno et al.

404 (2010a) found that a reduction in nest building effort was translated into increased
405 offspring fitness.

406 One interpretation of our results would support the role of sexual conflict in the
407 evolution of EPP. This interpretation would explain the patterns as caused by
408 experimental females being less able to escape from unwanted copulations with extra
409 pair males, thereby increasing their EPP rate (Plaza et al. 2019). This is in agreement
410 with a scenario in which the levels of EPP would be influenced by male coercion
411 instead of female choice (Björklund and Westman 1983) and it is consistent with the
412 results found in a non-experimental study by Moreno et al. (2015), where a positive
413 association between wing loading and EPP was reported. In contrast, an adaptive mate
414 choice explanation would support the interpretation that improved body condition in
415 experimental females led to increases in female condition and extra time, allowing them
416 to seek out EPC by spending more time in extra-territorial forays and evading their
417 social mate's guarding. None of these two options can be discarded. An alternative
418 explanation would predict a potentially enhanced experimental female attractiveness
419 due to the improved body condition translated into a higher capacity to lay a large
420 number of high quality eggs (increased fecundity and fitness perception). In this case,
421 males paired to experimental females would increase mate-guarding and copulation rate
422 (Pilastro et al. 2002; Griggio et al. 2003; Griggio et al. 2005) leading to lower levels of
423 EPP. We can now rule out this hypothesis as our results do not support it.

424 During the fertile period of the female, the social male would face a compromise
425 between mate guarding and searching for potential EPCs. We consider that this
426 compromise would not be affected by our treatment, as female fertility (Lifjeld et al.
427 1997b) could be easily perceived by the male through female behavioral signals (e.g.
428 solicitations) rather than from the state of nest completion. Some individual

429 characteristics expressing phenotypic quality may influence a male's ability or
430 willingness to perform mate guarding. However, male dorsal blackness as an index of
431 social dominance did not affect EPP. Furthermore, the extent of the male wing patch as
432 another potential male social signal showed no association with EPP (Spearman's rank
433 correlation: $r_{57} = -0.06$, $P = 0.63$).

434 Previous studies in pied flycatchers did not detect differences between extra pair
435 and within-pair males in age, size or ornamentation (Moreno et al. 2010b) and there is
436 no evidence of indirect benefits for extra-pair offspring in terms of good genes, as
437 measured by microsatellite heterozygosity or body condition (Lifjeld et al. 1997a;
438 Moreno et al. 2013). Although there is evidence of good-gene effects in other species
439 accrued thorough EPP (e.g. Kempenaers et al. 1992; Blomqvist et al. 2002), the picture
440 is not so clear and recent analyses of the evolution of infidelity in monogamous
441 passerines suggest that EPP is not adaptive for females in some species and that it may
442 be the result of strong selection in males (Arnqvist and Kirkpatrick 2005; Forstmeier et
443 al. 2011). However, there might be benefits for female extra-pair behaviour that
444 researchers just have not investigated or thought of yet (Mennerat et al. 2018).

445 The extent of white on female pied flycatcher wings has been proposed as a
446 signal of dominance through its association with testosterone levels (Moreno et al.
447 2014; Cantarero et al. 2015a). Moreover, female vigilance and dominance behaviours
448 are positively associated with the size of this patch (Plaza et al. 2018). Thus, dominant
449 females with larger patches may enforce their dominant status through signalling, being
450 more able to resist unwanted males and thereby negatively interacting with EPP
451 occurrence. This result supports previous evidence in the same population regarding
452 female age (Moreno et al. 2015). That old and dominant females (more experienced)
453 exhibit lower EPP values contradicts the presumption that EPP is the result of adaptive

454 female choice as precisely these females would be in a better position to select extra-
455 pair sires and resist mate guarding by their social mates.

456 Values found in brood EPP occurrence are similar to others in the same
457 population and slightly higher in the percentage of nestlings affected (22.4 and 7.5% in
458 2003, Moreno et al. 2010a; 28.8 and 13.1% in 2010, Moreno et al. 2013; 38.3 and
459 17.6% in 2015, Moreno et al. 2015). They are also similar to those found in another
460 Iberian population studied by (Canal et al. 2011) (39 and 20% respectively), and to the
461 medium EPP rate in socially monogamous passerines which is above 25%. The
462 importance of breeding synchrony and density on the interspecific variation in EPP has
463 previously been reported (Stutchbury 1995; Griffith et al. 2002). It is assumed that
464 temporal availability of reproductively active individuals may differ across the breeding
465 season. In our highly synchronous breeding population (Griffith et al. 2002; Moreno et
466 al. 2013), density of males not yet involved in parental duties may markedly decline
467 throughout the season. As a consequence, the pressure of males seeking EPC may
468 decrease, resulting in the negative relation between laying date and the incidence of
469 EPP. Previous studies in the same population showed no relation (Moreno et al. 2015)
470 or a negative relation (Moreno et al. 2013) between EPY and laying date. In fact, Canal
471 et al. (2012) described for the same species a decrease in EPP values during the days
472 before the laying date, followed by an increase during egg laying and incubation, and no
473 EPC occurring after those periods, suggesting that the demands of paternal care
474 decreased the availability of males for EPCs. This pattern is in accordance with the
475 general negative relation we found.

476 To conclude, we have found that females with a higher body mass during the
477 fertile period display higher EPP levels. The evolution of mass change strategies in
478 breeding altricial birds (Moreno 1989) has thus implications for EPP patterns. More

479 body reserves at laying mean not only a higher potential fecundity but a higher level of
480 EPP as well. This interaction had not previously received due attention but should be
481 considered in future studies of avian breeding strategies. If female condition at laying
482 denotes a high EPP for their partners, the possible negative consequences of a good
483 breeding condition for females in terms of reduced mate incubation feeding (Cantarero
484 et al. 2014) or help with nestling provisioning would merit further studies (Arnqvist and
485 Kirkpatrick 2005). We also found that females with signals of higher social dominance
486 show lower EPP values. Those results underline the role of female social traits in the
487 evolution of avian EPP.

488

489 **Compliance with ethical standards**

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495

496 **Conflict of interest** MP has received research FPI grant from ‘Ministerio de Ciencia,
497 Innovación y Universidades’. AC declares that he has no conflict of interest. JM
498 declares that he has no conflict of interest.

499

500 **Ethical approval**

501 We were legally authorized to capture and handle pied flycatchers by Consejería de
502 Medio Ambiente de Castilla y León (competent regional authority, protocol number
503 EP/SG/706/2016, according to Royal Decree 53/2013), and by J. Donés, director of

504 “Centro Montes de Valsain”, to work in the study area. All applicable international,
505 national, and/or institutional guidelines for the care and use of animals were followed.
506 The experiments comply with current Spanish laws, and grant holder and field
507 researchers were officially licensed for animal manipulation following current EU
508 regulations on animal manipulation (authorization types C and D with reference
509 numbers CAP-T-0123-15 and CAP-T-0121-15). The study was ethically approved by
510 the Ethical Committee of the ‘Consejo Superior de Investigaciones Científicas’ (CSIC).

511

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698

699 **FIGURE CAPTIONS**

700

701 **Table 1.** Average (\pm SE) values for breeding variables and female measurements of pied
 702 flycatchers in the “Experimental” and “Control” groups and results of Mann–Whitney
 703 *U*-test and Unpaired *t*-test analyses (day 1 = April 1) (N=59).

	Control	Experimental	Statistic	<i>P</i>
Hatching date	67.91 \pm 0.40	67.52 \pm 0.50	<i>U</i> = 20	0.62
Clutch size	5.91 \pm 0.09	5.86 \pm 0.11	<i>U</i> = 207	0.40
Female wing length (mm)	77.38 \pm 0.32	76.86 \pm 0.40	<i>t</i> = 0.99	0.32
Female age	2.72 \pm 0.23	3.00 \pm 0.31	<i>U</i> = 371	0.50
Female wing patch size	1.35 \pm 0.04	1.41 \pm 0.07	<i>t</i> = -0.61	0.53
Male dorsal blackness	0.86 \pm 1.31	0.87 \pm 1.93	<i>U</i> = 356	0.76
Female mass (g)	14.14 \pm 0.13	14.74 \pm 0.16	<i>t</i> = -2.79	0.007
Female wing loading (g/dm ²)	0.23 \pm 0.002	0.24 \pm 0.003	<i>t</i> = -3.07	0.03

704

705 **Table 2.** Results of two univariate GLM models with EPP (absence/presence) and
 706 proportion of EPY (number EPY / number total young) in a population of pied
 707 flycatchers as dependent variables and experimental treatment, laying date, extent of
 708 female white wing patch and male dorsal blackness as independent variables (N=59).

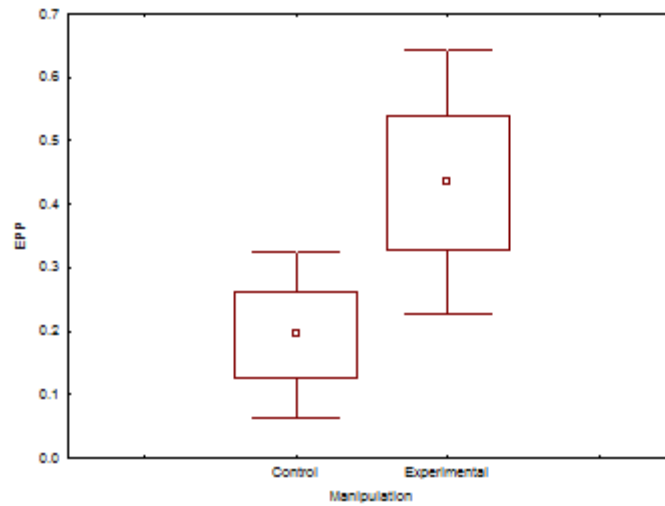
709

	Treatment (control)			Laying date			Female wing patch			Male dorsal blackness		
	Estimate	χ^2	<i>P</i>	Estimate	χ^2	<i>P</i>	Estimate	χ^2	<i>P</i>	Estimate	χ^2	<i>P</i>
EPP	1.61	5.94	0.01	0.36	5.28	0.02	2.33	4.91	0.02	0.00	0.03	0.86
EPY	-1.36	5.43	0.01	-0.62	19.75	<0.05	-2.25	6.50	0.01	0.01	0.19	0.66

710

711 **Figure 1.** Proportion of pied flycatcher nests with EPP in the “Experimental” and
712 “Control” groups (central points represent mean values, boxes represent Standard Errors
713 and whiskers represent 95% confidence intervals, N=59).

714



715