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Title: Complex effects of helper relatedness on female extra-pair reproduction in a cooperative breeder

Abbreviated title: Helper relatedness and female extra-pair reproduction

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Gabriela K Hajduk is no longer at the University of Edinburgh, but is now an independent scholar with no institutional affiliation.

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Data Accessibility Statement

Data used in the analyses presented in the manuscript will be archived in Dryad upon publication of the manuscript.

Lay Summary (70 words)

In superb fairy-wrens, unrelated helpers in a group increased extra-pair paternity, both within and (unexpectedly) outside the group. The presence of helpers who were sons of the breeding female had no effect on mating patterns in this cooperatively breeding species. The results undermine a long-standing explanation for extra-pair paternity, the 'constrained female hypothesis', which suggests that females assisted by helpers have more freedom to mate outside of their social partnership.

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Abstract

In cooperatively-breeding species, the presence of male helpers in a group often reduces the breeding female's fidelity to her social partner, possibly because there is more than one potential sire in the group. Using a long-term study of cooperatively-breeding superb fairy-wrens (*Malurus cyaneus*) and records of paternity in 1936 broods, we show that the effect of helpers on rates of extra-pair paternity varied according to the helpers' relatedness to the breeding female. The presence of unrelated male helpers in a group increased average rates of extra-pair paternity, from 57% for groups with no unrelated helpers, to 74% with one unrelated helper, to 86% with 2+ unrelated helpers. However, this increase was due in equal part to helpers within the group and males in other groups achieving increased paternity. In contrast, helpers who were sons of the breeding female did not gain paternity, nor did they affect the level of extra-group paternity (which occurred at rates of 60%, 58%, 61% in the presence of 0, 1, 2+ helper-sons respectively). There was no evidence of effects of helpers' relatedness to the female on nest productivity or nestling performance. Because the presence of helpers *per se* did not elevate extra-pair reproduction rates, our results undermine the 'constrained female hypothesis' explanation for an increase in extra-pair paternity with helper number in cooperative breeders. However, they indicate that dominant males are disadvantaged by breeding in 'cooperative' groups. The reasons why the presence of unrelated helpers, but not of helper-sons, results in higher rates of extra-group reproduction are not clear.

1 Introduction

2

3 In cooperatively-breeding species, breeding pairs are often assisted by subordinate ‘helpers’
4 who may make substantial contributions to offspring care, most obviously through
5 provisioning of young (Koenig and Dickinson, 2016), but also via other aspects such as nest
6 defense (Austad and Rabenold, 1985; Mumme, 1992) or even thermoregulation (Arnold
7 1990; Du Plessis 2004). Helping often occurs within kin groups of related individuals
8 (Dickinson and Hatchwell, 2004), and levels of helping may therefore vary with the helpers’
9 relatedness to the breeding pair and the resulting offspring: for example, a recent
10 comparative analysis of cooperatively-breeding bird species showed that helpers who are
11 more closely related typically – though not always – provide more care than do unrelated
12 helpers (Green et al. 2016). The presence and relatedness of helpers may also have important
13 implications for other aspects of group life such as the mating patterns of the breeding
14 individuals. In particular, females in many cooperative breeding species may take advantage
15 of the presence of additional subordinate males in their social group to mate with them
16 (Cockburn 2004). In several species it has also been shown that such extra-pair paternity
17 provides an incentive for subordinates to provision at the nest (Burke et al. 1989; Cockburn
18 2004).

19

20 More remarkably, it has also been shown that the presence of helpers within a group can
21 increase the rate of extra-group paternity (Cousseau et al. 2020). In some species with high
22 rates of natal philopatry, closely related males and females can form pair bonds, and extra-
23 group mating has been hypothesized to result from inbreeding avoidance (Brooker et al.
24 1990; Koenig and Haydock 2004). However, this seems unlikely to explain the mating patterns
25 of the genus which consistently shows the highest incidence of extra-group mating among
26 socially monogamous birds, fairy-wrens (genus *Malurus*). This is because *Malurus* species
27 with both high and very low rates of incestuous pairing consistently mate extra-group (Mulder
28 et al. 1994; Cockburn et al. 2013, 2016; Brouwer et al. 2017; Hajduk et al. 2018; Lichtenauer
29 et al. 2019). As an alternative, Mulder et al. (1994) suggested that in the superb fairy-wren,
30 *Malurus cyaneus*, female reliance on provisioning of the dominant male to rear young
31 successfully was reduced by the presence of additional carers, allowing her to increase her
32 rate of cuckoldry. A general form of this hypothesis has since been called the ‘constrained

33 female hypothesis', and argues that females face a trade-off between choice of mates,
34 genetic quality and their value in providing parental care (Gowaty 1996, 1997; Bennett and
35 Owens 2002).

36

37 Here we take advantage of a long-term study of parentage in the species that provoked the
38 constrained female hypothesis. Superb fairy-wrens (*M. cyaneus*) are socially monogamous,
39 with a single breeding female on each territory socially paired to the senior male on the
40 territory. These breeding pairs may be assisted by up to four (very rarely five) subordinate
41 males, 'helpers', though unassisted pairs are also common. Helpers are behaviorally
42 subordinate to the dominant male, but are reproductively mature: they can sire young and
43 their testosterone levels are no different from those of dominant males living in pairs (Peters
44 et al. 2001). Helpers assist with provisioning of young and defense of the territory (Cockburn
45 et al. 2008b), and their presence increases nestling mass (Kruuk et al. 2015; Hajduk et al.
46 2018).

47

48 We consider here the effects of the presence of different types of helpers on extra-pair
49 paternity, where we categorize helpers based on their relatedness to the breeding female:
50 sons of the breeding female ('helper-sons') vs others ('unrelated helpers'). Superb fairy-wrens
51 have among the highest rate of extra-pair and extra-group paternity among birds: ~83% of
52 broods have at least one extra-pair offspring (Hajduk et al. 2018), the majority of whom are
53 also extra-group, i.e. sired by males in other social groups (Mulder et al. (1994); see Box 1 for
54 definitions). Extra-pair reproduction is controlled by the female, who initiates extra-group
55 matings on the territory of her preferred male (Double and Cockburn 2000; Cockburn et al.
56 2009). In *M. cyaneus* and other *Malurus* species, rates of EPP increase with increasing
57 numbers of helpers (Mulder et al. 1994; Cockburn et al. 2016; Brouwer et al. 2017; Hajduk et
58 al. 2018). In this study we used data from our 26-year study of superb fairy-wrens in south-
59 eastern Australia (Cockburn et al. 2016) to investigate this effect in more detail, and to
60 determine the effect of the number of related versus unrelated helpers on the rates of both
61 within-group and extra-group extra-pair paternity.

62

63

64 **Methods**

65

66 **Study system**

67

68 The study population consisted of a color-banded population of superb fairy-wrens living in a
69 ~60 ha area located in and around the Australian National Botanic Gardens, Canberra,
70 Australia (35 ° 16 S, 149 ° 06 E). The study population was censused all 52 weeks of the year
71 (Cockburn et al. 2003), and census records included details of the presence and identity of
72 helpers on each territory. During the breeding season the progress of all nests was monitored,
73 with nestlings banded 5-8 days post-hatching, at which point blood samples were taken for
74 parentage analysis using microsatellite genotypes (see methods in Hajduk et al. (2018)). Fairy-
75 wrens are multi-brooded: due to heavy nest predation, a female may initiate up to eight
76 clutches in a given year, but will only ever raise a maximum of three broods to fledging.
77 Clutches may contain 1-5 eggs, with a strong mode at 3 eggs (Cockburn et al. 2008b).

78

79 Male superb fairy-wrens are philopatric. Most males will queue for dominance on their natal
80 territory, but about a third will end up dispersing to vacant breeding positions on adjacent
81 territories where all the previous males have died (Cockburn et al. 2008a). In contrast, females
82 always disperse before breeding for the first time (usually over three or more territories), so
83 when a female first establishes herself as dominant in a vacant territory, she is unrelated to
84 the dominant male and any subordinate males already present in the group. However, after
85 two or three years of breeding on a territory, a female is likely to have accumulated helpers
86 who are sons. Overall, helpers will therefore vary in their relatedness to the breeding female
87 on the territory, with nearly half of helpers being her sons (47% for the data used here; see
88 also Cockburn et al. 2016). Females never mate with their sons, either dominant or helper
89 (Cockburn et al. 2003; Hajduk et al. 2018). Both unrelated helpers and helper-sons provision
90 the young, and there is no evidence of any difference in their rates of doing so (Dunn et al.
91 (1995), N. Margraf and A. Cockburn, unpubl.).

92

93 We categorized all offspring in a brood as to whether they were the result of *within-pair*,
94 *within-group extra-pair*, or *extra-group* paternity: see Box 1 for definitions. We then
95 compared rates of (a) extra-pair vs within-pair paternity; and (b) extra-group vs within-group

96 paternity across groups with different composition and numbers of helpers. We did not use
97 any finer-scale measure of relatedness between individuals than whether a given helper was
98 the son of the dominant female, as our previous work investigating the associations between
99 the kinship of the social partners and extra-pair reproduction indicated that females never
100 mate with their sons but do not distinguish lower levels of relatedness (Hajduk et al. 2018).
101 These results suggest that females are not able to distinguish finer levels of relatedness, and
102 we therefore considered just two categories of ‘unrelated helpers’ vs ‘helper-sons’.

103

104 Social pairings between mothers and sons occurred in ~4% of all breeding events in our study
105 population, and resulted in 100% female extra-pair reproduction (i.e. all offspring were extra-
106 pair (Cockburn et al. 2003; Hajduk et al. 2018)). Furthermore, in all but one of these 73
107 breeding events for mother-son pairings in the dataset, all helpers were sons of the dominant
108 female. As mother-son pairings appear to be special cases in which mating patterns are
109 determined by extreme inbreeding avoidance (Hajduk et al. 2018), for clarity, we excluded
110 them from the current analyses. The remaining data spanned 26 years (1988-2013) and
111 contained a total of 1936 broods and 5485 nestlings. Sample sizes for models may deviate
112 from that due to removal of missing values from fixed predictors.

113

114 Throughout, we treat each brood (rather than each offspring) as a data point. Thus we present
115 mean percentages in each brood for different paternity types (see Box 1). These values are
116 very similar to, but not exactly the same, as percentages calculated across individual nestlings
117 of each paternity type, but they more closely reflect the patterns of paternity by different
118 females, as large broods do not contribute more than smaller broods.

119

BOX 1: TYPES OF PATERNITY

- **within-pair (WP) paternity:** offspring sired by the breeding female's social partner, the dominant male on the focal territory
- **within-group extra-pair (WGEP) paternity:** offspring sired by a helper within the focal social group
- **extra-group (EG) paternity:** offspring sired by a male from outside the social group that reared the offspring
- **within-group (WG) paternity:** offspring sired either by the social partner of the breeding female, or by helpers within the focal social group [WG = WP + WGEP]
- **extra-pair (EP) paternity:** offspring sired by any male other than the breeding female's social partner (either within- or extra-group) [EP = WGEP + EG]

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Statistical analyses

All analyses were carried out in R version 3.3.1 (Development Core Team, 2011) using MCMCglmm (Hadfield, 2010) to fit generalized linear mixed models (GLMMs), with a logit link function, using *family = multinomial2*. Fixed effects were considered statistically significant when the 95% credible intervals (CIs) did not span zero and the pMCMC values were < 0.05 . In MCMCglmm models we also aimed for an effective sample size of 2000 and autocorrelation below 0.1 for each model, ensuring that the resulting effective sample sizes are above 1000. Parameter expanded priors were used throughout. For detailed iteration and prior information for MCMCglmm models see the Supplementary Information.

We ran two GLMMs, to consider: (a) the proportion of *extra-pair* offspring in a brood (i.e. EP vs WP); and (b) the proportion of *extra-group* offspring in a brood (i.e. EG vs WG; see Box 1 for definitions). Both models had the same fixed and random structures. The fixed effects were: for each brood, covariates of the *number of helper-sons* (0-4) and the *number of unrelated helpers* (0-4) attending each brood, and *mother's age* and the age of the dominant male on the territory (*social father's age*). Age was initially fitted as a two-level factor of one-year-old vs older. We used this dichotomous classification for several reasons. First, investment in all aspects of reproduction by females increases sharply between the first and second year of age, but tends to asymptote thereafter (Cockburn et al 1998; Cockburn et al. 2008b; Kruuk et al 2015). Second, first year females cannot be paired to their sons. Third, females generally form their first pair bond after a dispersal movement just before the breeding season. This means that they lack information about important features of the males with which they choose to mate, including what appears to be the chief criterion for extra-group mate choice, the amount of time before the breeding season that the male has nuptial plumage (Cockburn et al. 2008c). Finally, this enabled us to include information from the class of individuals whose birth cohort was unknown, but which were known to be older than one-year-old. However, we also repeated the analyses using age as a continuous variable, which did not change conclusions (see Supplementary Information, Tables B3, B4, and B5). For random effects, we fitted *mother ID* and *social father ID* to account for multiple observations,

154 and *cohort* as a multilevel factor to account for year-to-year variation (1988-2013; each
155 cohort consists of nestlings from one breeding season, so for example 1998 denotes August
156 1998 to March 1999).

157

158 Additionally, we re-ran both of the above models on randomly-selected subsets of 50% of the
159 dataset ten times, to generate within-study replication (see the Supplementary Information
160 for details).

161

162 As a final component, because the ‘constrained female’ hypothesis relies on the contributions
163 of helpers to offspring care, we also tested for any effect of helpers of different relatedness
164 (sons or unrelated) on nestling performance or brood productivity. We assessed offspring
165 performance by modelling offspring survival to measurement age (5-8 days after hatching),
166 and nestling mass. We also investigated whether the relatedness of the helpers to the mother
167 affected her investment in the breeding attempt, as assessed by the clutch size (number of
168 eggs) of each brood. We fitted a model disentangling helper number from helpers’
169 relatedness to the breeding female to ensure that our main results are not being biased by
170 the known correlation between the number of helpers and nestling mass.

171

172 **Results**

173

174 Superb fairy-wren breeding events varied both in the presence and relatedness of
175 subordinate helper males in the group, and in the fidelity of the breeding female to her social
176 partner. The dominant pair on the territory was assisted by one or more helpers in 879 out of
177 the total 1936 (45%) broods. Of these, 363 were assisted exclusively by helper-sons, 435 were
178 assisted exclusively by unrelated helpers, and 81 were assisted by a mix of unrelated helpers
179 and helper-sons. As in previous work on this species, extra-pair reproduction levels were very
180 high: across all broods, 62% of offspring were extra-pair.

181

182 Our models of the effect of social environment showed that the proportion of extra-pair
183 offspring per brood increased substantially with increasing numbers of unrelated helpers, but
184 did not change with numbers of helper-sons (Figure 1a; Table 1a). These results were robust
185 to sub-sampling and confirmed in a replication study (Supplementary Information, Figure A1).

186 Further, rates of extra-*group* paternity also increased with numbers of unrelated helpers, but
187 again did not change with numbers of helper sons (Figure 1b; Table 1b). The GLMMs also
188 showed that older females produced a higher proportion of both EP and EG offspring (Table
189 1). In contrast, there was evidence in this model that older ‘social fathers’ (i.e. dominant
190 males) had a lower proportion of EG offspring than did one-year-olds. However, this effect
191 should be interpreted with caution, as it was not supported either in replicate analyses of
192 50% subsets of the data (Figure A1), nor if the social male’s age was fitted as a continuous
193 covariate (Tables B4, B5). Fitting parental age as a continuous covariate did not change the
194 conclusions regarding effects of numbers of helper sons or unrelated helpers (see
195 Supplementary Information, Tables B3, B4, and B5). There was also substantial variance
196 between females and between dominant males in the rates of both EP and EG paternity in
197 each brood (random effects variance components in Table 1).

198

199 Across all broods, the average proportion of nestlings resulting from extra-group (EG)
200 paternity was 60% and EG accounted for the majority of paternity across all further
201 categorizations, regardless of the presence of helpers and their relatedness to the breeding
202 female (Table 2a). Groups with at least one unrelated helper had an average of 10% within-
203 group extra-pair (WGEP) paternity (Table 2b, second row). WGEP was 0% in broods with only
204 helper-sons, confirming the observation that females do not mate with their sons, and also –
205 necessarily - 0% in broods with no helpers. Groups with at least one unrelated helper had an
206 average of 67% extra-group paternity (Table 2b, second row), as compared to 57% EG in
207 groups with no unrelated helpers (Figure 1b, top panel: this comprises both unassisted groups
208 and those with just helper-sons). In summary, the presence of unrelated helpers in a group
209 was associated with increased rates of EPP through a 10% increase in *within-group* EPP plus
210 a 10% increase in *extra-group* EPP. Thus, the reduced success of dominant males in the
211 presence of unrelated helpers in the group was due to the paternity being allocated away
212 from the group as much as to the helpers within the group.

213

214

215 There was no indication of any difference between the effects of helper-sons versus unrelated
216 helpers on nestling performance or productivity (see Supplementary Information). In brief,
217 nestling mass increased with numbers of both helper-sons and unrelated helpers, to almost

218 exactly the same degree (Table 3a). Adding a factor to test for differences between groups of
219 different composition (mixed groups, vs all sons, vs all unrelated) also showed no indication
220 of any difference in nestling mass ($p = 0.665$, Table A2), nor was there any association between
221 the numbers of helpers of different relatedness-type and survival to measurement age (Table
222 3b). Finally, we found no association between the effects of different levels of helper
223 relatedness and clutch size (Table A4).

224

225

226 **Discussion**

227

228 In this study, we used a long-term dataset on the cooperatively-breeding superb fairy-wren
229 to investigate how female extra-pair reproduction changed according to the social
230 environment. Extra-pair paternity was always greater than 50% of offspring regardless of the
231 type and number of helpers in a group, and increased further with the number of helpers in
232 a group, but only when these helpers were unrelated to the breeding female. Both extra-pair
233 and extra-group reproduction contributed to this increase, by very similar amounts. Our
234 results are based on records of nearly two thousand breeding events over twenty-six years,
235 and were robust to replication by sub-sampling. Below we first discuss their implications for
236 the understanding of extra-pair paternity, and then consider possible explanations for the
237 novel observation of differences between effects of helpers of different relatedness.

238

239 The reasons for extra-pair paternity (EPP) in socially-monogamous species remain the subject
240 of ongoing debate (Griffith et al. 2002; Westneat and Stewart 2003; Eliassen and Jorgensen,
241 2014; Forstmeier et al. 2014; Hsu et al. 2015), not least because of the challenges inherent in
242 empirical tests of competing hypotheses. In *Malurus* (fairy-wrens), inbreeding avoidance was
243 originally hypothesized to be the evolutionary force driving extra-group mating, as in some
244 populations incestuous social pairings are common (Brooker et al. 1990). This now seems
245 unlikely for at least two reasons. First, incestuous social pairings vary from extremely rare to
246 over 20% of pairings, yet this does not affect the extra-group mating rate (Cockburn et al.
247 2013, 2016; Brouwer et al. 2017). Not least, in this study population of *M. cyaneus*, 61% of
248 individuals are extra-pair, with 82.8% of broods have at least one extra-pair young, but only
249 4.2% of social pairings are between close relatives (Hajduk et al. 2018). Secondly, in the

250 species with the highest rate of extra-group matings, the red-winged fairy-wren (*M. elegans*),
251 nuclear family inbreeding occurs and is costly, but is equally likely to arise from extra-group
252 and within-group fertilisations (Lichtenauer et al. 2019).

253

254 An alternative scenario generating selection for EPP, the ‘constrained female hypothesis’
255 (Mulder et al. 1994; Gowaty, 1996, 1997), proposes that female extra-pair reproduction in
256 socially-monogamous species is more likely when the need for paternal care of offspring is
257 reduced. As a result, in cooperatively breeding systems, females assisted by helpers may be
258 predicted to have higher rates of extra-pair reproduction than unassisted females, because
259 they will be less dependent on their male partner for assistance in raising the young.
260 Increased rates of extra-pair paternity in the presence of helpers have been interpreted as
261 support for the constrained female hypothesis in the cooperatively breeding placid greenbul
262 *Phyllastrephus placidus* (Cousseau et al. 2020), although there was no change in EPP rates
263 with numbers of helpers in Seychelles warblers (Raj Pant et al. 2020). In superb fairy-wrens,
264 although the presence of helpers is not a prerequisite for breeding, females usually cannot
265 raise young without male assistance in some form; they will also abandon breeding attempts
266 if the number of helpers is unexpectedly reduced (Dunn and Cockburn, 1996). This suggests
267 that helpers provide valuable support during breeding, and hence that the constrained female
268 hypothesis might explain the increased EPP observed in the presence of helpers (Mulder et
269 al. 1994; Brouwer et al. 2011), although possibly not the ubiquitously high average rates of
270 EPP regardless of helper presence.

271

272 Our analyses here, however, showed that while overall rates of extra-pair reproduction were
273 very high, extra-pair paternity only increased when females were assisted by unrelated
274 helpers, and did not change when they were assisted by helper-sons. Thus these results would
275 only be compatible with the ‘constrained female hypothesis’ if unrelated helpers provide
276 useful assistance (lifting the ‘constraint’ of requiring additional care) but helper-sons do not.
277 The notion is difficult to test directly, but we found no evidence of any associations between
278 helper relatedness and any measures of nest productivity or offspring performance (nestling
279 mass and survival; see Supplementary Information, and also Cooper et al. 2020b), suggesting
280 no differences in care levels. There is also no evidence that helper-sons and unrelated helpers
281 provision nestlings at different rates (Dunn et al. (1995); N. Margraf and A. Cockburn,

282 unpubl.). Thus our results appear to be at odds with the constrained female hypothesis as an
283 explanation for variation in extra-pair paternity with helper presence in superb fairy-wrens.

284

285 The most plausible explanation for the occurrence of EPP in this species may be that a female
286 uses it to realize her optimum mate choice without the limitation of needing to be socially
287 paired to him (Cockburn et al. 2013) - but that somehow the distribution of paternity is
288 differentially affected by, or at least associated with, the presence of unrelated helpers.
289 Below, we consider possible explanations for how this might occur.

290

291 An immediate explanation for increased extra-*pair* paternity in the presence of unrelated
292 helpers is that these helpers sire offspring within their groups: when a group has at least one
293 unrelated helper, the helper(s) will sire on average 30% of the total within-group young (i.e.
294 WP + WGEP; Table 2). Helpers are sexually mature (Mulder and Cockburn, 1993; Peters et al.
295 2001) and fertile, and can themselves secure extra-group paternity relatively often (Double
296 and Cockburn, 2003; Cockburn et al. 2009) – even if their lower social status and (typically)
297 younger age means their within-group reproductive success is usually lower than that of
298 dominants (Cockburn et al. 2008a; Cooper et al. 2000a). Nevertheless, most extra-pair
299 offspring were sired by males from other groups, regardless of helper numbers or relatedness
300 (Table 2, EG). Increased within-group extra-pair paternity therefore cannot explain the
301 similar-sized increase in the proportion of extra-*group* offspring in broods assisted by
302 unrelated helpers.

303

304 What then explains the unexpected contrast between an increase in extra-group paternity
305 with numbers of unrelated helpers, but not with numbers of helper-sons? We can speculate
306 about possible direct and indirect explanations. Direct explanations invoke effects of the
307 presence of unrelated helpers on the behavior or physiology of the dominant male and/or
308 the female in ways that change the distribution of paternity. Helper-sons pose no threat (as
309 females do not mate with their sons), but dominant males in groups with unrelated helpers
310 could be adjusting their behavior in an attempt to reduce within-group extra-pair matings.
311 There is some evidence in this population of mate guarding: the dominant male defends the
312 female vigorously against helpers in a 30-minute window after she has returned from the
313 early-morning extra-group forays during which extra-group copulations occur (Cockburn et

314 al. 2016), in ways that may even involve extreme violence. Such behavior could delay his
315 subsequent mating with her, potentially biasing sperm competition, and hence fertilization,
316 in favor of the extra-group males with whom she has mated earlier. Dominant males in groups
317 with helpers have elevated testosterone levels relative to those without helpers, potentially
318 reflecting the need to assert dominance (Peters et al. 2001). We do not know whether
319 testosterone elevation occurs differentially in the presence of unrelated helpers, but our
320 observations suggest that dominant males are highly aggressive towards both unrelated
321 helpers and helper-sons.

322

323 We can also consider explanations based on indirect effects of confounding factors. Spatial
324 heterogeneity in environmental conditions may influence both group size and rates of extra-
325 pair reproduction. However, it is difficult to envisage any scenario whereby numbers of
326 unrelated helpers but not helper-sons could be spatially associated with rates of extra-pair
327 and extra-group copulation. This is especially so since superb fairy-wren females may travel
328 over relatively long distances (several territories (Double and Cockburn, 2000)) for extra-
329 group fertilizations. Secondly, temporal covariance in group composition and extra-pair
330 reproduction is also unlikely, because of the lack of any temporal variance in mating patterns,
331 as indicated by the small 'cohort' variance components in Table 1. Third, extra-pair
332 reproduction changes with both female and male age in cooperative breeders (e.g. Hsu et al.
333 2017; Part et al. 2019). Here, as outlined above, a female's age may be associated with both
334 her mating patterns and the helpers in her group, but as we included female age in our
335 models, any such effects are unlikely to be driving the association shown here. Dunn &
336 Cockburn (1999) found that females substantially older than their social mate tended to
337 cuckold him completely, and we suspect that this pattern is one explanation for the higher
338 cuckoldry of one-year old social mates, although we do not want to make too much of this
339 result, as it was not significant using different parameterisations of social male age, or in the
340 subset-replications (Supplementary Information, Figure A1 and Tables B4, B5). Unfortunately,
341 demographic changes associated with rainfall deficits and higher temperatures since 2001
342 mean that it is difficult to investigate this suggestion further, as the pairings where females
343 are substantially older than their social mates have become rarer, and are dominated by the
344 cases where the female is paired to her son. One final possibility is the role of behavioral
345 differences between females: for instance, if females that are more likely to move territories

346 between breeding events (and so end up with unrelated helpers) are also more likely to mate
347 with extra-group males. However, this scenario would not explain the increase in EPP and
348 EPG between one and 2+ unrelated helpers. In summary, at present, the reasons for the
349 difference between the effect of helper-sons vs unrelated helpers are difficult to pin down.

350

351 The results raise the question as to why dominant males tolerate unrelated helpers in their
352 group, when female extra-pair reproduction increases in their presence? There is no apparent
353 advantage of having helpers in terms of attracting potential mates, since larger aggregations
354 of males during the dawn chorus do not result in more *per capita* visits from females
355 (Cockburn et al. 2009), and although helpers lower the rate at which dominant males
356 provision nestlings, this does not lead to increased survival for the male (Cockburn et al.
357 2008b). Any indirect benefits via helpers' additional provisioning of chicks also do not appear
358 to be sufficient to counter the loss of paternity. We suspect that the answer lies in the
359 asymmetry of extra-group fertilizations, which are skewed towards a few attractive males
360 (Cockburn et al. 2016). Most dominant males only gain paternity through within-pair mating
361 with their social partner, and our previous work indicates that eviction of helpers could
362 jeopardize this pathway to reproductive success: experimental reduction of group size by
363 temporary removal of helpers during the breeding season can lead the female to divorce the
364 male (Dunn and Cockburn 2006). Dominant males may therefore be obliged to 'make the
365 worst of a good job' and to accept the presence of helpers as the safest route to at least some
366 reproductive success (Cockburn et al. 2016).

367

368 In conclusion, our study illustrates how relatedness between individuals can potentially affect
369 important aspects of group dynamics and mating patterns in complex animal groups. We
370 observed a substantial increase in rates of extra-group paternity in the presence of unrelated
371 helpers, but not in the presence of helper-sons. The result undermines the 'constrained
372 female' explanation for extra-pair reproduction increasing with helper number due to the
373 female being able to express "free choice" in the presence of helpers, an observation which
374 is now ripe for testing in other species (see Brouwer et al. (2017) for synthesis of related work
375 done to date in *Malurus*). The reason for the differential effects of helper relatedness is not
376 clear, although we can speculate on various potential direct or indirect explanations. More
377 generally, the results illustrate how intrinsic characteristics of conspecifics, rather than just

378 their numbers, can have important implications for multiple aspects of group dynamics. We
379 have shown here that particular characteristics of these conspecifics may play a crucial role
380 in shaping reproductive patterns.

References

- Arnold W. 1990. The evolution of marmot sociality: II. Costs and benefits of joint hibernation; *Behavioral Ecology and Sociobiology*; 27:239–246.
- Austad SN, Rabenold KN. 1985. Reproductive enhancement by helpers and an experimental inquiry into its mechanism in the bicolored wren; *Behavioral Ecology and Sociobiology*; 17:19–27.
- Brooker MG, Rowley I, Adams M, Baverstock PR. 1990. Promiscuity: an inbreeding avoidance mechanism in a socially monogamous species? *Behavioral Ecology and Sociobiology*; 26:191–200.
- Brouwer L, van de Pol M, Atema E, Cockburn A. 2011. Strategic promiscuity helps avoid inbreeding at multiple levels in a cooperative breeder where both sexes are philopatric; *Molecular Ecology*; 20:4796–4807.
- Brouwer L, van de Pol M, Hidalgo Aranzamendi N, Bain G, Baldassare DT, Brooker LC, Brooker MG, Colombelli-Negrel D, Enbody E, Gielow K, Hall ML, Johnson AE, Karubian J, Kingma SA, Kleindorfer S, Louter M, Mulder RA, Peters A, Pruett-Jones S, Tarvin KA, Thrasher DJ, Varian-Ramos C, Webster MS, Cockburn A. 2017. Multiple hypotheses explain variation in extra-pair paternity at different levels in a single bird family; *Molecular Ecology*; 26:6717–6729.
- Burke T, Davies NB, Bruford MW, Hatchwell BJ. 1989. Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting; *Nature*; 338:249–251.
- Cockburn A. 2004. Mating systems and sexual conflict. *Ecology and Evolution of Cooperative Breeding in Birds*. Walter D Koenig and JL Dickinson, Cambridge University Press: 81–101.
- Cockburn A, Brouwer L, Double MC, Margraf N, van de Pol M. 2013. Evolutionary origins and persistence of infidelity in Malurus: the least faithful birds; *Emu*; 113:208–217.
- Cockburn A, Brouwer L, Margraf N, Osmond HL, van de Pol M. 2016. Making the worst of a good job: cooperative breeding in superb fairy-wrens; in Koenig WD and Dickinson JL, eds., *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior*; Cambridge, UK: Cambridge University Press; 133–149.
- Cockburn A, Dalziell AH, Blackmore CJ, Double MC, Kokko H, Osmond HL, Beck NR, Head ML, Wells K. 2009. Superb fairy-wren males aggregate into hidden leks to solicit extra group fertilisations before dawn; *Behavioural Ecology*; 20:501–510.

Cockburn A, Osmond HL, Mulder RA, Double MC, Green DJ. 2008a. Demography of male reproductive queues in cooperatively breeding superb fairy-wrens *Malurus cyaneus*; *Journal of Animal Ecology*; 77:297–304.

Cockburn A, Osmond HL, Mulder RA, Green DJ, Double MC. 2003. Divorce, dispersal and incest avoidance in the cooperatively breeding superb fairy-wren *Malurus cyaneus*; *Journal of Animal Ecology*; 72:189–202.

Cockburn A, Sims RA, Osmond HL, Green DJ, Double MC, Mulder RA. 2008b. Can we measure the benefits of help in cooperatively breeding birds: the case of superb fairy-wrens *Malurus cyaneus*? *Journal of Animal Ecology*; 77:430–438.

Cockburn A, Osmond HL, Double MC. 2008c. Swingin' in the rain: condition dependence and sexual selection in a capricious world; *Proceedings of the Royal Society B*; 275:605–612.

Cooper E, Bonnet T, Osmond H, Cockburn A, Kruuk LEB. 2020a. Ageing and senescence across reproductive traits and survival in superb fairy-wrens (*Malurus cyaneus*); *American Naturalist*; in press.

Cooper E, Bonnet T, Cockburn A, Kruuk LEB. 2020b. Do the ages of parents or helpers affect offspring fitness in a cooperatively breeding bird? *Journal of Evolutionary Biology*; in press.

Cousseau L, Van de Loock D, Githiru M, Vangestel C, Lens L. 2020. Female need for paternal care shapes variation in extra-pair paternity in a cooperative breeder; *Behavioral Ecology*; ar2215.

Development Core Team. 2011. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Dickinson JL, Hatchwell BJ. 2004. Fitness consequences of helping; in Koenig WD and Dickinson JL, eds., *Ecology and Evolution of Cooperative Breeding in Birds*; 48–66.

Double MC, Cockburn A. 2000. Pre-dawn infidelity: females control extra-pair mating in superb fairy-wrens; *Proceedings of the Royal Society B*; 267:465–470.

Double MC, Cockburn A. 2003. Subordinate superb fairy-wrens (*Malurus cyaneus*) parasitize the reproductive success of attractive dominant males; *Proceedings of the Royal Society B*; 270:379–384.

Du Plessis MA. 2004. Physiological ecology; in Koenig WD and Dickinson JL, eds., *Ecology and Evolution of Cooperative Breeding in Birds*; 117–127.

Dunn PO, Cockburn A. 1996. Evolution of male paternal care in a bird with almost complete cuckoldry; *Evolution*; 50:2542–2548.

Dunn PO, Cockburn A. 1999. Extra-pair mate choice and honest signaling in cooperatively-breeding superb fairy-wrens; *Evolution*; 53:938-946.

Dunn PO, Cockburn A, Mulder RA. 1995. Fairy-wren helpers often care for young to which they are unrelated; *Proceedings of the Royal Society B*; 259:339–343.

Eliassen S, Jorgensen C. 2014. Extra-pair mating and evolution of cooperative neighbourhoods; *PLOS ONE*; 9:e99 878.

Forstmeier W, Nakagawa S, Griffith SC, Kempenaers B. 2014. Female extra-pair mating: adaptation or genetic constraint? *Trends in Ecology and Evolution*; 29:456–464.

Gowaty PA. 1996. Battles of the sexes and origins of monogamy; in Black JM, ed., *Partnerships in birds: The study of monogamy*; Oxford: Oxford University Press; 21–52.

Gowaty PA. 1997. Field studies of parental care in birds: New data focus questions on variation in females; in Snowdon CT and Rosenblatt JS, eds., *Advances in the study of behaviour*; New York: Academic Press; 476–531.

Green JP, Freckleton RP, Hatchwell BJ. 2016. Variation in helper effort among cooperatively breeding bird species is consistent with Hamilton’s Rule; *Nature Communications*; 7:12 663.

Griffith SC, Owens IPF, Thuman KA. 2002. Extra-pair paternity in birds: a review of interspecific variation and adaptive function; *Molecular Ecology*; 11:2195–2212.

Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package; *Journal of Statistical Software*; 33:1–22.

Hajduk GK, Cockburn A, Margraf N, Osmond HL, Walling CA, Kruuk LEB. 2018. Inbreeding, inbreeding depression, and infidelity in a cooperatively breeding bird; *Evolution*; 72:1500-1514.

Hsu Y-H, Schroeder J, Winney I, Burke T, Nakagawa S. 2015. Are extra-pair males different from cuckolded males? A case study and a meta-analytic examination; *Molecular Ecology*; 24:1558-1571.

Hsu Y-H, Simons MJP, Schroeder J, Girndt A, Winney IS, Burke T, Nakagawa S. 2017. Age-dependent trajectories differ between within-pair and extra-pair paternity success; *Journal of Evolutionary Biology*; 30:951-959.

Koenig WD, Dickinson JL. 2016. *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior*. Cambridge, Cambridge University Press.

Koenig WD, Haydock J. 2004. Incest and incest avoidance. in W. D. Koenig, and J. L. Dickinson, eds. *Ecology and evolution of cooperative breeding in birds*. Cambridge University Press, Cambridge and New York.

Kruuk LEB, Osmond HL, Cockburn A. 2015. Contrasting effects of climate on juvenile body size in a Southern Hemisphere passerine bird; *Global Change Biology*; 21:2929–2941.

Mulder RA, Cockburn A. 1993. Sperm competition and the reproductive anatomy of male superb fairy-wrens; *The Auk*; 110:588–593.

Mulder RA, Dunn PO, Cockburn A, Lazenby-Cohen KA, Howell MJ. 1994. Helpers liberate female fairy-wrens from constraints on extra-pair mate choice; *Proceedings of the Royal Society B*; 255:223229.

Mumme RL. 1992. Do helpers increase reproductive success? *Behavioral Ecology and Sociobiology*; 31:319–328.

Lichtenauer W, van de Pol M, Cockburn A, Brouwer L. 2019. Indirect fitness benefits through extra-pair mating are large for an inbred minority, but cannot explain widespread infidelity among red-winged fairy-wrens; *Evolution*; 73:467-480.

Peters A, Astheimer LB, Cockburn A. 2001. The annual testosterone profile in cooperatively breeding superb fairy-wrens, *Malurus cyaneus*, reflects their extreme infidelity; *Behavioral Ecology and Sociobiology*; 50:519–527.

Raj Pant S, Komdeur J, Burke TA, Dugdale HL, Richardson DS. 2019. Socio-ecological conditions and female infidelity in the Seychelles warbler; *Behavioral Ecology*; arz072.

Raj Pant S, Hammers M, Komdeur J, Burke TA, Dugdale HL, Richardson DS. 2020. Age-dependent changes in infidelity in Seychelles warblers; *Molecular Ecology*; 00:1-16.

Westneat DF, Stewart IRK. 2003. Extra-pair paternity in birds: Causes, correlates, and conflict; *Annual Review of Ecology, Evolution, and Systematics*; 34:365–396.

Figures

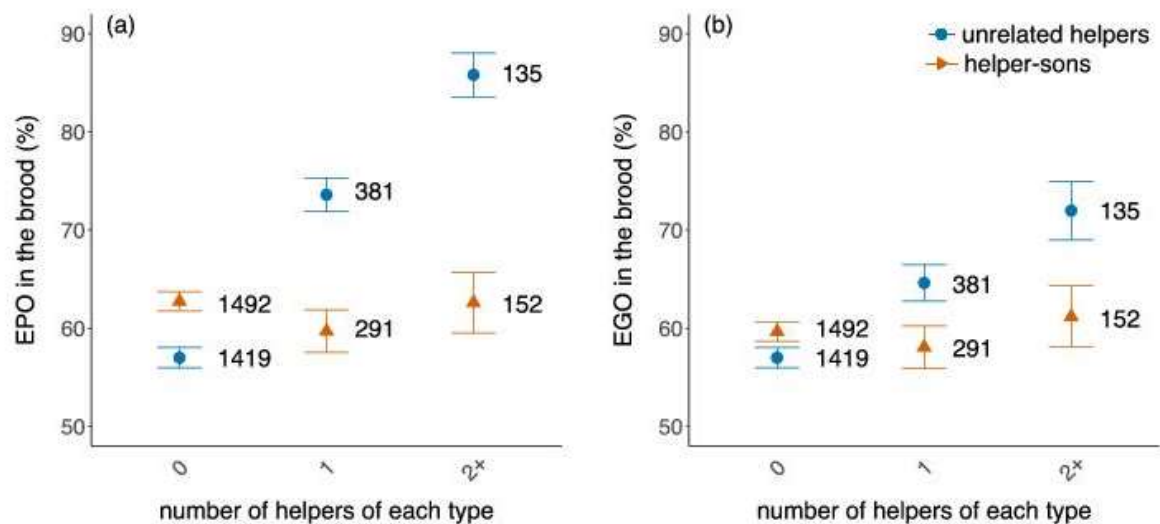


Figure 1. The effects of the numbers of helper-sons (orange triangles) and of unrelated helpers (blue dots) on percentages of (a) extra-pair offspring (EPO), and (b) extra-group offspring (EGO) in a brood. Points represent average % (± 1 SE) for EPO or EGO percentages in each brood (raw data for all broods) per helper category; there were few broods with 3 or 4 helpers of either type, so we combined these with broods with 2 helpers to create the '2+' category in the figure. Sample sizes (number of broods) are given next to the points. Note (i) that the y-axes start at 50%, reflecting the widespread extra-pair reproduction in the population; and (ii) the 'zero' category on the x-axes represents broods where there are no helpers of that particular type: this is therefore a combination of broods that are completely unassisted (and have the lowest rates of extra-pair paternity at ~57%, Table 2a) plus broods that are assisted only by the helpers of the other type.

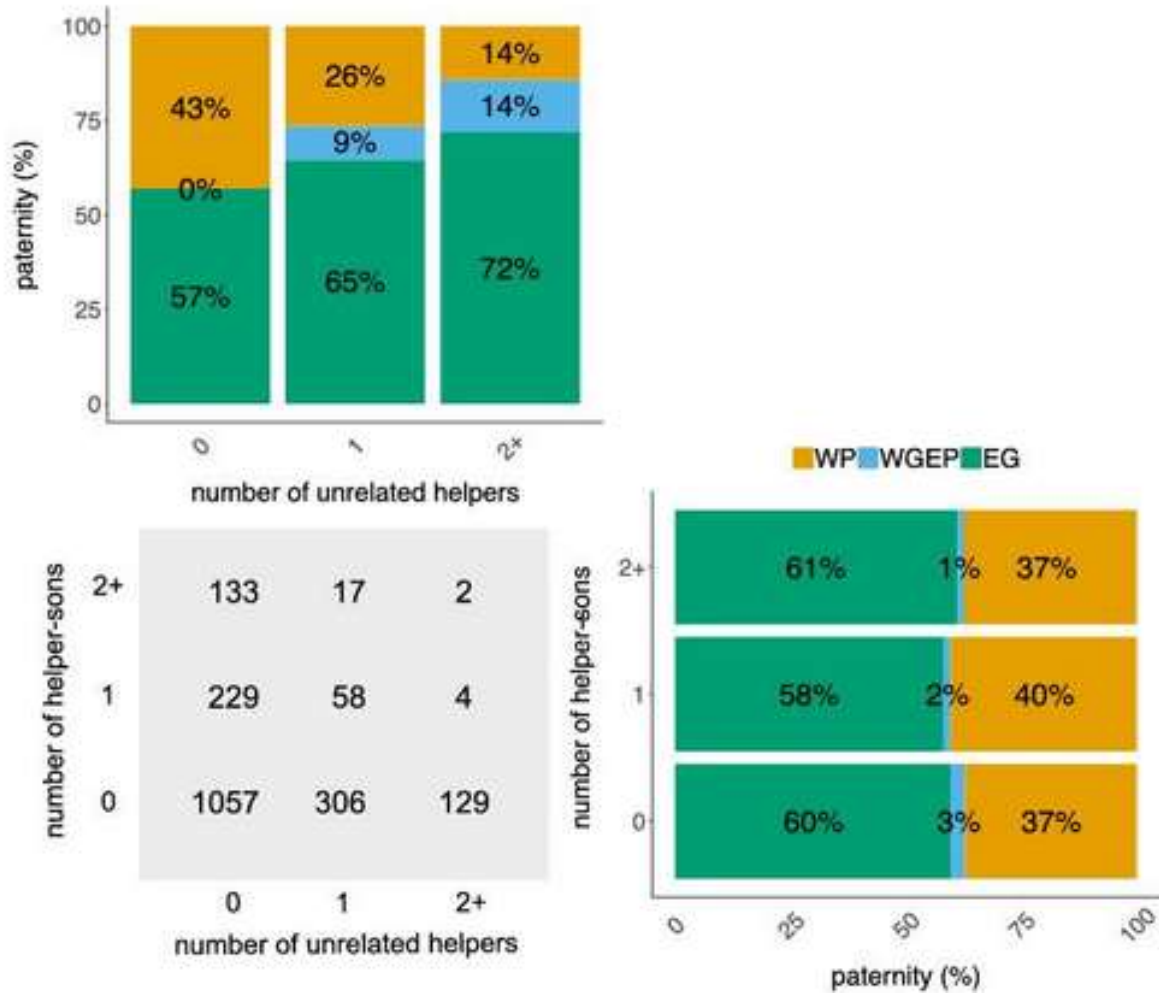


Figure 2. Matrix showing the numbers of broods of different types, with bar charts illustrating the percentage of within-pair (WP), within-group extra-pair (WGEP), and extra-group (EG) in each category. Note: (i) in the right-hand panel, the very low levels of WGEP (blue) will be paternity going to unrelated helpers also present in those groups; and (ii) in the top panel, WGEP = 0% in groups with no unrelated helpers, because WGEP always goes to unrelated helpers.

Tables and table legends

Table 1. GLMM of the influence of the numbers of helper-sons and unrelated helpers on the proportion of (a) extra-pair and (b) extra-group offspring in a brood. Analyses were carried out at the brood level for (a) numbers of extra-pair vs within-pair offspring in each brood; and (b) numbers of extra-group vs within-group offspring in each brood. Sample size indicates total numbers of broods, and cohort represents the 26 breeding seasons 1988-2013.

	response: proportion of			
	(a)		(b)	
	extra-pair offspring		extra-group offspring	
Fixed effects	Estimate (95% CI)	P	Estimate (95% CI)	P
intercept	0.37 (0.03, 0.72)	0.038	0.43 (0.06, 0.75)	0.014
mother age (1yo, older)				
older	0.39 (0.11, 0.64)	0.001	0.40 (0.14, 0.66)	0.004
social father age (1yo, older)				
older	-0.29 (-0.65, 0.03)	0.107	-0.38 (-0.72, -0.04)	0.030
number of helper-sons	0.09 (-0.08, 0.26)	0.304	0.10 (-0.07, 0.27)	0.272
number of unrelated helpers	1.02 (0.84, 1.22)	<0.001	0.47 (0.29, 0.65)	<0.001
Random effects	Variance (95% CI)		Variance (95% CI)	
mother ID	0.54 (0.21, 0.84)		0.58 (0.27, 0.90)	
social father ID	0.60 (0.30, 0.92)		0.54 (0.25, 0.83)	
cohort	0.01 (1.34 ⁻⁸ , 0.05)		0.02 (1.62 ⁻⁸ , 0.06)	
residual variance	1.75 (1.30, 2.17)		1.80 (1.37, 2.23)	
Sample size	1929		1929	

*

* Estimates and variance components reported are based on posterior means, with 95% credible intervals (CIs), on the logit link scale, from MCMCglmm models. P values were based on pMCMC (the number of simulations in which the parameter estimate was greater or less than zero, corrected for number of MCMC samples).

Table 2. Mean percentages of within-pair (WP), within-group extra-pair (WGEP) and extra-group (EG) paternity across broods, split by the presence and relatedness type of helpers (helper-sons vs unrelated helpers) in each group.

type of help	paternity (%)			sample size (<i>n</i> broods)
	WP	WGEP (extra-pair)	EG	
(a) all broods (n = 1936)				
all broods	37.7	2.7	59.6	1936
no helpers	43.1	0.0	56.9	1057
≥ 1 helper	31.1	6.0	62.8	879
(b) assisted broods (n = 879) - effect of helper relatedness				
≥ 1 helper-son	39.2	1.6 †	59.2	444
≥ 1 unrelated	23.2	10.3	66.5	516
(c) assisted broods (n = 879) - effect of group composition				
helper-sons	42.5	0.0	57.5	363
mixed	24.5	8.5	67.0	81
unrelated	23.0	10.6	66.5	435

† This reflects WGEP to an unrelated helper in a group that also had one or more helper-sons

*

* Values shown are the means of the percentages calculated for each brood. The data are presented as follows: (a) all broods; (b) assisted broods, showing the effect of helper relatedness type; (c) assisted broods, showing the effect of group composition with regard to helper relatedness type. Total n = 1936 broods, with 5485 nestlings, across 26 years.

Table 3. **(a)** Association between the numbers of helper-sons/unrelated helpers and nestling mass. Sample size indicates number of nestlings. **(b)** Effects of the numbers of helper-sons and unrelated helpers on survival of offspring to measurement age (5-8 days post-hatching). These analyses were carried out at the brood level in MCMCglmm.

	nestling mass		early survival	
	(a)		(b)	
Fixed effects	Estimate (SE)	P	Estimate (95% CI)	P
intercept	-3.69 (1.13)	0.002	-0.24 (-0.90, 0.36)	0.474
1992 (1992+, pre-1992)		<0.001	n/a	
pre-1992	0.58 (0.10)		n/a	
nestling age	2.10 (0.33)	<0.001	n/a	
nestling age ²	-0.08 (0.02)	<0.001	n/a	
brood size	-0.05 (0.02)	0.021	n/a	
sex (female, male)		<0.001	n/a	
male	0.15 (0.02)			
mother age (1yo, older)	n/a			
older	n/a		0.03 (-0.12, 0.17)	0.731
social father age (1yo, older)	n/a			
older	n/a		-0.01 (-0.20, 0.18)	0.939
average nestling age	n/a		0.01 (-0.07, 0.10)	0.845
inbreeding coefficient	-3.07 (1.18)	0.010	n/a	
number of helper-sons	0.10 (0.02)	<0.001	0.01 (-0.08, 0.10)	0.814
number of unrelated helpers	0.10 (0.02)	<0.001	0.005 (-0.09, 0.10)	0.928
Random effects	Variance (SE)		Variance (95% CI)	
mother ID	n/a		0.001 (2.35 ⁻¹⁰ , 0.004)	
social father ID	n/a		0.001 (2.13 ⁻¹⁰ , 0.004)	
cohort	0.01 (0.01)		0.001 (1.52 ⁻¹² , 0.005)	
nest ID	0.26 (0.01)		n/a	
hatch date	0.01 (0.01)		n/a	
residual variance	0.24 (0.01)		n/a	
Sample size	4910		1838	

*

* **(a)** This analysis was carried out at the level of individual offspring, in ASReml-R. Standard errors (SE) for the estimates and variance components are reported in brackets. See SI Section A2.1 for details of the model. **(b)** Estimates and variance components reported are based on posterior means and 95% credible intervals; note that MCMCglmm constrains variance components to be positive, thus the low values for random effects suggest that none of the phenotypic variation can be attributed to these random effects). See SI Section A2.1 for details of the model.