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11 **Multiple hypotheses explain variation in extra-pair paternity at different levels in a**
12 **single bird family**

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46

47

48 **Abstract**

49 Extra-pair paternity (EPP), where offspring are sired by a male other than the social male,
50 varies enormously both within and among species. Trying to explain this variation has proved
51 difficult because the majority of the interspecific variation is phylogenetically-based. Ideally,

52 variation in EPP should be investigated in closely related species, but clades with sufficient
53 variation are rare. We present a comprehensive multifactorial test to explain variation in EPP
54 among individuals in 20 populations of nine species over 89 years from a single bird family
55 (Maluridae). Females had higher EPP in the presence of more helpers, more neighbours, or if
56 paired incestuously. Furthermore, higher EPP occurred in years with many incestuous pairs,
57 populations with many helpers, and species with high male density or in which males provide
58 less care. Altogether, these variables accounted for 48% of the total and 89% of the
59 interspecific and inter-population variation in EPP. These findings indicate why consistent
60 patterns in EPP have been so challenging to detect and suggest that a single predictor is
61 unlikely to account for the enormous variation in EPP across levels of analysis. Nevertheless,
62 it also shows that existing hypotheses can explain the variation in EPP well and that the
63 density of males in particular is a good predictor to explain variation in EPP among species
64 when a large part of the confounding effect of phylogeny is excluded.

65 **Introduction**

66 It is now clear that complete genetic monogamy is the exception rather than the rule in
67 socially monogamous birds, and this discovery has revolutionized our view of mating
68 systems (Bennett & Owens 2002), not least because it changes our understanding of the way
69 selection works. The surge in studies investigating genetic mating systems revealed that
70 extra-pair paternity (EPP), where offspring are sired by a male other than the female's social
71 partner, occurs in over 70% of species that have been studied (reviewed in: Griffith *et al.*
72 2002).

73 Attempts to explain variation in EPP rates within species have explored a wide range
74 of factors including the role of ecology (Spottiswoode 2004; Taff *et al.* 2013; Schlicht *et al.*
75 2015), life-history (Richardson & Burke 1999; Bouwman *et al.* 2007) and genetic diversity
76 (Forstmeier *et al.* 2002; Foerster *et al.* 2003). Strikingly, despite 30 years of research, the
77 enormous amount of variation among species in the occurrence and levels of EPP remains
78 largely unexplained (Petrie & Kempenaers 1998; Griffith *et al.* 2002; Macedo *et al.* 2008),
79 other than that over 50% of the interspecific variation in EPP rates can be attributed to
80 phylogeny occurring at or above the family level (Arnold & Owens 2002; Griffith *et al.* 2002).
81 Thus, the main associations between ecology and EPP might be due to higher-level
82 phylogenetic history, and variation among species might not reflect current selective
83 pressures. Ideally, one should therefore study inter-specific variation in EPP between closely
84 related species.

85 The widespread occurrence of EPP among different clades of birds as well as the vast
86 number of proposed explanations (Griffith *et al.* 2002) suggest that multiple factors could
87 play a role in determining EPP rates. Thus far, most studies have focussed on testing the role
88 of a single or few alternative hypotheses, obscuring inferences about which factors are most
89 important. Another complexity is that EPP rates can vary at multiple levels, for example, over
90 time, among individuals in the same population, or among populations or species. Different
91 factors may predominate at different levels of variation. For example, breeding synchrony
92 correlates with variation in EPP rates among species (Stutchbury 1998; Spottiswoode 2004;
93 Bonier *et al.* 2014), but not among individuals in many species (e.g.: Weatherhead &
94 Yezerinac 1998; Saino *et al.* 1999; Kraaijeveld *et al.* 2004; Lindstedt *et al.* 2007). Ideally,
95 multiple hypotheses should be tested simultaneously at different levels of variation, as this
96 would allow for assessment of the relative importance of each hypothesis.

97 Whether a pattern is detected will also depend on the amount of variation in both EPP
98 rates and the explanatory factor. This raises a challenge: studies examining variation in EPP
99 ideally require closely-related species to avoid confounding effects of phylogeny, yet the
100 strong phylogenetic signal also means that variation in both EPP and the explanatory factor
101 are often limited within clades, hampering detection of patterns. There also are few clades for
102 which EPP data from multiple populations of multiple species are available.

103 Here we simultaneously test five hypotheses that have often been proposed in the
104 literature as possible explanations for variation in EPP: the breeding synchrony, density,
105 constrained female, inbreeding avoidance and life-history (male survival) hypotheses
106 (explained in Table 1, for review see: Griffith *et al.* 2002; Westneat and Stewart 2003).
107 Alternative hypotheses have been proposed that we have not considered here, either because
108 they do not lead to testable predictions or the data to test them are unavailable for the
109 Maluridae (see discussion). We test how well the five hypotheses explain individual,
110 temporal, inter-population, and inter-specific variation in EPP rates using data collected over
111 89 study years from nine species spanning 20 populations of a single family of birds, the
112 Maluridae (fairy-, emu- and grass-wrens). These species exhibit rates of EPP that span the
113 entire natural range: from complete genetic monogamy to extreme promiscuity (0%- 80% of
114 offspring; this study; Cockburn *et al.* 2013). In addition, Maluridae is probably the best
115 studied avian family with respect to genetic mating system (Cockburn *et al.* 2013), so there
116 are data on many species and populations. Finally, since species of this family are a model
117 system in behavioural and evolutionary ecology, detailed information on their behaviour, life-

118 history and ecology exists (Buchanan & Cockburn 2013), which also exhibits sufficient intra-
119 and interspecific variation to test key hypotheses in a meaningful way.

120

121 **Methods**

122 *Study system and data collection*

123 The Maluridae are endemic to Australia and Papua New Guinea and all species included here
124 (and most likely all species in the family) are facultative cooperative breeders, with multiple
125 subordinate males and sometimes also females often assisting the dominant pair to rear young
126 (Rowley & Russell 1997). All species maintain territories during the breeding season.

127 We collated published and unpublished data from 4,072 broods and 10,665 offspring
128 collected over 89 study years from nine species of Maluridae spanning 20 populations (see
129 Supporting Information A for an overview of the data). Our dataset included 7 populations of
130 superb fairy-wren (*Malurus cyaneus*; Double & Cockburn 2003; Colombelli-Négrel *et al.*
131 2009; Bain *et al.* 2014), two populations each of white-shouldered fairy-wren (*M.*
132 *alboscapulatus*; for details see Supporting Information A), red-winged fairy-wren (*M.*
133 *elegans*; Brouwer *et al.* 2014), variegated fairy-wren (*M. lamberti*; for details see Supporting
134 Information A; Johnson 2016), red-backed fairy-wren (*M. melanocephalus*; Varian-Ramos *et*
135 *al.* 2012; Baldassarre & Webster 2013), and splendid fairy-wren (*M. splendens*; Brooker *et*
136 *al.* 1990; Webster *et al.* 2004; Tarvin *et al.* 2005), and one population each of purple-crowned
137 fairy-wren (*M. coronatus*; Kingma *et al.* 2009), southern emu-wren (*Stipiturus malachurus*;
138 Maguire & Mulder 2008) and thick-billed grasswren (*Amytornis modestus*; Louter 2016).
139 Studies were included for all populations where genetic parentage analyses had been
140 conducted and sufficient data were available to estimate the majority of the predictors of
141 interest (see below). We report data on EPP here, but it should be noted that our estimates of
142 EPP are almost identical to the rate of extra-group paternity (i.e. paternity by males from
143 outside the social group), as within-group subordinates rarely gain paternity (Mulder *et al.*
144 1994; Webster *et al.* 2004; Brouwer *et al.* 2011). EPP data are based primarily on data
145 collected from nestlings between 2 and 8 days old, except for *M. alboscapulatus*, for which
146 fledglings were sampled. Starvation of nestlings is rare, and incomplete sampling is usually
147 due to predation. Genotyping was based on microsatellite data except for the population of *M.*
148 *splendens* from Perth which was genotyped using allozymes (Brooker *et al.* 1990), and *A.*
149 *modestus*, which was based on RAD sequencing (Louter 2016). Although these methods
150 differ in their ability to assign parentage to extra-group males, all of them are excellent in
151 determining mismatches with the territorial male, and hence should produce identical

152 estimates of EPP (methods are unbiased; Kaiser *et al.* 2017). Re-analysing the top-models
153 after excluding the *M. alboscapulatus* and *M. splendens* studies showed that the results
154 remain largely unchanged, although the association between EPP and the number of helpers
155 receives more support at the species rather than the population level (see Table S1).

156

157 *Defining and measuring predictors of EPP*

158 Each hypothesis resulted in a specific set of predictions with regard to patterns of individual,
159 temporal, inter-population and interspecific variation in EPP (explained in Table 1):

- 160 1. Breeding synchrony hypothesis: in Maluridae females have been shown to control extra-
161 pair mating by visiting the extra-pair male's territory at dawn (Double & Cockburn 2000)
162 and most commonly obtain EPP from neighbouring males (Double & Cockburn 2003;
163 Brouwer *et al.* 2011; Kingma *et al.* 2013). Furthermore, more synchronous broods
164 contained more EPP in *M. coronatus* (Kingma *et al.* 2013). Consequently, we used the
165 same approach as Kingma *et al.* (2013), and calculated breeding synchrony at the
166 individual level as the number of days between lay dates of a focal nest and the
167 immediate neighbour with the closest lay dates. In addition, breeding synchrony was also
168 calculated as the mean difference between lay dates of a focal nest with all its immediate
169 neighbours, but using this method did not change the results (Fig. A1). Since we do not
170 have such detailed spatial (territory border) data for all populations, we used a different
171 approach at the population level. For each population, an estimate of the proportion of
172 simultaneously fertile females was calculated as the variance of the proportion of
173 dominant females that started egg laying each month. By taking the variance this measure
174 also accounts for the length of the breeding season. In addition, we calculated a breeding
175 synchrony index following Kempnaers (1993). The mean of each measure per species
176 was used as a predictor at the species level.
- 177 2. Density hypothesis: at the individual level the number of adjacent neighbouring territories
178 was used as a proxy of density. Some species and populations inhabit riparian or
179 fragmented habitat in which territories are linearly arranged and only share boundaries at
180 the two extremes of the territory, whereas others occupy contiguous habitat, with
181 neighbours on all sides. An index of annual male population density was estimated by
182 dividing the median number of neighbouring dominant males for a given habitat type
183 (two for linear, four for contiguous habitat) by the average territory length of a given
184 population in a given year. We only included dominant males here since dominant males
185 gain the majority of EPP in most species (Double & Cockburn 2003; Webster *et al.* 2004;

186 Brouwer *et al.* 2011) and in this way we can disentangle density from a direct effect of
187 the number of helpers (constrained female hypothesis, see below). The index of male
188 density was fitted on a logarithmic scale. The means of annual male density per
189 population and per species were used as predictors at the population and species level
190 respectively, whereas the annual deviation of the population mean was used as a predictor
191 for temporal variation (within-subject centring; van de Pol & Wright 2009). In addition,
192 to investigate whether variation in EPP is explained by habitat geometry, geometry
193 (contiguous or linear) was used as a proxy for density at the population and species level
194 (Brouwer *et al.* 2014; Bain *et al.* 2014). Habitat geometry of a population did not
195 correlate significantly with our index of male density (Pearson $r = -0.36$, $P = 0.14$).

196 3. Constrained female hypothesis: the presence of helpers might reduce the dependency of
197 the female on care by the dominant male, as helpers can potentially compensate for
198 reduced investment or desertion by the dominant, allowing the female greater freedom to
199 pursue EPP (Mulder *et al.* 1994). Consequently, at the individual level we used the
200 number of male and female helpers per female as a predictor. The mean of the annual
201 number of helpers per population and per species were used as predictors at the
202 population and species level respectively, whereas the annual deviation of the population
203 mean number of helpers was used as a predictor for temporal variation. In addition, at the
204 population and species level we also used male care as a predictor, calculated as the
205 average proportion of provisioning rates made by males without helpers.

206 4. Inbreeding avoidance hypothesis: inbreeding avoidance via EPP is potentially most
207 beneficial in closely related social pairs, thus incestuous (between first order relatives)
208 social pairing was used as a predictor. For the *M. cyaneus* ACT population a pedigree was
209 used to determine whether a pair was incestuous or not. For other populations a pair was
210 considered incestuous when its pairwise relatedness (r) calculated from the molecular
211 markers (Lynch & Ritland 1999; Wang 2002) was within the range of the mean ± 1.5
212 S.D. of known first order relatives. We choose this measure rather than a fixed value (i.e.
213 $r = 0.5$) to account for genotyping errors and because relatedness values will vary
214 depending on the microsatellites used. Whether a pair was incestuous or not was used as a
215 predictor at the individual level. The means of the annual proportion of incestuous
216 pairings per population and per species were used as predictors at the population and
217 species level respectively, whereas the annual deviation of the population mean was used
218 as a predictor for temporal variation.

219 5. Life-history (survival) hypothesis: mean annual adult male survival per population and
220 per species were used as predictors for the population and species level, respectively.
221 Since male fairy-wrens are extremely philopatric (Margraf & Cockburn 2013), this
222 survival estimate is unlikely to suffer from problems associated with undetected dispersal,
223 as is often the case in other species.

224

225 *Statistical analyses*

226 We created two models. Temporal, population and interspecific variation in EPP rates were
227 analysed simultaneously in a single model. Individual variation in EPP was analysed in a
228 separate model, as for some studies a complete dataset with all predictors of interest was not
229 available at the individual level (but only available as an aggregate statistic on a subset of the
230 data, e.g. mean EPP for females with X neighbours). At the individual level, the number
231 extra-pair offspring / total number offspring) for groups of individuals with associated values
232 of the predictor of interest (e.g. number of neighbours) was fitted in a binomial regression
233 weighed by the total number of sampled offspring and identity of the population as a fixed
234 effect. Model selection (see below) was performed by comparing the models with and
235 without the predictor of interest.

236 To test which hypotheses could explain temporal, inter-population and interspecific
237 variation in EPP, the proportion of EPP per year in a population (number extra-pair offspring/
238 total number offspring sampled) was fitted as a binomial response in a generalized linear
239 mixed model (GLMM) weighted by the total number of sampled offspring. Year, population
240 and species identity were entered as nested random effects (intercepts) to account for the fact
241 that we have multiple data points from the same populations/species (see for R code
242 Supporting Information B). Since we do not have replicate populations for each species, the
243 predictors at the population level also contain information at the species level. Consequently,
244 to investigate whether variation among species is more important than variation among
245 populations, we also assessed whether the variable of interest averaged per species is a better
246 predictor than the population-averaged predictor.

247 For various reasons (e.g. data were not collected, experimental manipulations, or
248 limited project duration) not all predictor variables were available for each year/population
249 (see Supporting Information C). Missing values (9% missing) were assumed to be missing
250 completely at random and set to zero after transforming each variable to z-scores (Nakagawa
251 & Freckleton 2011). This enabled us to use the full dataset and test the different hypotheses

252 simultaneously with a multifactorial model selection approach. Testing the final model on a
253 dataset without missing values did not qualitatively change the results.

254 To select the most parsimonious model, we used Akaike's information criterion
255 corrected for sample size (AIC_c), with sample size conservatively set to the number of
256 populations ($N=20$) (Akaike 1973; Burnham & Anderson 2002). Models that are better
257 supported by the data result in lower AIC_c values. For the analyses on temporal, inter-
258 population and interspecific variation we used an all-subset approach with all possible
259 combinations of predictors (see Table 1) included as main effects, whereby predictors at the
260 level of the population and species were not included simultaneously (since these are partly
261 confounded). We reported the top models within two ΔAIC_c of the best supported model only
262 (out of model set of >10,000 models; see Table S2 for detailed model selection results).
263 Additionally, we report the Akaike weights to assess the relative likelihood of competing
264 models. The proportional change in variance between the null (without predictors) and the
265 final model was calculated to determine how much of the inter-population and inter-specific
266 variance can be attributed to the predictors included in the final model (Merlo 2005). Finally,
267 we calculate the R^2 (Snijders & Bosker 1999) to estimate the proportion of the total variance
268 explained at each level, by the best model and to assess the relative importance of different
269 variables. All statistical analyses were performed in R3.2.4 (R Development Core Team
270 2015) using RStudio (RStudio Team 2015) and packages lme4 (Bates *et al.* 2014), MuMIn
271 (Bartoń 2015) and mateable (Wagenius *et al.* 2016).

272 Although we studied closely related species from a single family, phylogenetic
273 patterns at a lower taxonomic level could still affect the results. To investigate whether our
274 results can be explained by phylogeny, the variables from the top model were fitted in a
275 phylogenetic mixed model approach using R package MCMCglmm (Hadfield 2010).
276 Unfortunately, the phylogeny of Maluridae has not been fully resolved, with the position of
277 *M. coronatus* being ambiguous (Cockburn *et al.* 2013). To account for phylogenetic
278 uncertainty we followed a similar approach as Ross *et al.* 2013. We downloaded 1,300
279 different trees from BirdTree.org (Jetz *et al.* 2012, see Supporting Information D) and
280 sampled a tree from the posterior distribution of trees at iteration t , running the MCMC model
281 for 1,000 iterations and saving the median from each run. This process was repeated for 1,300
282 iterations where we disposed of the first 300 as a burn-in. *A. modestus* has only recently been
283 considered as a different species from *A. textilis* (Black *et al.* 2010), but unfortunately this has
284 not been included in phylogenies yet. Consequently, we used the phylogenetic data for *A.*
285 *textilis* here. The results showed that after accounting for phylogeny, all variables from the

286 best supported model remained statistically significant and effect sizes barely changed, with
287 the phylogenetic signal being rather weak ($\lambda = 0.13$, Pagel 1999; see Supporting Information
288 A).

289

290 **Results**

291 *Variation in EPP across levels*

292 There was considerable variation in EPP rates at each of the different levels. EPP rates across
293 Maluridae varied between 0% and 80% of offspring (Fig. 1a). We compared observed rates
294 of EPP against those predicted from a binomial distribution that assumed that all
295 populations/species have the global average EPP of 0.57 (6097 out of 10,665 offspring; Fig.
296 1a). More than half of the populations were outside the 95% quantile, even for those in which
297 the power to detect such a departure was low because of small sample size. For the best-
298 studied species *M. cyaneus*, differences among the seven populations accounted for 24% of
299 the species' variation in EPP rates. Similarly, the annual rates of EPP for the longest-running
300 population study illustrate that there can be substantial inter-annual variation within a
301 population, as 28% of 25 annual means were outside the 95% quantile of a temporally
302 invariant binomial distribution (Fig. 1b).

303 Forty-six percent of the variation in EPP was at the temporal level and the other 54%
304 at the species and population level (with more variation at the species (47%) than at the
305 population level (7%)), but note that species and population are partly confounded).

306

307 *Variation among individuals*

308 Variation in EPP among individuals was most consistent with predictions of the density,
309 constrained female, and particularly the inbreeding avoidance hypothesis, but not the
310 breeding synchrony hypothesis. For the latter, although some populations appeared to have
311 higher and others lower EPP rates with increasing synchrony, there was no overall pattern,
312 and including synchrony reduced model support ($\Delta AIC_c = 1.9$; Fig. 2a). Support for the
313 density hypothesis comes from the association between EPP and the number of neighbouring
314 territories, but this association was non-linear and was strongest when there were few
315 neighbours (Fig. 2b). Indeed, fitting EPP as a logarithmic function of the number of
316 neighbours was best supported by the data ($\Delta AIC_c = -14$).

317 Consistent with the constrained female hypothesis, groups with more helpers
318 generally had higher EPP ($\Delta AIC_c = -80$), but primarily so in populations with overall higher

319 EPP levels (Fig. 2c; adding the interaction between the average EPP and the number of
320 helpers of a population yielded $\Delta AIC_c = -16$ compared to a linear effect of the number of
321 helpers). Finally, consistent with the inbreeding avoidance hypothesis, incestuous pairs had
322 higher levels of EPP than non-incestuous pairs in all nine populations for which data were
323 available (Fig. 2d; $\Delta AIC_c = -210$).

324

325 *Variation among years*

326 Temporal variation in EPP was consistent with the inbreeding avoidance hypothesis, but not
327 with the density and constrained female hypotheses (Fig. 3Ai-iii). Patterns at the temporal
328 level showed that only annual variation in the proportion of incestuous pairs was consistently
329 included in the top models (Table 2).

330

331 *Variation among populations*

332 Patterns at the population level were consistent with the constrained female hypothesis, but
333 not with the breeding synchrony, inbreeding avoidance and life-history hypotheses (Fig. 3Bi-
334 vii). Although populations with higher EPP were associated with higher density (Fig. 3Bii), a
335 model that included density as a predictor at the species level explained the variation in EPP
336 much better ($\Delta AIC_c = -9.6$), and therefore there was no evidence that density can explain
337 variation in EPP among populations. The constrained female hypothesis was supported,
338 because higher EPP was associated with populations with more helpers (Table 2, models 1-4;
339 Fig. 3Biv). Furthermore, there was some evidence for higher EPP in populations with
340 reduced male care (Table 2, models 2, 4 & 5; Fig. 3Bv), although this hypothesis was actually
341 better supported at the species level (see below).

342

343 *Variation among species*

344 Patterns at the species level were consistent with both the density and the constrained female
345 hypotheses, but not with the inbreeding avoidance or life-history hypotheses (Fig. 3Ci-vii).
346 There was also not much support for the breeding synchrony hypothesis, because adding
347 breeding synchrony to the top model increased AIC_c values (Table 2, model 1 vs. model 2 &
348 3). Replacing our breeding synchrony measure by the breeding synchrony index following
349 Kempnaers (1993) showed that the latter was not a better predictor for variation in EPP
350 (Table 2, model 6 vs. model 3). The density hypothesis was strongly supported as dominant
351 male density was consistently included in the top 182 models (Table S2), indicating that
352 Maluridae with a higher male density were associated with higher EPP rates (Fig. 3Cii). An

353 additional effect of habitat geometry was not supported by the data, as the addition of
354 geometry to the best supported model increased AIC_c values ($\Delta AIC_c = 2.5$, Fig. 3Ciii).
355 Support for the constrained female hypothesis came from the association that species with
356 reduced male care (Table 2, models 1, 3 & 6; Fig. 3v) had higher EPP. Although there was
357 some support for this hypothesis at the population level, replacing the population predictor
358 with the species predictor in the top models reduced AIC_c values (Table 2, model 4 vs. model
359 1 $\Delta AIC_c = -1.4$), indicating that there was little evidence for additional variation among
360 populations. There was no evidence that the number of helpers at the species level explained
361 variation in EPP better than the number of helpers at the population level (Table 2, model 5
362 vs models 1-4).

363

364 *Explanatory value and relative importance of hypotheses*

365 The six best-supported models to explain variation in EPP in Maluridae within 2 AIC_c units
366 of the top model (Table 2) account for 29% of the Akaike model weight. Overall, the best
367 supported model explained 48% of the total variation in EPP among years, populations and
368 species. Calculating the proportion of change in variance of the null versus the best supported
369 model showed that 89% of the among-population and among-species variation could be
370 attributed to variation in male density, male care and the number of helpers. Our
371 multifactorial analysis also allowed for assessing the relative importance of predictor
372 variables: of the seven predictors tested at the species level, male density was much more
373 important than male care, because it explained 2.2 times as much of the inter-specific
374 variation ($R^2_{\text{male care}} = 0.11$ vs. $R^2_{\text{density}} = 0.24$). Habitat geometry, number of helpers,
375 incestuous pairings, breeding synchrony and male survival only explained marginal amounts
376 of variation ($R^2 < 0.05$). The importance of our multifactorial approach is further exemplified
377 by the fact that it led to different results than a unifactorial approach. In a unifactorial
378 approach, at the population level the density hypothesis (Table S2, model 5845) and at the
379 species level the inbreeding avoidance hypothesis (Table S2, model 5751) would have
380 received support, whereas the proportion of male care would have been better supported at
381 the population rather than the species level (Table S2, model 4879 vs. model 6348).

382

383 **Discussion**

384 This is the first comprehensive analysis to simultaneously test multiple key hypotheses at
385 different taxonomic levels. Using data from possibly the best-studied family of birds with

386 respect to genetic mating system, we found that variation in EPP rates were consistent with
387 the inbreeding avoidance, constrained female and density, but not with the life-history or
388 breeding synchrony hypotheses. At the individual level, females had higher EPP if they had
389 more helpers, more neighbours, or were paired incestuously. Furthermore, years with many
390 incestuous pairs, populations with many helpers, and species with high male density and/or
391 low levels of male care were associated with higher EPP rates. Together, these factors
392 explained 48% of the total variation in EPP and even 89% of the variation among Maluridae
393 populations and species. In particular, the density of males was a good predictor of variation
394 in EPP among species in Maluridae, showing that existing hypotheses can explain the
395 variation in EPP well.

396

397 *Implications for key hypotheses and alternative explanations*

398 Density has received considerable attention in studies investigating variation in EPP, because
399 a higher encounter rate between individuals should facilitate EP mating (Westneat *et al.*
400 1990). Previous work comparing EPP among populations with different densities have shown
401 mixed results (Griffith *et al.* 2002). This may be because the number of populations
402 compared is usually small and the variation in both density and EPP are limited. A
403 comparative analysis on 72 species provided some evidence that density explains
404 intraspecific variation (Westneat & Sherman 1997) and a recent study on 13 populations of
405 the reed bunting (*Emberiza schoeniclus*) showed a positive association between density and
406 EPP both within- and among subpopulations (Mayer & Pasinelli 2013). Here we have
407 similarly shown that females living at higher density and species with a higher density of
408 dominant males were associated with higher EPP. Thus, there is emerging evidence that
409 density plays a key role in explaining inter-population and inter-specific variation in EPP
410 when considering studies that have sufficient power of detection. The geographical scale over
411 which extra-pair behaviour occurs (i.e. the distances females travel to mate) is needed to
412 interpret these density effects. Kingma *et al.* (2009) suggest that habitat configuration can
413 reduce the likelihood that a female encounters a male of sufficient quality to make cuckolding
414 her mate worthwhile, which may help explain why effects were most pronounced at low
415 densities in our analyses. Furthermore, species differ in how many territories females traverse
416 in order to mate, so that identifying a density metric that is both general and biologically
417 relevant is challenging (particularly in broad-scale comparative studies on species that vary
418 widely in their behaviour). We have used the density of immediate neighbours, which reflects
419 the modal distance of extra-pair sires in Maluridae for which this is known (Double &

420 Cockburn 2003; Brouwer *et al.* 2011; Kingma *et al.* 2013), but we cannot exclude the
421 possibility that some species travel further and that this may explain the mixed results among
422 species for individual-level density effects.

423 A general problem with the constrained female hypothesis is that the direction of
424 causality can be uncertain. Specifically, a reduced dependency on care will allow females to
425 pursue more EPP, but more EPP could also result in reduced investment by males. Evidence
426 exists for both pathways; for example experimental increase of cuckoldry risk reduced a
427 male's investment in paternal care in dung beetles (*Onthophagus taurus*, Hunt & Simmons
428 2002), whereas an increase in territory quality resulted in reduced dependency on male care
429 and increased EPP in serins (*Serinus serinus*, Hoi-Leitner *et al.* 1999). We found higher EPP
430 in species with less male care among dominant males, which can also be interpreted in both
431 ways as a driver or consequence of EPP. In contrast, our findings that females and
432 populations with more helpers had higher EPP supports the hypothesis that lowering female
433 constraints from male care favours higher EPP, as helpers provide care but rarely gain
434 paternity from their mothers in their own territory. Further support against a reversal of
435 causality comes from behavioural evidence that relatedness to the offspring does not predict a
436 male's provisioning rate in two *Malurus* species (Varian-Ramos *et al.* 2012; L. Brouwer,
437 unpublished data).

438 The inbreeding avoidance hypothesis was first proposed to explain the high incidence
439 of incestuous pairing and high levels of EPP in *M. splendens* (Brooker *et al.* 1990), although
440 this hypothesis is still hotly debated (e.g.: Arct *et al.* 2015; Forstmeier 2015; Nakagawa *et al.*
441 2015). Correlations between the occurrence of incestuous pairs and EPP could be the result of
442 other factors, like population density, or be a side-effect of males investing less in mate-
443 guarding when paired to a closely related female. However, there are several lines of
444 evidence which support the idea that extra-pair mating helps avoid inbreeding. First, the
445 proportion of incestuous pairings predicted variation in EPP better than density or the number
446 of helpers (Fig. 3Ai-iii). Second, in Maluridae females have been shown to control extra-pair
447 mating by visiting the extra-pair male's territory at dawn, making it unlikely that mate-
448 guarding plays a role in this system (Double & Cockburn 2000). Third, in *all* Maluridae
449 species and populations, incestuous pairs had higher EPP than non-incestuous pairs (Fig. 2d).
450 Furthermore, it has been shown that females were less related to extra-pair sires than to their
451 social mates (Tarvin *et al.* 2005; Brouwer *et al.* 2011; Kingma *et al.* 2013), and that
452 experimental manipulation of pair relatedness did affect EPP rates (Varian-Ramos & Webster
453 2012). Kin-recognition is likely to be the underlying mechanism of inbreeding avoidance

454 through EPP, although a role of sperm compatibility cannot be excluded. Nevertheless, it
455 seems unlikely that extra-pair mating primarily serves as an inbreeding avoidance
456 mechanism, because in many Maluridae populations/species the majority of females gain
457 EPP, while only a minority are paired incestuously. Some of us have even argued that cause
458 and effect of this association could be in the opposite direction: populations or species with
459 high levels of EPP would allow females to form incestuous social pairs (Cockburn *et al.*
460 2013).

461 Alternative (ultimate) hypotheses have been proposed that we have not considered
462 here, either because they do not lead to testable predictions or the data to test them are
463 unavailable for the Maluridae. For example, EPP has been suggested to be a by-product of
464 selection on other characteristics of the mating system (Arnqvist & Kirkpatrick 2005;
465 Forstmeier *et al.* 2011), a mechanism for females to choose their preferred (high quality)
466 mate (Møller 1992; Lifjeld *et al.* 1993) or genetically compatible males (Ball & Parker 2003;
467 Griffith & Immler 2009), when social mate choice is restricted. However, identifying suitable
468 predictor variables for these hypotheses and collecting the biological data for meaningful
469 tests is extremely challenging. Moreover, it is likely that some of these ideas, like male
470 quality and genetic compatibility, will be correlated with male density.

471
472 *Implications for how we study variation in EPP*

473 Strong phylogenetic signals prevent meaningful testing of hypotheses that explain
474 interspecific variation in EPP, highlighting the importance of intra-family comparisons.
475 However, investigation of the key hypotheses in closely-related species is often problematic
476 because variation in both EPP and the explanatory factors is generally limited, hampering
477 detection of patterns. Our study has several important implications. First, by studying a
478 family of birds that exhibits sufficient variation in both EPP and the predictors of interest, a
479 large part of the interspecific variation in EPP rates was explained. The idea that intra-family
480 comparison can lead to different insights is exemplified by the density hypothesis. Density is
481 typically correlated with many other factors, such as breeding system, and previous
482 comparative studies across species in many families did not find any evidence for a role of
483 density in interspecific variation in EPP (Westneat & Sherman 1997; Wink & Dyrce 1999).
484 By contrast, here we have shown that density does explain a large percentage of the
485 interspecific variation in EPP when comparing closely related species with relatively similar
486 breeding systems.

487 The second implication of our study is that investigating multiple hypotheses
488 simultaneously may lead to different insights than studying the role of single variables in
489 isolation. For example, a unifactorial approach showed support for the density hypothesis at
490 the population level, whereas this hypothesis was not supported in a multifactorial approach
491 after accounting for the constrained female hypothesis. Furthermore, both male density and
492 male care explained a substantial amount of the interspecific variation in EPP, but density
493 was relatively more important. Finally, we did not find evidence for a role of habitat
494 geometry in variation in EPP rates, which at first sight seems to contradict the result that
495 individuals with more neighbours had higher EPP. However, we found that male density
496 explained variation in EPP better than geometry, possibly because male density can still be
497 relatively low in contiguous habitat due to large territory sizes.

498 The third (although not very surprising) implication of our study is that it is premature
499 to reject hypotheses on the basis of analysis at only a single level of variation. While some
500 hypotheses enjoyed strong support at particular levels of analysis, no single factor was
501 associated with variation in EPP at all levels. Variation in EPP among species, which was
502 partly explained by male density, has been determined on a very different evolutionary time
503 scale compared to variation among years, which was best explained by the proportion of
504 incestuous pairings. Male density of a species will very much depend on habitat
505 characteristics, whereas the proportion of incestuous pairings will vary with the annual
506 dynamics of the population. The lack of support for a single hypothesis at all levels in our
507 study may help explain why previous studies have shown so many mixed results (Griffith *et*
508 *al.* 2002).

509 Finally, we showed that including different predictors for the same hypotheses
510 combined with a good understanding of the behaviour might help disentangle cause and
511 effect of correlations. Our interpretation that a reduction of female's constraints allows for
512 higher EPP was based on both the effect of a male's contribution to care, and the number of
513 helpers (see above). Experimental studies may provide an alternative way to disentangle
514 cause and effect. However experiments on EPP in the wild are often not straightforward and
515 additionally run the risk of unknowingly manipulating several variables rather than the
516 purported sole experimental variable. For example, by manipulating density of a population,
517 the resources available for a female might be affected too, altering her constraints in pursuing
518 EPP.

519 To conclude, our findings that different hypotheses play a role in explaining EPP at
520 different levels also indicates that these results are context dependent and thus will vary with

521 the specific characteristics of the study system. We studied a family of birds that is quite
522 atypical in that all species are cooperative breeders. The presence of helpers specifically,
523 reduces constraints for females to a much larger extent than could be expected in systems
524 without helpers. Nevertheless, additional comparative studies on closely-related species are
525 needed to confirm whether patterns generally are more apparent at the within-family level,
526 and whether a re-evaluation of the evidence provided by broad-scale comparative studies on
527 EPP is needed. However, there are impediments to assembling data from more families,
528 namely the need for sufficient knowledge of behaviour and variation in EPP and ecology, the
529 challenges to define biologically relevant predictors when species vary widely in their
530 behaviour, and the immense research effort needed for detailed field studies. Despite such an
531 arduous task that requires concerted research effort, there are substantial rewards of growing
532 insight into how and why EPP occurs.

533

534

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729

730 **Author contributions**

731 LB and MP developed the idea. LB collated the data, performed the analyses and wrote the
732 paper. LB, MP and AC discussed the results and implications. All authors contributed to data
733 collection and commented on the manuscript.

734

735 **Data Accessibility**

736 Data is provided in Supporting Information C and Supporting Information D.

737

738 **Figure legends:**

739

740 **Figure 1.** The proportion of extra-pair paternity versus the number of offspring sampled for
741 a) 20 different Maluridae populations and b) 25 years of a single *M. cyaneus* population. The
742 quantiles are derived by sampling from a binomial distribution with an average of 0.57 (a)
743 and 0.66 (b) respectively.

744

745 **Figure 2.** The proportion of extra-pair paternity (number extra-pair offspring/ total number
746 offspring at that category level) for females from different Maluridae populations in relation

747 to a) breeding asynchrony, b) the number of neighbouring territories, c) the number of helpers
748 in a group and d) social pairing. Regression lines for which the 95% CI of the slope did not
749 overlap with zero are depicted by solid lines. The size of symbols is proportional to the cube
750 root of the sample size. For legend see Figure 1.

751

752 **Figure 3.** The variation in proportion of EPP in Maluridae at the A) temporal, B) inter-
753 population and C) interspecific level in relation to predictors of the breeding synchrony,
754 density, constrained female, inbreeding avoidance and life-history hypotheses. The size of
755 symbols are proportional to the cube root of the sample size. Estimates for trendlines were
756 derived from Table 2, those of predictors which received support by the data are shown in
757 solid, whereas those that were not supported are dashed. Note that in Cii) the mean habitat
758 geometry of a species can vary between 0 (contiguous) and 1 (linear) due to populations of a
759 single species having different geometries. For legend see Figure 1

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Table 1. Hypotheses proposed for variation in EPP together with their predictions, and predictors used to test them in this study at the level of the individual, year and population/species. Predictors shown underlined received support in our analyses.

Hypothesis	Explanation	Prediction	Level of variation		
			Individual	Temporal	Inter-population/ Interspecific
Breeding synchrony: a. Male assessment b. Male trade-off	a. Breeding synchronously facilitates simultaneous comparison of different males (Westneat et al. 1990). b. Synchrony results in trade-off for males between mate guarding and EP mating (Stutchbury & Morton 1995)	a. Breeding more synchronously will result in higher EPP rates. b. Breeding more synchronously will result in lower EPP rates.	Breeding synchrony	–	Breeding synchrony
Density	The encounter rates between individuals affect the rate of EPP (Westneat et al. 1990).	Higher population or breeding density increases the rate of EPP.	<u>No. neighbours</u>	Male density	<u>Male density (sp)</u> & Habitat geometry
Constrained female	Females are constrained in pursuing EPP, because it can result in retaliation by the male, leading to reduced paternal care when the male loses confidence in paternity (Birkhead & Møller 1996).	Reduced dependency on care by the male (more helpers or population where males contribute less) will result in higher EPP.	<u>No. helpers</u>	No. helpers	<u>No. helpers</u> & <u>Proportion male care</u>
Inbreeding	Inbreeding can be reduced by mating with an extrapair partner (Brooker et al. 1990; Pusey &	EPP rates will increase with higher rates of pairings between highly	<u>Incestuous</u>	<u>Proportion incestuous</u>	Proportion incestuous

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avoidance	Wolf 1996).	related individuals.	<u>pairing</u>	<u>pairings</u>	pairings
Life-history (male survival)	Risk of retaliation by males with a short lifespan is low, as it is not adaptive for them to abandon a reproductive event. (Wink & Dyrz 1999)	Lower survival will result in higher EPP.	-	-	Male survival

Table 2. Summary of model selection results testing the key hypotheses to explain temporal, inter-population and interspecific variation in EPP. Coefficients are shown with SE's based on standardized predictor variables (z-scores) and are on the logit scale. N.a. means that predictor variables were either not available, or that the variable does not vary at that level of investigation; “-” means that predictor variable was not fitted in that particular model. N = 89 years from 20 populations of 9 species. The null model with random effects only had a $\Delta AIC_c = 27$, $\sigma^2_{Species} = 1.56$, $\sigma^2_{Population} = 0.25$, $\sigma^2_{Year} = 0.03$

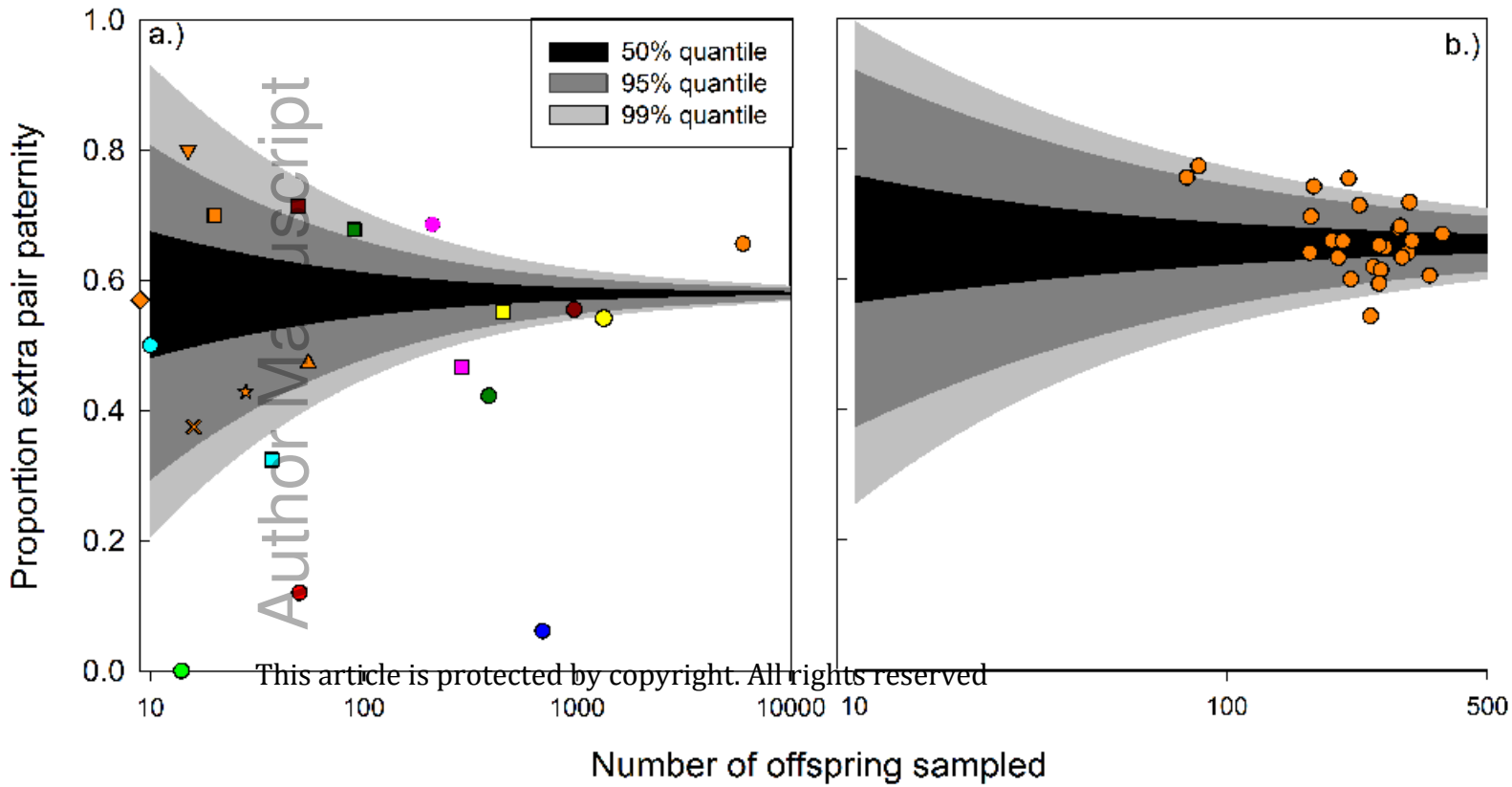
Model	ΔAIC_c	Model weight	Intercept	σ^2_{Spp}	σ^2_{Po}	σ^2_{Yr}	Hypothesis	Breeding synchrony		Density		Constrained female		Inbreeding avoidance	Life history
								Level of variation	Var(prop ortion fertile females)	breeding synchrony index	Log Male density	Habitat geometr y*	No. helpers	Proportio n male care	Proportion incestuous pairs
1	0	0.08	-0.22±0.12	0.0	0.17	0.02	Temporal	n.a.	n.a.	-	n.a.	-	n.a.	0.13±0.04	n.a.
							Inter- population	-	-	-	-	0.49±0.10	-	-	-
							Interspecific	-	-	0.83±0.16	-	-	-0.65±0.13	-	-
2	0.7	0.06	-0.20±0.09	0.0	0.07	0.02	Temporal	n.a.	n.a.	-	n.a.	-	n.a.	0.14±0.04	n.a.
							Inter- population	-	-	-	-	0.28±0.09	-0.47±0.09	-	-

							Interspecific	0.36±0.12	-	0.67±0.11	-	-	-	-	-
3	1.3	0.04	-0.21±0.11	0.0	0.09	0.02	Temporal	n.a.	n.a.	-	n.a.	-	n.a.	0.14±0.04	n.a.
							Inter-population	-	-	-	-	0.33±0.10	-	-	-
							Interspecific	0.31±0.13	-	0.73±0.15	-	-	-0.54±0.12	-	-
4	1.4	0.04	-0.21±0.13	0.0	0.18	0.02	Temporal	n.a.	n.a.	-	n.a.	-	n.a.	0.13±0.04	n.a.
							Inter-population	-	-	-	-	0.45±0.10	-0.57±0.12	-	-
							Interspecific	-	-	0.78±0.16	-	-	-	-	-
5	1.4	0.04	-0.19±0.14	0.04	0.15	0.02	Temporal	n.a.	n.a.	-	n.a.	-	n.a.	0.13±0.04	n.a.
							Inter-population	-	-	-	-	-	-0.66±0.14	-	-
							Interspecific	-	-	0.79±0.19	-	0.49±0.12	-	-	-
6	1.8	0.03	-0.21±0.10	0.0	0.10	0.02	Temporal	n.a.	n.a.	-	n.a.	-	n.a.	0.14±0.04	n.a.
							Inter-population	-	-	-	-	0.41±0.09	-	-	-
							Interspecific	-	0.35±0.15	0.66±0.13	-	-	-0.66±0.11	-	-

*Reference category is contiguous habitat.

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Species	Population							
	I	II	III	IV	V	VI	VII	average
<i>A. modestus</i>	●							⬡
<i>M. alboscapulatus</i>	○	□						⬡
<i>M. coronatus</i>	●							⬡
<i>M. cyaneus</i>	○	□	◇	▽	☆	△	✕	⬡
<i>M. elegans</i>	●	■						⬡
<i>M. lamberti</i>	○	□						⬡
<i>M. melanocephalus</i>	○	□						⬡
<i>M. splendens</i>	●	■						⬡
<i>S. malachurus</i>	●							⬡

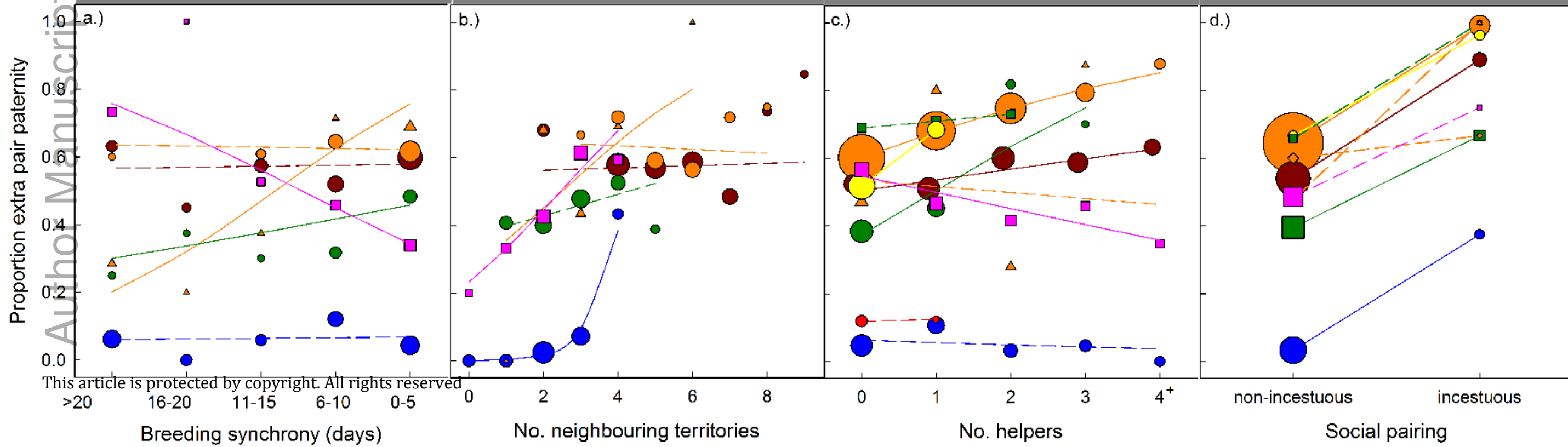


Breeding synchrony

Density

Constrained female

Inbreeding avoidance





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Title:

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