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

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Abstract

Extra-pair paternity (EPP), where offspring are sired by a male other than the social male, varies enormously both within and among species. Trying to explain this variation has proved difficult because the majority of the interspecific variation is phylogenetically-based. Ideally, variation in EPP should be investigated in closely related species, but clades with sufficient variation are rare. We present a comprehensive multifactorial test to explain variation in EPP among individuals in 20 populations of nine species over 89 years from a single bird family (Maluridae). Females had higher EPP in the presence of more helpers, more neighbours, or if paired incestuously. Furthermore, higher EPP occurred in years with many incestuous pairs, populations with many helpers, and species with high male density or in which males provide less care. Altogether, these variables accounted for 48% of the total and 89% of the interspecific and inter-population variation in EPP. These findings indicate why consistent patterns in EPP have been so challenging to detect and suggest that a single predictor is unlikely to account for the enormous variation in EPP across levels of analysis. Nevertheless, it also shows that existing hypotheses can explain the variation in EPP well and that the density of males in particular is a good predictor to explain variation in EPP among species when a large part of the confounding effect of phylogeny is excluded.

Introduction

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

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

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

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

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

Methods

Study system and data collection

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

We collated published and unpublished data from 4,072 broods and 10,665 offspring collected over 89 study years from nine species of Maluridae spanning 20 populations (see Supporting Information A for an overview of the data). Our dataset included 7 populations of superb fairy-wren (*Malurus cyaneus*; Double & Cockburn 2003; Colombelli-Négrel *et al.* 2009; Bain *et al.* 2014), two populations each of white-shouldered fairy-wren (*M. alboscapulatus;* for details see Supporting Information A), red-winged fairy-wren (*M. elegans*; Brouwer *et al.* 2014), variegated fairy-wren (*M. lamberti*; for details see Supporting Information A; Johnson 2016), red-backed fairy-wren (*M. melanocephalus*; Varian-Ramos *et al.* 2012; Baldassarre & Webster 2013), and splendid fairy-wren (*M. splendens*; Brooker *et al.* 1990; Webster *et al.* 2004; Tarvin *et al.* 2005), and one population each of purple-crowned fairy-wren (*M. coronatus*; Kingma *et al.* 2009), southern emu-wren (*Stipiturus malachurus*; Maguire & Mulder 2008) and thick-billed grasswren (*Amytornis modestus*; Louter 2016). Studies were included for all populations where genetic parentage analyses had been conducted and sufficient data were available to estimate the majority of the predictors of interest (see below). We report data on EPP here, but it should be noted that our estimates of EPP are almost identical to the rate of extra-group paternity (i.e. paternity by males from outside the social group), as within-group subordinates rarely gain paternity (Mulder *et al.* 1994; Webster *et al.* 2004; Brouwer *et al.* 2011). EPP data are based primarily on data collected from nestlings between 2 and 8 days old, except for *M. alboscapulatus,* for which fledglings were sampled. Starvation of nestlings is rare, and incomplete sampling is usually due to predation. Genotyping was based on microsatellite data except for the population of *M. splendens* from Perth which was genotyped using allozymes (Brooker *et al.* 1990), and *A. modestus,* which was based on RAD sequencing (Louter 2016). Although these methods differ in their ability to assign parentage to extra-group males, all of them are excellent in determining mismatches with the territorial male, and hence should produce identical estimates of EPP (methods are unbiased; Kaiser *et al.* 2017). Re-analysing the top-models after excluding the *M. alboscapulatus* and *M. splendens* studies showed that the results remain largely unchanged, although the association between EPP and the number of helpers receives more support at the species rather than the population level (see Table S1).

Defining and measuring predictors of EPP

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

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

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

3. Constrained female hypothesis: the presence of helpers might reduce the dependency of the female on care by the dominant male, as helpers can potentially compensate for reduced investment or desertion by the dominant, allowing the female greater freedom to pursue EPP (Mulder *et al.* 1994). Consequently, at the individual level we used the number of male and female helpers per female as a predictor. The mean of the annual number of helpers per population and per species were used as predictors at the population and species level respectively, whereas the annual deviation of the population mean number of helpers was used as a predictor for temporal variation. In addition, at the population and species level we also used male care as a predictor, calculated as the average proportion of provisioning rates made by males without helpers.
4. Inbreeding avoidance hypothesis: inbreeding avoidance via EPP is potentially most beneficial in closely related social pairs, thus incestuous (between first order relatives) social pairing was used as a predictor. For the *M. cyaneus* ACT population a pedigree was used to determine whether a pair was incestuous or not. For other populations a pair was considered incestuous when its pairwise relatedness (r) calculated from the molecular markers (Lynch & Ritland 1999; Wang 2002) was within the range of the mean ± 1.5 S.D. of known first order relatives. We choose this measure rather than a fixed value (i.e. r = 0.5) to account for genotyping errors and because relatedness values will vary depending on the microsatellites used. Whether a pair was incestuous or not was used as a predictor at the individual level. The means of the annual proportion of incestuous pairings per population and per species were used as predictors at the population and species level respectively, whereas the annual deviation of the population mean was used as a predictor for temporal variation.

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

Statistical analyses

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

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

For various reasons (e.g. data were not collected, experimental manipulations, or limited project duration) not all predictor variables were available for each year/population (see Supporting Information C). Missing values (9% missing) were assumed to be missing completely at random and set to zero after transforming each variable to z-scores (Nakagawa & Freckleton 2011). This enabled us to use the full dataset and test the different hypotheses simultaneously with a multifactorial model selection approach. Testing the final model on a dataset without missing values did not qualitatively change the results.

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

Although we studied closely related species from a single family, phylogenetic patterns at a lower taxonomic level could still affect the results. To investigate whether our results can be explained by phylogeny, the variables from the top model were fitted in a phylogenetic mixed model approach using R package MCMCglmm (Hadfield 2010). Unfortunately, the phylogeny of Maluridae has not been fully resolved, with the position of *M. coronatus* being ambiguous (Cockburn *et al.* 2013). To account for phylogenetic uncertainty we followed a similar approach as Ross *et al.* 2013. We downloaded 1,300 different trees from BirdTree.org (Jetz *et al.* 2012, see Supporting Information D) and sampled a tree from the posterior distribution of trees at iteration *t*, running the MCMC model for 1,000 iterations and saving the median from each run. This process was repeated for 1,300 iterations where we disposed of the first 300 as a burn-in. *A. modestus* has only recently been considered as a different species from *A. textilis* (Black *et al.* 2010), but unfortunately this has not been included in phylogenies yet. Consequently, we used the phylogenetic data for *A. textilis* here. The results showed that after accounting for phylogeny, all variables from the best supported model remained statistically significant and effect sizes barely changed, with the phylogenetic signal being rather weak (λ = 0.13, Pagel 1999; see Supporting Information A).

Results

Variation in EPP across levels

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

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

Variation among individuals

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

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

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

Variation among years

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

Variation among populations

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

Variation among species

Patterns at the species level were consistent with both the density and the constrained female hypotheses, but not with the inbreeding avoidance or life-history hypotheses (Fig. 3Ci-vii). There was also not much support for the breeding synchrony hypothesis, because adding breeding synchrony to the top model increased AIC_c values (Table 2, model 1 vs. model 2 & 3). Replacing our breeding synchrony measure by the breeding synchrony index following Kempenaers (1993) showed that the latter was not a better predictor for variation in EPP (Table 2, model 6 vs. model 3). The density hypothesis was strongly supported as dominant male density was consistently included in the top 182 models (Table S2), indicating that Maluridae with a higher male density were associated with higher EPP rates (Fig. 3Cii). An additional effect of habitat geometry was not supported by the data, as the addition of geometry to the best supported model increased AIC$_c$ values (ΔAIC$_c$ = 2.5, Fig. 3Ciii). Support for the constrained female hypothesis came from the association that species with reduced male care (Table 2, models 1, 3 & 6; Fig. 3v) had higher EPP. Although there was some support for this hypothesis at the population level, replacing the population predictor with the species predictor in the top models reduced AICc values (Table 2, model 4 vs. model 1 ΔAIC$_c$ = -1.4), indicating that there was little evidence for additional variation among populations. There was no evidence that the number of helpers at the species level explained variation in EPP better than the number of helpers at the population level (Table 2, model 5 vs models 1-4).

Explanatory value and relative importance of hypotheses

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

Discussion

This is the first comprehensive analysis to simultaneously test multiple key hypotheses at different taxonomic levels. Using data from possibly the best-studied family of birds with respect to genetic mating system, we found that variation in EPP rates were consistent with the inbreeding avoidance, constrained female and density, but not with the life-history or breeding synchrony hypotheses. At the individual level, females had higher EPP if they had more helpers, more neighbours, or were paired incestuously. Furthermore, years with many incestuous pairs, populations with many helpers, and species with high male density and/or low levels of male care were associated with higher EPP rates. Together, these factors explained 48% of the total variation in EPP and even 89% of the variation among Maluridae populations and species. In particular, the density of males was a good predictor of variation in EPP among species in Maluridae, showing that existing hypotheses can explain the variation in EPP well.

Implications for key hypotheses and alternative explanations

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

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

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

The inbreeding avoidance hypothesis was first proposed to explain the high incidence of incestuous pairing and high levels of EPP in *M. splendens* (Brooker *et al.* 1990), although this hypothesis is still hotly debated (e.g.: Arct *et al.* 2015; Forstmeier 2015; Nakagawa *et al.* 2015). Correlations between the occurrence of incestuous pairs and EPP could be the result of other factors, like population density, or be a side-effect of males investing less in mate-guarding when paired to a closely related female. However, there are several lines of evidence which support the idea that extra-pair mating helps avoid inbreeding. First, the proportion of incestuous pairings predicted variation in EPP better than density or the number of helpers (Fig. 3Ai-iii). Second, in Maluridae females have been shown to control extra-pair mating by visiting the extra-pair male's territory at dawn, making it unlikely that mate-guarding plays a role in this system (Double & Cockburn 2000). Third, in *all* Maluridae species and populations, incestuous pairs had higher EPP than non-incestuous pairs (Fig. 2d). Furthermore, it has been shown that females were less related to extra-pair sires than to their social mates (Tarvin *et al.* 2005; Brouwer *et al.* 2011; Kingma *et al.* 2013), and that experimental manipulation of pair relatedness did affect EPP rates (Varian-Ramos & Webster 2012). Kin-recognition is likely to be the underlying mechanism of inbreeding avoidance through EPP, although a role of sperm compatibility cannot be excluded. Nevertheless, it seems unlikely that extra-pair mating primarily serves as an inbreeding avoidance mechanism, because in many Maluridae populations/species the majority of females gain EPP, while only a minority are paired incestuously. Some of us have even argued that cause and effect of this association could be in the opposite direction: populations or species with high levels of EPP would allow females to form incestuous social pairs (Cockburn *et al.* 2013).

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

Implications for how we study variation in EPP

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

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

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

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

To conclude, our findings that different hypotheses play a role in explaining EPP at different levels also indicates that these results are context dependent and thus will vary with the specific characteristics of the study system. We studied a family of birds that is quite atypical in that all species are cooperative breeders. The presence of helpers specifically, reduces constraints for females to a much larger extent than could be expected in systems without helpers. Nevertheless, additional comparative studies on closely-related species are needed to confirm whether patterns generally are more apparent at the within-family level, and whether a re-evaluation of the evidence provided by broad-scale comparative studies on EPP is needed. However, there are impediments to assembling data from more families, namely the need for sufficient knowledge of behaviour and variation in EPP and ecology, the challenges to define biologically relevant predictors when species vary widely in their behaviour, and the immense research effort needed for detailed field studies. Despite such an arduous task that requires concerted research effort, there are substantial rewards of growing insight into how and why EPP occurs.

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Author contributions

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

Data Accessibility

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

Figure legends:

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

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

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

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

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.

			Level of variation		
Hypothesis	**Explanation**	**Prediction**	**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.	No. neighbours	Male density	Male density (sp) & 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.	No. helpers	No. helpers	No. helpers & Proportion male care
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	Incestuous	Proportion incestuous	Proportion incestuous

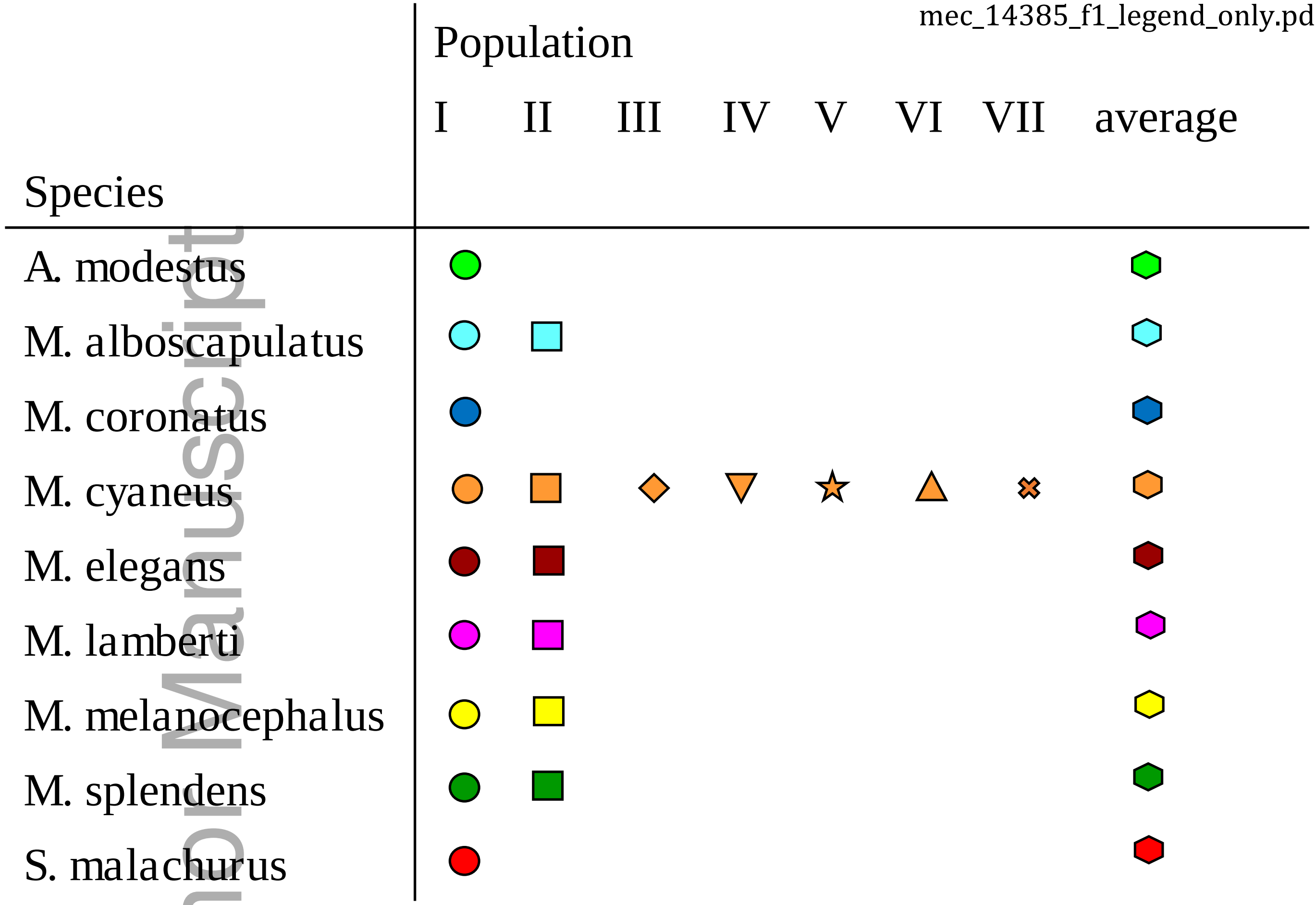
avoidance	Wolf 1996).	related individuals.	pairing	pairings	pairings
Life-history (male survival)	Risk of retaliation by males with a short lifespan is low, as it is not adaptive for them to abandon a reproductive event. (Wink & Dyrcz 1999)	Lower survival will result in higher EPP.	–	–	Male survival

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 $\Delta AIC_c = 27$, $\sigma^2_{Species} = 1.56$, $\sigma^2_{Population} = 0.25$, $\sigma^2_{Year} = 0.03$

							Hypothesis	**Breeding synchrony**		**Density**		**Constrained female**		**Inbreeding avoidance**	**Life history**
Model	**ΔAIC_c**	**Model weight**	**Intercept**	**σ^2_{Spp}**	**σ^2_{Pop}**	**σ^2_{Yr}**	**Level of variation**	**Var(proportion fertile females)**	**breeding synchrony index**	**Log Male density**	**Habitat geometry***	**No. helpers**	**Proportion male care**	**Proportion incestuous pairs**	**Male survival**
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.



	Population							
Species	I	II	III	IV	V	VI	VII	average
A. modestus	●							⬢
M. alboscapulatus	●	■						⬢
M. coronatus	●							⬢
M. cyaneus	●	■	◆	▼	★	▲	✖	⬢
M. elegans	●	■						⬢
M. lamberti	●	■						⬢
M. melanocephalus	●	■						⬢
M. splendens	●	■						⬢
S. malachurus	●							⬢

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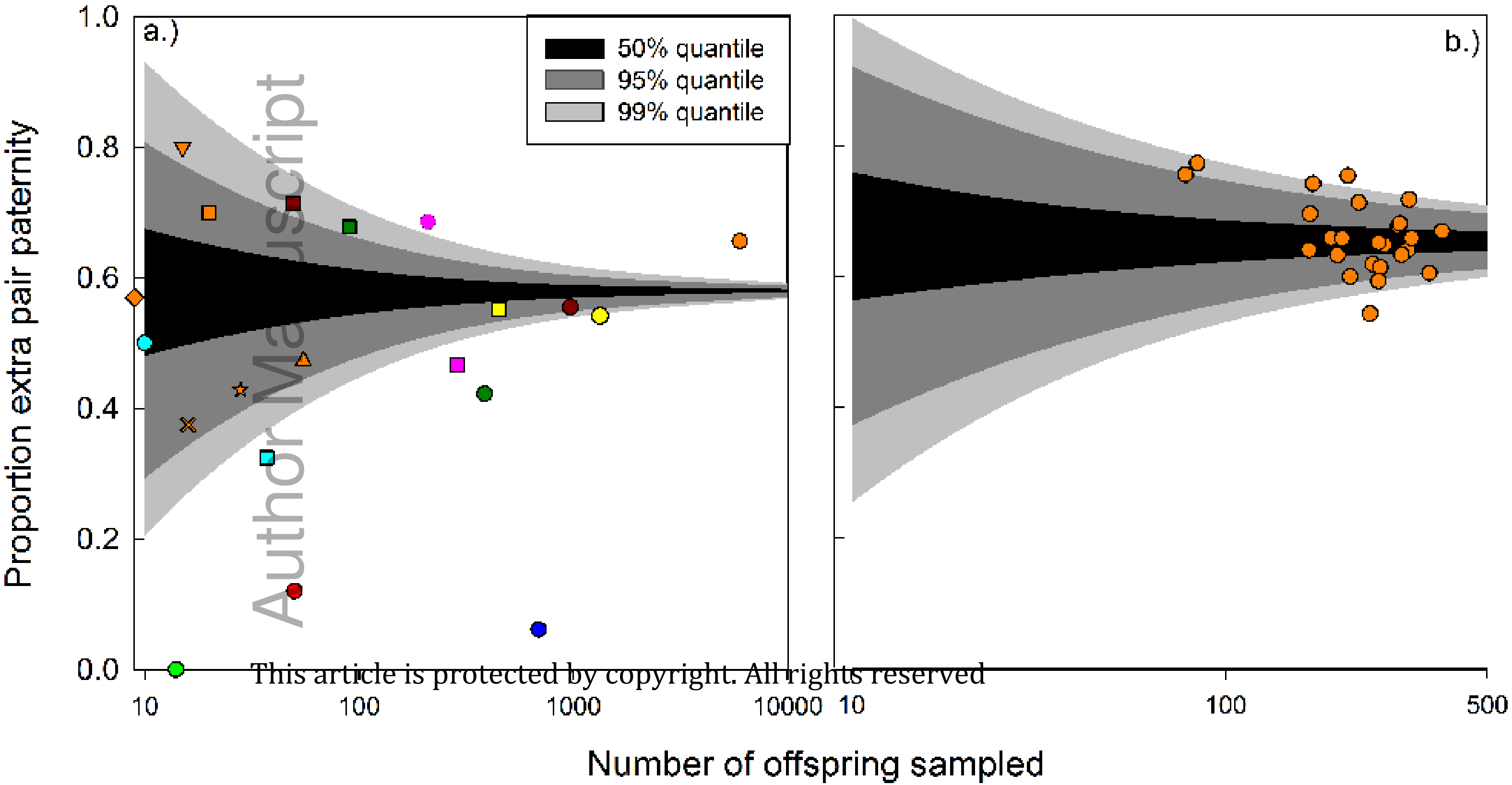

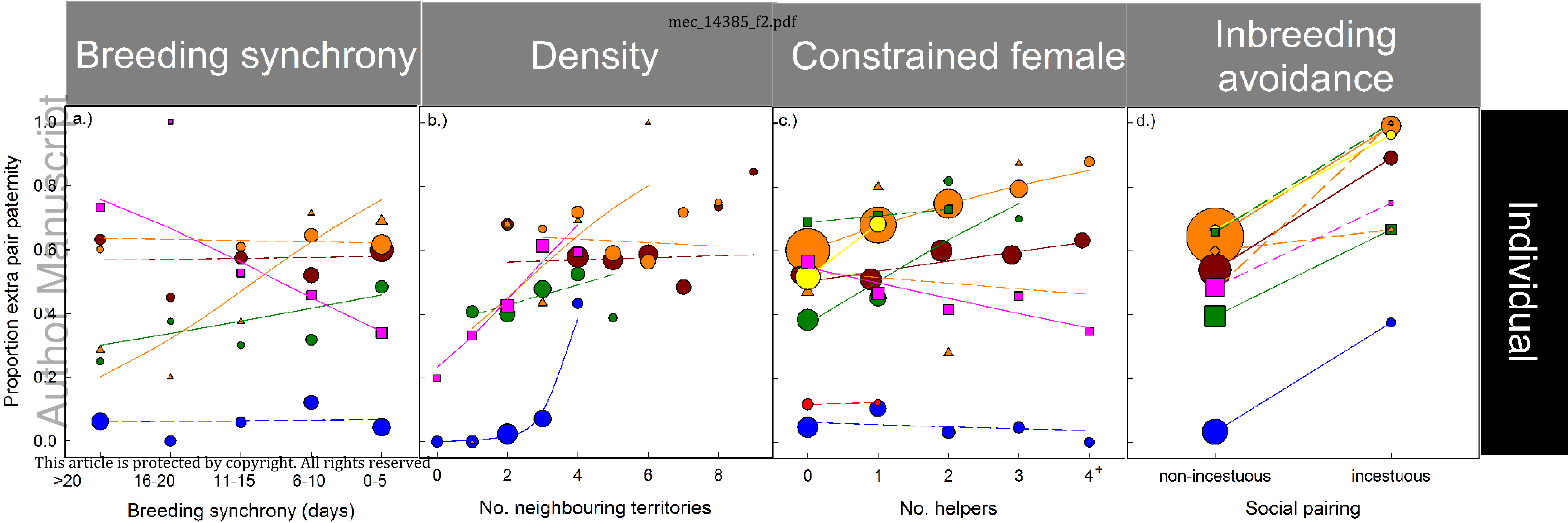
Breeding synchrony
Density
Constrained female
Inbreeding avoidance
Individual
a.)
b.)
c.)
d.)
Proportion extra pair paternity
0.0
0.2
0.4
0.6
0.8
1.0
>20
16-20
11-15
6-10
0-5
Breeding synchrony (days)
0
2
4
6
8
No. neighbouring territories
0
1
2
3
4+
No. helpers
non-incestuous
incestuous
Social pairing