




# Annual egg productivity predicts female-biased mortality in avian species

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Among avian species, the differential cost entailed by either sex in competition for mates has been regarded as the main evolutionary

influence on sex differences in mortality rates. However, empirical evidence suggests that sex-biased adult mortality is mainly related to differential energy investment in gamete production, with a greater annual mass devoted to egg production leading to higher female mortality. We explicitly tested the generality of this pattern in a comparative framework. Annual egg production can be relatively large in some species (up to 200% of female body mass) and annual mortality is generally biased toward females. We showed that greater annual egg productivity resulted in higher mortality rates of females relative to males. Mating system was secondarily important, with species in which males were more involved in mating competition having more equal mortality rates between the sexes. However, both traits explained only a limited fraction of the interspecific variation in female-biased mortality. Other traits, such as sexual size dimorphism and parental care, had much weaker influences on female-biased mortality. Our results suggest that both annual mass devoted to gamete production by females and mating system contribute to the evolution of the fundamental life-history trade-off between reproduction and survival in avian taxa.

**KEY WORDS:** Birds, comparative analysis, egg productivity, path analysis, reproduction cost, sex-biased mortality, trade-off.

In life-history theory, trade-offs occur whenever an increase in the expression of one fitness-related trait is counterbalanced by a decrease in the expression of other traits (Stearns 1992; Roff 2002). The most prominent life-history trade-offs involve reproduction (Reznick 1985; Stearns 1992; Roff 2002; Harshman and Zera 2007). Indeed, natural selection optimizes the balance between the reproductive (the fraction of the total energy budget of an organism that is devoted to reproductive processes) and somatic efforts (the fraction of the total energy budget of an organism that

is devoted to somatic growth and maintenance) that produce the greatest individual fitness (Hirshfield and Tinkle 1975). One of the central tenets of life-history theory is thus that current reproductive effort may compromise survival and future reproduction, and these reductions contribute to the so-called cost of reproduction (Williams 1966; Tuomi et al. 1983; Reznick 1985; Nur 1988; Lindén and Møller 1989; Stearns 1992; Roff 2002; Harshman and Zera 2007). Trade-offs between reproductive effort and survival have been addressed by many experimental and correla-

tive studies concerning single populations/species (e.g., Gustafsson and Sutherland 1988; Nilsson and Svensson 1996; Cox et al. 2010), but also in comparative and meta-analytic investigations (e.g., Promislow and Harvey 1990; Shine and Schwarzkopf 1992; Owens and Bennett 1994; Martin 1995; Promislow 2003; Dobson and Jouventin 2010; Santos and Nakagawa 2012; Sibly et al. 2012; Székely et al. 2014). Overall, these studies generally provided clear evidence—from both physiological and evolutionary standpoints (Reznick 1985)—of a negative relationship between these two competing energy-demanding activities. Notwithstanding, its generality, pervasiveness, and strength have been questioned (e.g., Alerstam and Högstedt 1984; Both et al. 1998; Santos and Nakagawa 2012).

Considering that males and females play different roles in sexual reproduction and usually invest a different amount of energy and resources in a given reproductive episode, sex differences in reproductive costs may be expected. Indeed, the differential costs of reproductive activities between the sexes have often been associated with sex-biased adult mortalities (Promislow 1992, 2003; Promislow et al. 1992; Owens and Bennett 1994; Moore and Wilson 2002; Liker and Székely 2005; Székely et al. 2014).

Adult mortality may be affected by multiple time- and energy-demanding processes related to reproduction, often occurring at different times in the life cycle. Mortality may be biased toward males when the intensity of sexual selection and the extent of mating competition is higher among males than females (Promislow 1992, 2003; Promislow et al. 1992; Moore and Wilson 2002; Roff 2002; Liker and Székely 2005; Christe et al. 2006), or toward females in those species in which females are the sex that invests the most in caring for the progeny (Williams 1966; Owens and Bennett 1994; Promislow 2003; Liker and Székely 2005; Székely et al. 2014).

Results of comparative analyses of sex-biased mortality were consistent with either or both of these predictions (Owens and Bennett 1994; Promislow 2003; Liker and Székely 2005; Székely et al. 2014). However, most previous comparative studies on the mortality effects of reproduction did not explicitly focus on interspecific variation in energetic investment in gamete production (Owens and Bennett 1994; Liker and Székely 2005; but see Martin 1995; Székely et al. 2014). This is unfortunate because in many anisogamous and oviparous taxa, like birds, gamete production can represent an important form of reproductive investment (Monaghan and Nager 1997; Williams 2005). Although male energy investment in spermatozoa production might be non-negligible in species with intense sperm competition (review in Lemaître et al. 2020), it is widely acknowledged that females of oviparous taxa show a larger energy investment in gamete production compared to males (Trivers 1972; see also Monaghan and Nager 1997; Williams 2005). In these taxa, the amount of energy devoted to gamete production clearly

represents a strong candidate for shaping sex differences in life-history traits, including annual mortality rates. The lack of comparative studies explicitly investigating the influence of gamete production on sex-biased mortality is surprising, because this trait has been at the very core of early studies of evolutionary trade-offs. For instance, the question as to why females of avian species do not lay more eggs than they actually do was posed decades ago (e.g., Lack 1947; Perrins 1965; Williams 1966; Charnov and Krebs 1974), subsequently triggering vast amounts of empirical research about life-history trade-offs mediated by egg production (e.g., clutch size vs. egg size and quality: Blackburn 1991; Nager et al. 2000; Williams 2001; Martin et al. 2006; Sibly et al. 2012; clutch size vs. future reproduction: Monaghan et al. 1998; Nager et al. 2001; de Heij et al. 2006; clutch size vs. survival: Nur 1988; Martin 1995; Ghalambor and Martin 2001; Descamps et al. 2009).

We performed a phylogenetic comparative analysis of life-histories among bird species that was aimed specifically at investigating the influences of the mass that females devote annually to egg production (relative to female body mass; Sibly et al. 2012; hereafter, annual egg productivity) on interspecific variation in female-biased mortality. We relied on a more comprehensive and accurate measure of female egg productivity than previous studies (e.g., Székely et al. 2014) by also including the annual component, that is, the (average) total number of clutches laid by females in a given year (Sibly et al. 2012). Both in seasonal and in nonseasonal environments, some females lay a single clutch per year, whereas others lay multiple clutches. Hence, laying multiple clutches in a given year can be a crucial factor in determining annual egg productivity (Martin 1995; Sibly et al. 2012).

Based on the available empirical and theoretical arguments outlined above, we predicted that, across species, a greater annual egg productivity by females should be associated with a stronger bias in mortality rates toward females. Birds represent ideal candidates for investigating whether interspecific differences in the energy investment in gamete production shape sex-biased mortality for several reasons: (1) they show strong anisogamy (female gametes are several orders of magnitude larger than those of males), implying that female investment in gamete production is much larger compared to that of males (the total mass of eggs laid by female birds in a given reproductive season may even be double compared to female body mass; e.g., Perrins 1965); (2) they show widespread but varying female-biased mortality; and (3) data on sex-specific mortality and life-history are available for a large number of species.

We also examined possible influences of species-typical mating systems. We expected that the positive correlation between annual egg productivity and the extent of female-biased mortality could be moderated by mating system because polygynous bird species tend to show more equal mortality rates

between the sexes, likely due to larger energy expenditure and/or greater predation risk in mating competition by males relative to females (Liker and Székely 2005; Székely et al. 2014).

We considered other potentially confounding factors that may covary with either interspecific variation in female-biased mortality or annual egg productivity (or both), such as the extent of female contribution to parental care (Székely et al. 2014) or nest position (cavity-nesting vs. non-cavity-nesting species; Martin and Li 1992; Arnold et al. 2012). Finally, we performed a phylogenetic path analysis (von Hardenberg and Gonzalez-Voyer 2013; Gonzalez-Voyer and von Hardenberg 2014) to test for different evolutionary scenarios linking potential predictors to sex-biased mortality, focusing on the hypothesis that annual egg productivity should have a direct influence on interspecific variation in sex-biased mortality.

## Methods

### MORTALITY RATES AND FEMALE-BIASED MORTALITY

Data on sex-specific annual mortality rates were taken from Székely et al. (2014), who derived them from field studies reporting mortality rates for both adult males and adult females in the same population and obtained with the same methodology (capture-recapture, ring recoveries, or local return rates). Similar to Székely et al. (2014), we expressed sex-biased mortality, hereafter referred to as “female-biased mortality,” as  $\text{logit}(\text{adult female mortality rate}) - \text{logit}(\text{adult male mortality rate})$ , with positive values indicating female-biased mortality, whereas negative values indicating male-biased mortality. Although previous studies (e.g., Székely et al. 2014) used log-transformation of mortality rates, we decided to rely on logit-transformation because it led to an improved distribution of residuals in subsequent regression analyses. Importantly, because this variable is calculated considering the annual mortality rate of one sex relative to the annual mortality rate of the other sex, this measure is independent of interspecific variation in life-span.

### ANNUAL EGG PRODUCTIVITY

Annual egg production consists of three components: mean egg mass, mean clutch size, and mean seasonal number of clutches laid (for multi-brooded species). Hence, annual egg production can be conveniently computed as (mean egg mass  $\times$  mean number of eggs per clutch  $\times$  mean number of clutches per year). Data on annual egg production were retrieved from Sibly et al. (2012), who performed a large-scale analysis of life-history trait coevolution in avian species and reported estimates of annual egg production for 980 species. Because interspecific variation in annual egg production largely reflects interspecific differences in body mass

(the correlation between annual egg production and body mass, both  $\log_{10}$ -transformed, is  $r = 0.93$ ), in subsequent analyses we relied on an index of annual egg productivity (in line with Sibly et al. 2012), computed as the ratio of annual egg production to female body mass, that is, (annual egg production) / (female body mass), expressed in  $\text{year}^{-1}$ . Data on female body mass were taken from Lislevand et al. (2007). Although it is well-known that ratios may have undesirable statistical properties, especially related to their intrinsic dependence on the value of the denominator and their undefined variance (e.g., Atchley et al. 1976), the annual egg productivity index proposed by Sibly et al. (2012) is justified both on theoretical and empirical grounds (for details, see Brown and Sibly 2006, Sibly and Brown 2007; Meiri et al. 2012; Sibly et al. 2012). Of particular interest to the present study is that the dependence on the denominator should be alleviated when the correlation between numerator and denominator is high (Smith 1999) ( $r = 0.93$  in the present dataset). The main potential issue of using annual egg productivity for our study is indeed the difficulty in partitioning out the influence of annual egg production per se from the influence of body size on female-biased mortality, which we tackled using three different approaches (see PHYLOGENETIC REGRESSION MODELS section).

Overall, we could match annual egg productivity and female-biased mortality for 178 out of the 265 species for which we had estimates of sex-specific mortality rates (hereafter, test dataset).

### OTHER TRAITS POTENTIALLY AFFECTING FEMALE-BIASED MORTALITY

#### *Mating system bias*

We scored the mating system of each sex as the frequency of polygamy on a 5-point scale (0 = no [or very rare] polygamy [ $<0.1\%$  of individuals]; 1 = rare polygamy [0.1%–1%]; 2 = uncommon polygamy [1%–5%]; 3 = moderate polygamy [5%–20%]; 4 = common polygamy [ $>20\%$ ]) (Liker et al. 2013; Székely et al. 2014). Mating system bias was calculated as the difference between male and female scores (Liker et al. 2013; Székely et al. 2014); hence, positive values indicate a tendency toward polygyny, whereas negative values a tendency toward polyandry. This variable was available for 177 species of the test dataset.

#### *Sexual size dimorphism*

Sexual dimorphism in body size may affect female-biased mortality and confound the influence of interspecific variation in annual egg productivity on female-biased mortality. On the one hand, male-biased size dimorphism (i.e., relatively large male compared to female body size) may result from intense sexual selection via intrasexual competition, which may impose mortality costs on males (Promislow et al. 1992; Owens and Bennett

1994; Owens and Hartley 1998). In addition, a larger body size requires more energy to sustain physiological activities, thus increasing vulnerability to starvation. Hence, male-biased size dimorphism should be associated with a lower female-biased mortality. On the other hand, mortality rates are expected to increase with decreasing body size, consistently with life-history theory (Székely et al. 2014). Thus, sex differences in mortality rates are expected to covary with sex differences in body size: specifically, we expect mortality to be more biased toward females when females are smaller relative to males, that is, when sexual dimorphism is male-biased. Sexual size dimorphism was expressed as  $\log_{10}(\text{male mass}) - \log_{10}(\text{female mass})$  (Smith 1999), based on data from Lislevand et al. (2007). This variable was available for all the 178 species in the test dataset.

### *Female parental care*

Female-biased mortality may be affected by differential patterns of parental care by males and females, both pre- and posthatching (Székely et al. 2014). Sex differences in parental care were retrieved from Liker et al. (2015), which provides a composite score of sex bias in parental care. This score summarizes information on six components of avian parental care: nest building, incubation, nest guarding (guarding and defending the nest during incubation), chick brooding, feeding, and guarding (guarding and defending the brood after hatching) (for details, see Liker et al. 2015). Sex differences in parental care were obtained by scoring female participation relative to male for each of the above six components. The relative participation by females was scored on a 5-point scale (−1: no female care; −0.5: 1%–33% female care; 0: 34%–66% female care; 0.5: 67%–99% female care; 1: 100% female care). Hence, a score of 0 indicates similar effort by both sexes, whereas a score of 1 (or −1) indicates female (or male) uniparental care (note that these values are the opposite compared to Liker et al. 2015, which expressed it as relative male participation in parental care). Female prehatching parental care was calculated as the mean score value for nest building, incubation, and nest guarding, whereas female posthatching care was calculated as the mean score values of chick brooding, chick feeding, and chick guarding before fledging (Liker et al. 2015). Data were available for 178 and 177 species in the test dataset, respectively. Because female pre- and posthatching parental care were strongly positively correlated ( $r = 0.70$ ,  $n = 177$ ), were measured on the same scale, and we had no clue as to which of these two components was the strongest predictor of female-biased mortality (actually, neither of these two variables significantly predicted female-biased mortality in a large-scale comparative study; Székely et al. 2014), we computed the mean score of female pre- and posthatching parental care as a general index of female participation in parental care relative to males (female parental care hereafter).

### *Cavity nesting*

A further potentially relevant factor that may affect female-biased mortality is nest site. Indeed, most female mortality may occur during incubation in some species (Arnold et al. 2012), and females of species nesting in cavities may suffer lower mortality compared to those of species not breeding in cavities (Martin and Li 1992). Hence, we scored cavity nesting for all species of the test dataset (0 = non-cavity nesting; 1 = cavity nesting).

### **PHYLOGENY**

In comparative analyses, phylogenetic relationships among species should be controlled for (Felsenstein 1985; Harvey and Pagel 1991; Freckleton et al. 2002). To this end, we retrieved a complete phylogeny of the set of species included in this study from the BirdTree website ([www.birdtree.org](http://www.birdtree.org)) (Jetz et al. 2012). Following Rubolini et al. (2015), we downloaded 1000 trees from both the “Hackett” and “Ericson” phylogenies (for details, see Jetz et al. 2012), which we used as phylogenetic hypotheses. We based all the subsequent analyses on the “Ericson” phylogenetic reconstruction, but results using the “Hackett” phylogeny were qualitatively identical (details not shown). The “Ericson” tree set was summarized into a 50% majority-rule consensus tree (Rubolini et al. 2015) by means of the SumTrees program, part of DendroPy, a Python library for phylogenetic computing (Sukumaran and Holder 2010). The resulting tree is shown in Figure S1.

### **PHYLOGENETIC REGRESSION MODELS**

To investigate the influence of annual egg productivity on female-biased mortality, we relied on phylogenetically corrected generalized least-square regression models (PGLS) using the `pgls` function of the R library “caper” (version 0.5.2) (Orme et al. 2011), with the Pagel’s  $\lambda$  parameter, a commonly used metric of the phylogenetic signal (Freckleton et al. 2002), estimated using maximum likelihood. We first ran a univariate PGLS model of sex-biased mortality versus annual egg productivity on the test dataset ( $n = 178$  species). Annual egg productivity was  $\log_{10}$ -transformed in all analyses due to the highly skewed distribution (see also Sibly et al. 2012 for details). We then fitted a multiple PGLS model including annual egg productivity, sexual size dimorphism, mating system bias, female parental care, and cavity nesting as predictors. This analysis was based on data from 177 species (out of 178 of the test dataset). Multicollinearity did not affect this multiple PGLS model (Variance Inflation Factor,  $VIF, \leq 1.27$  for all variables;  $VIF$  values calculated with R package “performance” version 0.7.0; Lüdtke et al. 2021).

A potential issue of using the annual egg productivity index proposed by Sibly et al. (2012) as a predictor in PGLS models of female-biased mortality is the difficulty in partitioning out the effects of productivity per se from the effects of body size

(female body mass) on female-biased mortality. Due to the negative scaling of annual egg productivity with female body mass (Sibly et al. 2012), leading to a strong correlation of female body mass with annual egg productivity ( $r = -0.79$ ; see also *Results*), it was unfeasible to include both annual egg productivity and female body mass as independent predictors in the analyses because coefficients may be biased by collinearity (Dormann et al. 2013). To investigate the independent effects of body size and annual egg production/productivity on female-biased mortality, we thus adopted three different approaches: (1) we fitted alternative PGLS models of female-biased mortality including either annual egg productivity or female body mass as predictors, and checked which model was best supported by the data (according to AIC values); (2) we computed the residuals of the linear regression of annual egg production on female body mass and included these residuals as an index of annual egg productivity in PGLS models of female-biased mortality; and (3) we applied a principal component analysis (PCA) to annual egg production and female body mass, and extracted two principal components (PCs); PC1 was expected to be an index of body size, whereas PC2 should reflect residual variation in annual egg production after accounting for body size, that is, a further index of annual egg productivity. The results of these approaches were then compared to assess the consistency of the results.

We checked the robustness of PGLS models with respect to phylogenetic uncertainty, as reflected by the fact that BirdTree tree sets constitute a sample of equally plausible Markov Chain Monte Carlo (MCMC) trees sampled in proportion to their posterior probability (Jetz et al. 2012), with individual trees showing variation either in topology or branch length. For each of the 1000 downloaded trees, we therefore ran a PGLS model and then computed the average parameter estimates and standard errors across all models. Model-averaged parameter estimates are reported with their associated 95% confidence intervals (CIs). All analyses were run using R 4.0.4 (R Core Team 2020).

#### PHYLOGENETIC CONFIRMATORY PATH ANALYSIS AND FORMULATION OF PATH MODELS

Phylogenetic confirmatory path analysis was conducted following von Hardenberg and Gonzalez-Voyer (2013) and Gonzalez-Voyer and von Hardenberg (2014). Different hypotheses, representing different possible causal relationships between traits, were graphically represented by means of directed acyclic graphs, which can be mathematically expressed as a set of structural equations. Path models were built based on available knowledge on the relationships between traits likely involved in predicting female-biased mortality (see below).

The conditional probabilistic independencies implied in the hypothesized path models were tested by means of the d-sep test. The path model is considered supported by the data if the

d-sep test, as assessed by the Fisher's  $C$  statistic, is nonsignificant (Shipley 2000). All path models included the same set of 177 species for which we had complete information about the possible predictors of female-biased mortality. The best-fitting path models were selected by an information-theoretic approach, as recommended by von Hardenberg and Gonzalez-Voyer (2013), ranking models according to the  $C$ -statistic Information Criterion corrected for small samples (CICc). This procedure is formally equivalent to standard AIC-based model selection (Shipley 2013). Standardized path coefficients (calculated on standardized variables) were averaged among equally well-supported path models (i.e., those models having a  $\Delta$ CICc below 2 points from the best-fitting model; von Hardenberg and Gonzalez-Voyer 2013) according to their CICc weight (see Symonds and Moussali 2011). Model-averaged direct path coefficients are reported with their associated 95% CIs.

Based on previous studies and our working hypothesis (i.e., that annual egg productivity directly and positively affects female-biased mortality), we built path models relating female-biased mortality (FB), annual egg productivity (PR), mating system bias (MS), sexual size dimorphism (SD), female parental care (FC), and cavity nesting (CN).

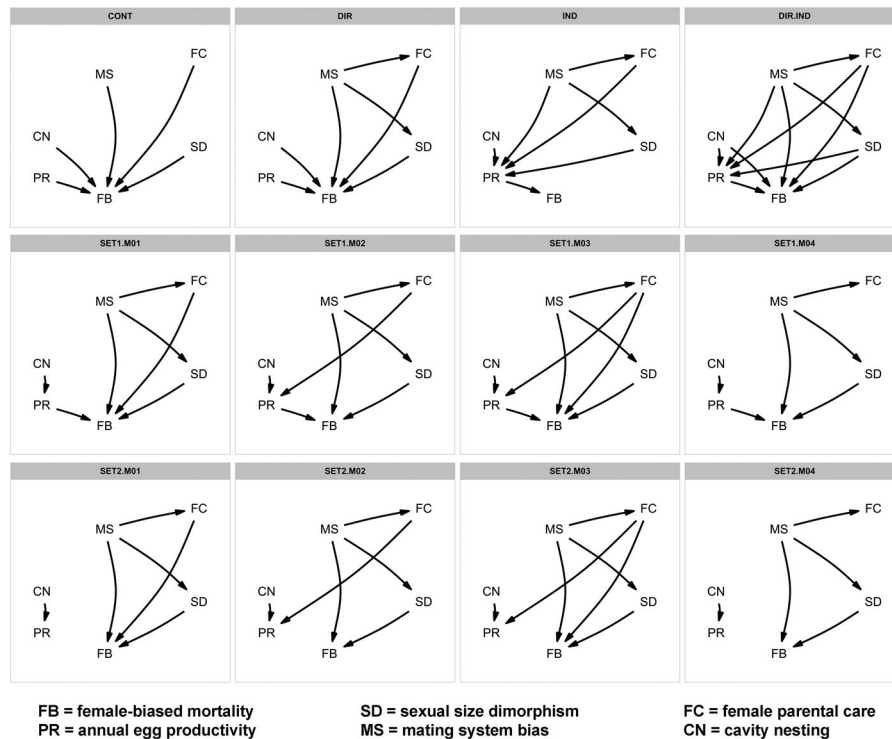
MS was previously shown to significantly predict both FB (Liker and Székely 2005; Székely et al. 2014) and PR (Sibly et al. 2012). Furthermore, MS is well-known to predict both FC (Székely et al. 2013, 2014) and SD (Owens and Hartley 1998; Dunn et al. 2001), and the latter may thus also predict FB. Hence, MS influences on FB can be direct and/or indirect (via FC and/or SD/PR).

We may further expect FC to have a direct influence on FB, because a higher investment in parental care by females may translate into stronger FB (but see Liker and Székely 2005; Székely et al. 2014). We therefore built models envisaging a direct and/or indirect (via PR) influence of FC on FB.

Finally, empirical evidence suggests that cavity nesting birds have lower annual fecundity and higher interannual survival than non-cavity nesters (e.g., Martin and Li 1992; Martin 1995). We thus expected CN to be associated with lower PR. In most path models, any influence of CN on FB was thus expected to be indirect and mediated by PR (see below).

Based on the above theoretical arguments and empirical evidence, we first built a reasonable basic path structure relating MS, SD, FC, and CN (i.e., those variables that were not at the main focus of our study), whereby we hypothesized  $MS \rightarrow FC$  and  $MS \rightarrow SD$  (other paths among these variables were unlikely) (Fig. 1).

Relying on this basic path structure, we then built three sets of models (Fig. 1), envisaging, respectively: SET0—PR and all the above variables were directly and independently affecting FB (DIR); PR was directly affecting FB, and all the above



**Figure 1.** Sets of path models testing the influence of annual egg productivity (PR) on female-biased mortality (FB) including different potential evolutionary paths by which cavity nesting (CN), mating system bias (MS), female parental care (FC), and sexual size dimorphism (SD) may relate to FB and/or PR. Upper row: SET0 models; middle row: SET1 models; lower row: SET2 models (see *Methods* for details). SET2 models are the same models as those in SET1, but without any direct influence of PR on FB.

variables were indirectly affecting FB (via PR) (IND); PR and all the above variables were directly and indirectly (via PR) affecting FB (DIR.IND); in this set, we also included a control model (CONT) envisaging direct and independent influences of all variables on FB (which we expected to be the least supported model); SET1—direct influences of PR, MS, and SD on FB, indirect influences of CN on FB (via PR), and combinations of direct and indirect (via PR) influences of FC on FB ( $n = 4$  models); SET2—same as SET1, but without any direct influence of PR on FB ( $n = 4$  models). According to our working hypothesis, SET2 models (not including a direct influence of PR on FB) were expected to be far less supported than SET1 models.

Path models were fitted using the “phylopath” R package (version 1.1.2) (van der Bijl 2018).

## Results

### TRAITS INFLUENCING FEMALE-BIASED MORTALITY

Females had greater mortality than males in 106 out of 178 species of the test dataset (59.5%). Female-biased mortality varied greatly among species, ranging from  $-1.84$  (i.e., strongly male-biased; *Selasphorus platycercus*) to  $+1.73$  (i.e., strongly

female biased; *Seiurus aurocapilla*) and was on average  $+0.19$  ( $0.47$  SD) across species (i.e., slightly female biased).

Interspecific variation in female-biased mortality was significantly influenced by annual egg productivity, with species with greater productivity showing a larger female bias in adult mortality in a bivariate PGLS model fitted on the test dataset ( $n = 178$  species) (Table 1). The variance explained was rather small (about 7%), implying a considerable amount of unexplained among-species variation (Table 1). The intercept of the above PGLS model, re-fitted using centered annual egg productivity, was significantly larger than 0, implying that, at the mean value of annual egg productivity and accounting for phylogenetic effects, mortality was significantly female-biased in the test dataset (estimate:  $0.19$  [ $0.03$  SE],  $t_{176} = 5.70$ ,  $P < 0.001$ ,  $\lambda = 0$ ).

The multiple PGLS model confirmed the positive and statistically significant influence of annual egg productivity on female-biased mortality (Table 1; Fig. 2). As expected, this latter model confirmed a significant negative association between mating system bias and female-biased mortality, with species in which males are more involved in mating competition relative to females showing more equal mortality rates between the sexes (Table 1; Fig. 2). Other predictors had a much weaker influence on female-biased mortality (Table 1). Model-averaged parameter

**Table 1.** PGLS models of among-species variation in female-biased mortality (positive values denote higher female than male mortality, and negative values the opposite). Model-averaged estimates and their 95% CIs (accounting for phylogenetic uncertainty) are also shown (see *Methods* for details). Coefficients are from centered and standardized ( $SD = 1$ ) variables (both dependent and predictors) for ease of comparisons of effect sizes.

Trait	Estimate (SE)	<i>t</i>	<i>P</i>	Model-averaged estimates [95% CIs]
Univariate PGLS model ( $n = 178$ species; $\lambda = 0$ ; $R^2 = 0.074$ )				
Annual egg productivity	0.27 (0.07)	3.75	<0.001	0.27 [0.13 to 0.41]
Multiple PGLS model ( $n = 177$ species; $\lambda = 0$ ; $R^2 = 0.098$ )				
Annual egg productivity	0.29 (0.08)	3.68	<0.001	0.28 [0.13 to 0.44]
Mating system bias	-0.17 (0.08)	2.13	0.035	-0.17 [-0.33 to -0.01]
Sexual size dimorphism	0.05 (0.08)	0.65	0.52	0.05 [-0.10 to 0.20]
Female parental care	0.08 (0.08)	1.00	0.32	0.08 [-0.08 to 0.24]
Cavity nesting	-0.03 (0.07)	0.36	0.72	-0.03 [-0.17 to 0.12]

**Table 2.** Statistics of the fitted path models (see Fig. 1 for a graphical representation). Fisher's *C* statistic is reported together with its associated degrees of freedom, *P*-value, and the C-statistic Information Criterion (corrected for small samples) (CICc). Models are sorted according to their CICc values (with lowest values representing the best-fitting models). The set of best-fitting path models ( $\Delta$ CICc < 2) is highlighted in boldface.

Model	<i>C</i>	df	<i>P</i>	CICc	$\Delta$ CICc
<b>DIR.IND</b>	<b>12.0</b>	<b>8</b>	<b>0.152</b>	<b>49.8</b>	<b>0.00</b>
<b>SET1.M04</b>	<b>24.5</b>	<b>18</b>	<b>0.141</b>	<b>50.4</b>	<b>0.52</b>
<b>SET1.M01</b>	<b>22.2</b>	<b>16</b>	<b>0.137</b>	<b>50.4</b>	<b>0.58</b>
SET1.M02	24.2	16	0.086	52.4	2.56
SET1.M03	21.9	14	0.081	52.5	2.65
IND	25.4	16	0.063	53.7	3.82
DIR	28.9	16	0.025	57.1	7.27
SET2.M01	37.8	18	0.004	63.7	13.90
SET2.M02	39.6	18	0.002	65.5	15.70
SET2.M04	42.2	20	0.003	65.8	15.93
SET2.M03	37.6	16	0.002	65.8	15.95
CONT	70.6	20	<0.001	94.2	44.31

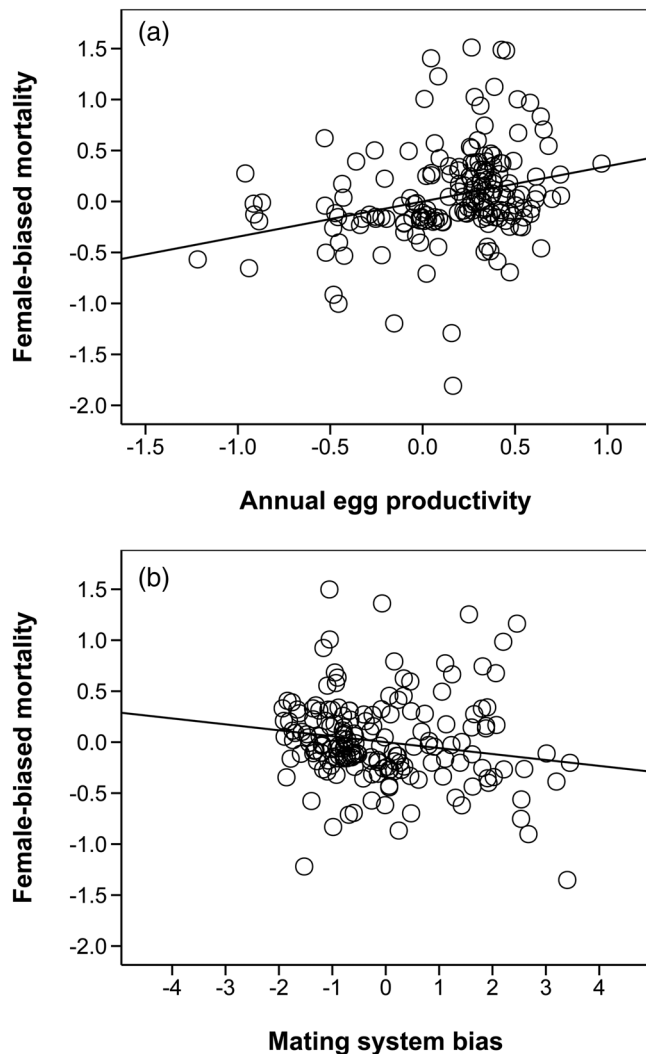
estimates suggested that accounting for phylogenetic uncertainty had a negligible effect on the conclusions (Table 1).

Due to the negative scaling of annual egg productivity with female body mass (see also Sibly et al. 2012) (estimate: -0.41 [0.03 SE],  $t_{176} = 13.64$ ,  $P < 0.001$ ,  $\lambda = 0.88$ ; PGLS with  $\log_{10}$ -transformed female body mass as a predictor), the association between female-biased mortality and annual egg productivity could be confounded by interspecific differences in body size. As reported in Appendix A, all the three alternative approaches we adopted to partition out the relative influences of body size and annual egg productivity on female-biased mortality yielded qualitatively similar conclusions, confirming a positive and statistically significant association between female-biased mortality and annual egg productivity, while at the same time pointing out a much weaker and nonsignificant influence of female body mass

on female-biased mortality. Hence, interspecific variation in body size appeared to have a limited influence on the association between annual egg productivity and female-biased mortality, in line with previous findings (Székely et al. 2014).

### COMPARING PATH MODELS

Conditional independencies were fulfilled in several models, as gauged by the nonsignificant d-sep test (Table 2). However, some causal models were better supported by the data than others. Specifically, the three top-ranking models ( $\Delta$ CICc < 2) all shared a direct influence of PR on FB (Fig. 1; Table 2). The model-averaged standardized path coefficients indeed showed that the strongest direct predictor of FB was PR, followed by MS (Fig. 3). Other predictors had much weaker and nonsignificant direct



**Figure 2.** Partial regression plots of female-biased mortality in avian species ( $\text{logit}(\text{adult female mortality rate}) - \text{logit}(\text{adult male mortality rate})$ , residuals; positive values indicate higher mortality of females relative to males) in relation to (a) annual egg productivity ( $\text{year}^{-1}$ ,  $\log_{10}$ -transformed values, residuals), with species showing relatively larger annual egg productivity also suffering relatively larger female-biased mortality, and (b) mating system bias (see *Methods*; residuals), with species in which males are more involved in mating competition relative to females showing smaller female-biased mortality. Lines show fitted linear regressions from the multiple PGLS model shown in Table 1 ( $n = 177$  species with complete information on all predictors) (residuals calculated from the multiple PGLS model based on unstandardized variables).

influences on FB (Fig. 3). Direct path coefficients from best-fitting path models also supported a significant influence of MS on both FC and SD. In addition, CN was associated with higher (rather than lower, as expected) PR, and SD was found to be negatively associated with PR, with more sexual size dimorphic

species showing a lower annual egg productivity (see Fig. 3 for details).

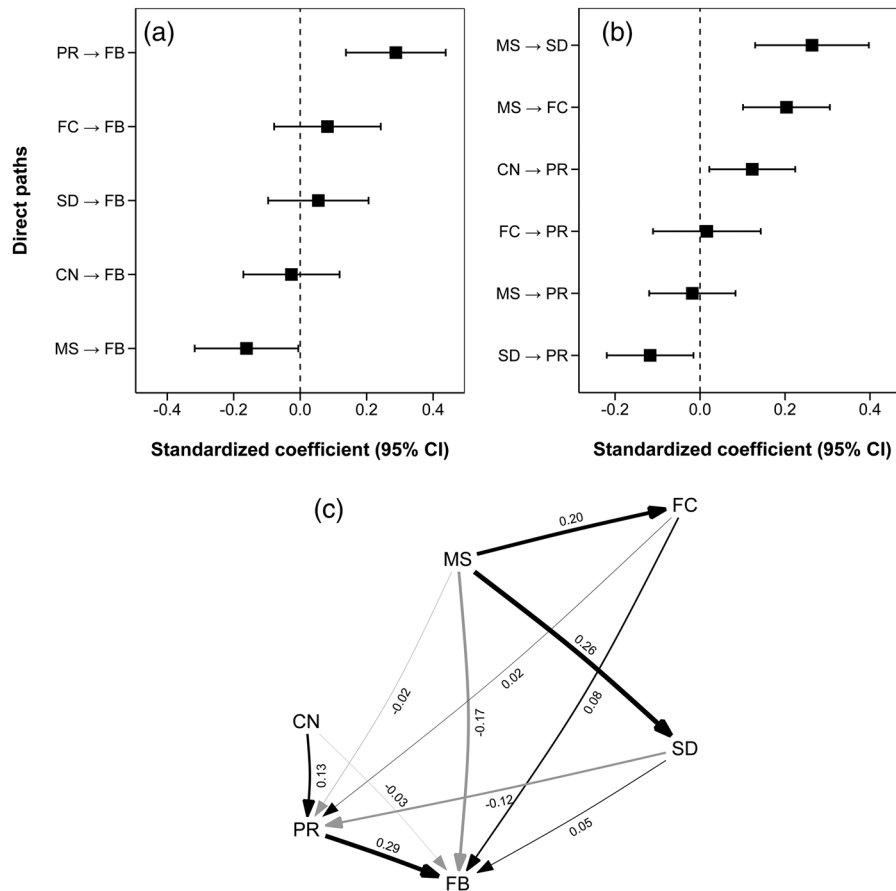
Based on our working hypothesis, we had anticipated that SET2 models (not implying any influence of PR on FB; Fig. 1) should be less supported by the data than the other models. This was indeed the case, as the best-fitting model within this subset had a  $\Delta\text{CICc} > 10$  from the best-supported one (Table 2).

## Discussion

In our comparative study of avian species, annual egg productivity emerged as the best supported factor influencing female-biased mortality. The effect of this variable persisted when accounting for possible co-influencing factors, which were previously suggested to affect the extent of female-biased mortality, such as mating system and relative female contribution to parental care (Owens and Bennett 1994; Liker and Székely 2005; Sibly et al. 2012; Székely et al. 2014). This association was supported by phylogenetic path analyses, as all best-fitting path models shared a direct influence of annual egg productivity on female-biased mortality. Furthermore, body size, which could confound the association between annual egg productivity and female-biased mortality, emerged as having a minor influence only on female-biased mortality (see also Székely et al. 2014). The variance in female-biased mortality explained by annual egg productivity was low (about 7%), implying that a large fraction of interspecific differences in female-biased mortality remains unexplained. This value is, however, similar to the average amount of variance explained by the main factor of interest in ecological and evolutionary studies (2.51%–5.42%; Møller and Jennions 2002).

Consistent with previous studies of vertebrates (Promislow et al. 1992, 1994; Promislow 1992; Liker and Székely 2005; Székely et al. 2014), our results suggest that sexual selection pressures, as estimated by mating system bias (but not sexual size dimorphism), are a further factor influencing differential mortality between males and females. As expected, species in which male competition for access to mates is more intense (i.e., highly polygynous ones) exhibit lower female-biased mortality than those in which male-male competition at the premating stage is weak or females compete for mates (i.e., highly polyandrous species). Mating system also had a relatively strong direct influence on sexual dimorphism, in accordance with the hypothesis that male competition for mates has an influence on sex differences in body size, whereby strong male-male competition for access to females should select for relatively large male versus female body size (Dunn et al. 2001). Our analyses further suggest that relative female pre- and posthatching parental investment, including nest building, incubation, and offspring care, plays a negligible role in affecting female-biased mortality patterns, in





**Figure 3.** Model-averaged standardized path coefficients from the best-fitting path models for (a) direct causal paths affecting female-biased mortality (FB) and (b) direct causal paths affecting traits other than FB. Path coefficients are sorted top to bottom according to their sign (from positive to negative) and strength. The directed acyclic graph showing the sign and strength of all model-averaged path coefficients is shown in panel (c) (positive coefficients: black; negative coefficients: gray; width of vertices is proportional to effect size). CN = cavity nesting; FC = female parental care; MS = mating system bias; PR = annual egg productivity; SD = sexual size dimorphism

line with previous evidence (Liker and Székely 2005; Székely et al 2014).

On the whole, these findings are consistent with the “traditional” view that greater investment in gamete production by females contributes to explain interspecific variation in female-biased avian mortality, in accordance with previous broad-scale phylogenetic studies of avian reproduction (Martin 1995; Székely et al. 2014).

Proximately, several mechanisms can explain the association between egg productivity and female-biased mortality (reviewed by Monaghan and Nager 1997; Williams 2005). First, egg production and laying are highly energy-demanding activities that impose a considerable increase in metabolic energy expenditure (Carey 1996; Stevenson and Bryant 2000; Nilsson and Råberg 2001; Vézina and Williams 2002). Such a high nutrient demand during egg production can be sustained by the depletion of protein or lipid from various tissues (Houston et al. 1995; Williams and Martyniuk 2000; Gauthier et al. 2003), including body

reserves and pectoral flight muscles, which in turn can compromise individual condition and make females more susceptible to parasite infection (Gustafsson et al. 1994; Oppliger et al. 1997), which may impair survival. Moreover, egg-laying increases body mass and impairs flight performance, thus exposing females to high predation risk (Lee et al. 1996; Kullberg et al. 2002). Moreover, egg-laying females may be highly susceptible to parasite infection (Gustafsson et al. 1994; Oppliger et al. 1997), which can increase mortality. Finally, egg production is intimately associated with other reproductive costs, such as incubation and offspring rearing, because females laying more and larger clutches necessitate also to invest more resources in offspring rearing. The few experimental studies separately manipulating egg laying and offspring rearing (Nelson 1964; Moreno et al. 1991; Heaney and Monaghan 1995; Visser and Lessells 2001) showed a higher cost of the former than the latter. However, the cost of both laying additional eggs and rearing additional offspring is higher than that imposed by either laying additional eggs or rearing additional

offspring (Milonoff and Paananen 1993; Heaney and Monaghan 1995). Despite such evidence in single populations/species, our comparative analyses show that the extent of parental care seems to play a minor role in influencing sex-biased mortality compared to annual egg productivity (see also Székely et al. 2014).

In conclusion, our study provided evidence that annual egg productivity has an influence, although relatively weak, on differential mortality between the sexes in birds, and that mating system might have contributed an additional influence, with species in which males are more involved in mating competition showing a tendency for a more equal mortality rate between the sexes. Although path analysis suggested the existence of plausible evolutionary causal paths linking annual egg productivity to female mortality rates, we note that the comparative approach is intimately correlational. Hence, it cannot be ruled out that traits underlying sex differences in mortality patterns might concomitantly influence reproductive effort, with reproductive and mortality patterns coevolving due to evolutionary constraints. Nevertheless, our results suggest that both annual energy investment in gamete production by females and mating system might contribute to the evolution and maintenance of fundamental life-history trade-offs between reproduction and survival among avian taxa.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### AUTHOR CONTRIBUTIONS

DR, AL, and APM conceived the study. DR and RA performed the analyses. AR and DR wrote the manuscript. AL, AR, DR, and GB collected part of the data. APM, AL, and RA interpreted the results and contributed to writing the final draft.

#### DATA ARCHIVING

Data are available at Dryad: <https://doi.org/10.5061/dryad.bnzs7h4dw>.

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## Appendix A

### Partitioning out the influence of body mass and annual egg productivity on female-biased mortality

#### Alternative PGLS model of female-biased mortality including female body mass instead of annual egg productivity as a predictor

We fitted to the test dataset (178 species) an alternative PGLS model of female-biased mortality including female body mass ( $\log_{10}$ -transformed) instead of annual egg productivity as a predictor. This PGLS model showed a poorer fit compared to the model with annual egg productivity as a predictor ( $\Delta\text{AIC} = 5.4$ ), and the influence of female body mass on female-biased mortality was nonsignificant (PGLS model fitted on centered and standardized [ $\text{SD} = 1$ ] variables, estimate  $-0.17$  (0.11 SE),  $t_{176} = 1.57$ ,  $P = 0.12$ ,  $\lambda = 0.16$ ). Hence, body mass was a weaker predictor of female-biased mortality than annual egg productivity, suggesting that the association between annual egg productivity and female-biased mortality was not confounded by interspecific differences in body size. All models were robust to phylogenetic uncertainty (details not shown for brevity).

#### Computing annual egg productivity as residuals of annual egg production on female mass

Using residuals as predictors in regression analyses is generally discouraged (e.g., Garcia-Berthou 2001; Freckleton 2002), and even more so in the case of comparative analyses, because residuals do not represent biological features of a given species, but rather depend on the characteristics of a dataset. Hence, residuals are by definition dataset dependent (i.e., a given species may be characterized by different values of residuals conditional on the other species being included in the regression analyses) (Smith 1999).

In spite of these issues, residuals may allow to remove, at least partly, the effect of female body mass from annual egg production. We thus obtained residuals from a linear regression of annual egg production ( $\log_{10}$ -transformed) on female body mass ( $\log_{10}$ -transformed) fitted to the test dataset. The correlation between these two variables was very high ( $r = 0.93$ ). Residuals ( $\text{PR}_{\text{res}}$ ) were positively correlated with annual egg productivity ( $r = 0.61$ ) and were included as an alternative indicator of annual egg productivity in simple and multiple PGLS models of female-biased mortality (the multiple model including mating system

bias, sexual size dimorphism, female parental care, and cavity nesting as other predictors).

$PR_{res}$  significantly and positively predicted female-biased mortality in the simple PGLS model fitted to the test dataset (PGLS model fitted on centered and standardized [ $SD = 1$ ] variables, estimate 0.21 [0.09 SE],  $t_{176} = 2.44$ ,  $P = 0.016$ ,  $\lambda = 0.12$ ). Similarly, the multiple PGLS model indicated a statistically significant and positive effect of  $PR_{res}$  on female-biased mortality (estimate 0.20 [0.10 SE],  $t_{171} = 2.12$ ,  $P = 0.035$ ), whereas the effects of the other predictors were nonsignificant (details not shown), with the exception of mating system bias (estimate  $-0.22$  [0.09 SE],  $t_{171} = 2.49$ ,  $P = 0.014$ ). All models were robust to phylogenetic uncertainty (details not shown for brevity).

### Using PCA to obtain a body-size-independent index of annual egg productivity

A further potential way to obtain a size-independent index of annual egg productivity is through PCA (Rohlf and Bookstein 1987), although even the application of PCA for size correction has well-known issues. Indeed, PCA may generate statistical artifacts, even when all traits are tightly correlated with overall size, especially when the magnitude of variance is heterogeneous among the traits, and when the traits under study are few (Berner 2011).

However, to further check the robustness of our findings to alternative measures of annual egg productivity, we applied a PCA to female body mass ( $\log_{10}$ -transformed) and annual egg production ( $\log_{10}$ -transformed) using the “FactoMineR” R package. The first PC (PC1) accounted for most of the variance (96.3%) and reflected an axis of variation of body size across species, as it was positively correlated with both female body mass and annual egg production ( $r = 0.98$  in both cases). PC2 accounted for the residual variance in the two variables (3.7%) and was positively correlated with annual egg production ( $r = 0.19$ ) and negatively with female body mass ( $r = -0.19$ ). Hence, PC2 could be considered as a size-independent measure of annual egg production. Indeed, PC2 was strongly positively correlated with annual egg productivity ( $r = 0.76$ ) and with  $PR_{res}$  ( $r = 0.98$ ). PC2 was thus included as an indicator of annual egg productivity

in simple and multiple PGLS models of female-biased mortality (the multiple model including mating system bias, sexual size dimorphism, female parental care, and cavity nesting as other predictors).

PC2 significantly and positively predicted female-biased mortality in the simple PGLS model fitted to the test dataset (PGLS model fitted on centered and standardized [ $SD = 1$ ] variables, estimate 0.23 [0.08 SE],  $t_{176} = 2.72$ ,  $P = 0.007$ ,  $\lambda = 0.09$ ). An alternative simple PGLS model including PC1 (instead of PC2) showed that PC1 did not significantly influence female-biased mortality (estimate  $-0.12$  [0.12 SE],  $t_{176} = 0.98$ ,  $P = 0.33$ ,  $\lambda = 0.21$ ) and resulted in a significantly poorer model fit ( $\Delta AIC = 5.2$ ).

As in previous analyses, the multiple PGLS model highlighted a statistically significant and positive effect of PC2 on female-biased mortality (estimate 0.21 [0.09 SE],  $t_{171} = 2.32$ ,  $P = 0.021$ ), whereas the effects of the other predictors were nonsignificant (details not shown), with the exception of mating system bias (estimate  $-0.22$  [0.09 SE],  $t_{171} = 2.48$ ,  $P = 0.014$ ). All models were robust to phylogenetic uncertainty (details not shown for brevity).

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### Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Phylogenetic hypothesis used for comparative analyses (test dataset,  $n = 178$  species). The phylogenetic tree is a 50% majority-rule consensus tree obtained from 1000 random trees downloaded from the BirdTree.org website ([www.birdtree.org](http://www.birdtree.org)) (“Ericson” phylogeny) (see Jetz et al. 2012).