EG = extra-group  
WP = within-pair

**Introduction**

Background

* Deciphering how parents influence the fitness of their offspring is central to understanding evolution by natural selection. Parents can influence their offspring through both germ-line and environment.
* In iteroparous animals, effects of both parental germ-line, and environment can change with age. Parental age may act on offspring fitness through several different mechanisms:
  + Epigenetic changes resulting in germline deterioration
  + Physiological senescence resulting in poorer parental care
  + Improvement of parental care abilities through experience, or accumulation of resources
* These mechanisms are not mutually exclusive and are likely to have contrasting influences on offspring fitness. However, the relative importance of these different mechanisms of parental age effects are poorly understood as most previous research has either isolated only germ-line effects, or quantified both germ-line and environmental effects combined (but see Schroeder et al. 2015). To date, the age-related effects of parental germline, parental care, and parental environment have never been isolated and quantified within a single population observationally, without the manipulation of cross-fostering.
  + This is due to logistical constraints. Individuals of known-age parents need to be tracked throughout life. The parents need to be tracked until their death as well in order to differentiate associations between age and quality (i.e. selective (dis)appearance) from true effects of ageing.
* In cooperative breeders, the conditions of the social environment can also be associated with parental age if older parents are more likely to have more helpers. Recently, senescence of parental care with age have been shown to be buffered by increased helper presence in cooperative breeders (Berger et al. 2018; Hammers et al. 2019). Investigating parental age effects in cooperative breeders can provide insight into the fitness benefits of the social structure and resulting effects on population demography and life-history.

Superb fairy-wrens

* Non-obligate cooperative breeders, helpers help with young provisioning and territory defense
* Year-round territories occupied by breeding female, dominant male, and between zero and five helper males.
* As a dominant male gets older, his territory is likely to accumulate more helpers as males queue for dominance. As such, parental age is associated with a change in social environment that is quantifiable (as a count of the number of helpers) that may have a positive effect on offspring fitness. We can measure the effects of parental age (intrinsic improvement in parenting with age) and helper number (extrinsic improvement in environment associated with age).
* High rate of infidelity, with females commonly mating with males outside her territory, and so paternal germ-line and paternal environmental effects can be measured in isolation (for EG chicks), and together (for WP chicks)

Aims

1. Disentangle germ-line and parental care age-related effects by quantifying:

* Age-related changes in paternal care (age of social fathers of EG chicks)
* Age-related changes in germ-line (age of genetic father of EG chicks)
* Combined age-related changes of parental care and germ-line (mother age (of all chicks), and father age of WP chicks)

1. Quantify age-related effects that are not due to age *per se,* but instead are a result of a correlation between lifespan and average offspring fitness by controlling for all parents lifespans.
2. Investigate whether age-related effects are a result of intrinsic improvements in parental care, or changes in the social environment with age, by re-running models including the number of helpers present on the natal territory.

**Methods**

Study population

* Long term study in and around ANBG intensively monitored since 1988
* Life-history of each individual is tracked, including hatching date, both chick and adult survival, immigration, emigration, territory composition and borders
* Chicks color-banded and a blood sample taken to assign parentage during the nestling stage approximately 7 days after hatching

Data selection

* used data from the 1988 to the 2018 breeding season (30 cohorts)
* Included only birds hatched within the study area with a known hatch date, and whose parents were of known age at their hatching and also known age of death. For chicks sired extra-pair this includes the information for both fathers (genetic and social).
* We excluded chicks who’s social father was the son of the dominant female (141 birds, 3% of initial sample)
* We excluded chicks who’s genetic father was a helper on their natal territory (165 birds/3% of initial sample)
* Early life trait sample size: 4361, with 53% male and 47% female
* Recruitment into the breeding population could only be measured in males since death cannot be distinguished from emigration from the study area for females during their first year of life. Males are highly philopatric and easily tracked. After excluding males for which emigration or death was uncertain due to living close to the study area border, 2278 males were used in this analysis.

Statistical analysis

* Each of the 3 fitness-related traits in chicks were modelled separately using mixed effects models with an appropriate error distribution. Random effects for each parental ID (mother, social father, and genetic father) were included to control for the non-independence of repeated measures from the same parents across chicks. For male recruitment, social father was nested within mother in order to avoid over-parameterization of this model given it’s relatively smaller sample size. Cohort was included as a random effect to control for unmeasured heterogeneity in environmental conditions between years.
* *Weight:* The majority of weights were taken 7 days after hatching, but sometimes day(s) earlier or later and so age in days at weighing was controlled for in this model. At the time of weighing, chicks had not yet fledged from their natal nest and relied completely on others for food. Pre-1992 was also included to control for a change in the time of day chicks were weighed from this year forward. Weight followed a normal distribution and so a linear model was used.
* *Survival:* Early-life survival was measured from the late nestling stage (approximately 7 days old, when chicks are banded and blood sampled), until 4 weeks after fledging when they reach independence from their parents and may then disperse to another territory. Residual values from the chick weight regressed against age at weighing and the pre-1992 variable (see *weight* above) were included as a variable in the model. Controlling for the weight of each chick as a nestling allows us to distinguish direct effects of parental ages on survival from secondary effects resultant from any parental age effects on weight. Survival was modeled using a Bernoulli distribution (fitted with a logit-link function).
* To investigate sex differences in the parental-age effects on these traits, a second model with an in interaction between each parental age and chick sex was used.
* *Recruitment:* Survival from nestling to recruitment (measured as being alive at the start of the next breeding season) was measured in only males (see *data selection* above). Recruitment probability was modeled using a Bernoulli distribution (fitted with a logit-link function).
* For each trait, the predictor variables of interest were maternal age, and paternal age. For EG chicks, this includes two paternal ages (social and genetic). In order to estimate both EG and WP paternal age effects within the same model, interactions between the binary variable of EG (0 or 1) and social and genetic father age, as well as an interaction between WP (0 or 1) and genetic father age were included in each model.
* Incubation date controlled for in each model, as it can have considerable effects on both early and late life fitness.
* In order to address our second aim (Quantify age-related effects that are not due to age *per se,* but instead are a result of a correlation between lifespan and average offspring fitness), we included the lifespans of the mother and father(s) in all models.
* In order to address our third aim (Investigate whether age-related effects are a result of intrinsic improvements in parental care, or an improvement in parental environment with age), we ran each model a second time including a categorical variable for the number of helpers (zero, one, two or more) on the natal territory.
* All analysis done in R version 3.5.0, LMMs/GLMMs fitted using lme4

**Results**

Weight

* The weight of chicks sired within-pair increases with increasing father age, while for chicks sired extra-group, there is no effect of either the age of the social or the genetic father (table 1).
* Chick weight improves with the number of helpers on the territory, and this improvement largely drives the within-pair father age effect (table 2).
* There was a significant interaction between maternal lifespan and sex (supplementary), where males had a non-significant increase in weight, and females had a non-significant decrease in weight with increasing maternal lifespan. None of the other paternal age or lifespan effects had a significant interaction with sex (supplementary).

**Table 1.** Effects of parental ages and lifespans on chick weight as a nestling (approximately 7 days post-hatching) from a linear mixed-model.

|  |  |  |  |
| --- | --- | --- | --- |
| Predictors | Estimates | CI | p |
| Intercept | 0.700 | 0.443 – 0.957 | **<0.001** |
| Mother Age | -0.003 | -0.025 – 0.020 | 0.810 |
| Mother Lifespan | 0.003 | -0.019 – 0.025 | 0.795 |
| Extra-pair Genetic Father Age | -0.009 | -0.032 – 0.014 | 0.449 |
| Extra-pair Genetic Father Lifespan | -0.012 | -0.034 – 0.010 | 0.282 |
| Cuckolded Social Father Age | 0.008 | -0.015 – 0.032 | 0.490 |
| Cuckolded Social Father Lifespan | -0.004 | -0.026 – 0.018 | 0.714 |
| Within-pair Father Age | 0.029 | 0.002 – 0.056 | **0.036** |
| Within-pair Father Lifespan | 0.002 | -0.024 – 0.027 | 0.894 |
| Extra-Group [yes] | 0.235 | 0.065 – 0.404 | **0.007** |
| Incubation Date | 0.322 | 0.189 – 0.455 | **<0.001** |
| Age at Weighing | 0.850 | 0.822 – 0.879 | **<0.001** |
| Pre-1992 | 0.413 | 0.085 – 0.742 | **0.015** |

Note: Sample size is 4361 individual chicks. The model includes random effects of mother ID (n = 534, τ = 0.08), social father ID (n = 484, τ = 0.05), genetic father ID (n = 565, τ = 0.04), and cohort (n= 30, τ = 0.02). The residual variance is 0.45. A value of 1 for the binomial variable ‘extra-group’ indicates the chick was sired by a male outside the natal territory, while a value of 0 indicates the sire was the within-pair social mate. All extra-pair father terms are interaction terms between the effect and the extra-pair binomial variable. Both within-pair father terms are also interaction terms, with each effect interacting with a second dummy variable for within-pair sire (0/1).

**Table 2.** Effects of parental ages, parental lifespans, and number of helpers residing on the natal territory on chick weight as a nestling (approximately 7 days post-hatching) from a linear mixed-model.

|  |  |  |  |
| --- | --- | --- | --- |
| Predictors | Estimates | CI | p |
| Intercept | 0.708 | 0.451 – 0.965 | **<0.001** |
| Mother Age | -0.005 | -0.027 – 0.017 | 0.664 |
| Mother Lifespan | 0.002 | -0.021 – 0.024 | 0.871 |
| Extra-pair Genetic Father Age | -0.009 | -0.032 – 0.014 | 0.433 |
| Extra-pair Genetic Father Lifespan | -0.012 | -0.034 – 0.010 | 0.294 |
| Cuckolded Social Father Age | 0.002 | -0.022 – 0.026 | 0.854 |
| Cuckolded Social Father Lifespan | -0.005 | -0.027 – 0.017 | 0.655 |
| Within-pair Father Age | 0.022 | -0.005 – 0.049 | 0.114 |
| Within-pair Father Lifespan | 0.001 | -0.024 – 0.026 | 0.937 |
| Extra-Group [yes] | 0.227 | 0.058 – 0.396 | **0.009** |
| Incubation Date | 0.326 | 0.193 – 0.459 | **<0.001** |
| Helpers [one] | 0.098 | 0.033 – 0.163 | **0.003** |
| Helpers [two+] | 0.096 | 0.012 – 0.181 | **0.026** |
| Age at Weighing | 0.850 | 0.821 – 0.878 | **<0.001** |
| Pre-1992 | 0.406 | 0.075 – 0.736 | **0.018** |

Note: Sample size is 4361 individual chicks. The model includes random effects of mother ID (n = 534, τ = 0.08), social father ID (n = 484, τ = 0.05), genetic father ID (n = 565, τ = 0.04), and cohort (n = 30, τ = 0.02). The residual variance is 0.45. A value of 1 for the binomial variable ‘extra-group’ indicates the chick was sired by a male outside the natal territory, while a value of 0 indicates the sire was the within-pair social mate. All extra-pair father terms are interaction terms between the effect and the extra-pair binomial variable. Both within-pair father terms are also interaction terms, with each effect interacting with a second dummy variable for within-pair sire (0/1).

Survival

* Similarly to weight, increasing within-pair father age increases the probability of chick survival while extra-pair genetic and social father ages have no effects (table 3).
* In contrast to weight, survival probability is not significantly associated with helper number, and the positive effects of within-pair father age are not diminished when helper number is controlled for (table 4)
* Chick survival improves with their mother’s lifespan, but the current age of their mother shows a negative association, approaching significance (table 3, table 4).
* None of the parental age or lifespan effects had sex-specific effects (supplementary).

**Table 3.** Effects of parental ages and lifespans on chick survival to potential independence (four weeks post-fledging) from a generalized linear mixed-model (Bernoulli distribution, logit-link function).

|  |  |  |  |
| --- | --- | --- | --- |
| Predictors | Log-Odds | CI | p |
| Intercept | -2.125 | -2.684 – -1.566 | **<0.001** |
| Mother Age | -0.055 | -0.132 – 0.023 | 0.166 |
| Mother Lifespan | 0.101 | 0.023 – 0.178 | **0.011** |
| Extra-pair Genetic Father Age | -0.054 | -0.133 – 0.025 | 0.181 |
| Extra-pair Genetic Father Lifespan | 0.026 | -0.046 – 0.098 | 0.474 |
| Cuckolded Social Father Age | -0.012 | -0.093 – 0.069 | 0.778 |
| Cuckolded Social Father Lifespan | -0.037 | -0.115 – 0.042 | 0.363 |
| Within-Pair Father Age | 0.115 | 0.023 – 0.208 | **0.015** |
| Within-pair Father Lifespan | -0.045 | -0.131 – 0.042 | 0.309 |
| Extra-Group [yes] | 0.299 | -0.280 – 0.879 | 0.311 |
| Incubation Date | 3.353 | 2.863 – 3.843 | **<0.001** |
| Weight (Residual) | 0.213 | 0.109 – 0.317 | **<0.00**1 |

Note: Sample size is 4361 individual chicks. The model includes random effects of mother ID (n = 534, τ = 0.85), social father ID (n = 484, τ = 0.83), genetic father ID (n = 565, τ = 0.15), and cohort (n = 30, τ = 0.10). The residual variance is 3.29. A value of 1 for the binomial variable ‘extra-group’ indicates the chick was sired by a male outside the natal territory, while a value of 0 indicates the sire was the within-pair social mate. All extra-pair father terms are interaction terms between the effect and the extra-pair binomial variable. Both within-pair father terms are also interaction terms, with each effect interacting with a second dummy variable for within-pair sire (0/1).

**Table 4.** Effects of parental ages, parental lifespans, and number of helpers residing on the natal territory on chick survival to potential independence (four weeks post-fledging) from a generalized linear mixed-model (Bernoulli distribution, logit-link function).

|  |  |  |  |
| --- | --- | --- | --- |
| Predictors | Log-Odds | CI | p |
| Intercept | -2.129 | -2.688 – -1.570 | **<0.001** |
| Mother Age | -0.056 | -0.134 – 0.021 | 0.155 |
| Mother Lifespan | 0.100 | 0.023 – 0.178 | **0.012** |
| Extra-pair Genetic Father Age | -0.056 | -0.135 – 0.023 | 0.165 |
| Extra-pair Genetic Father Lifespan | 0.027 | -0.045 – 0.099 | 0.460 |
| Cuckolded Social Father Age | -0.018 | -0.100 – 0.064 | 0.670 |
| Cuckolded Social Father Lifespan | -0.034 | -0.113 – 0.045 | 0.399 |
| Within-pair Father Age | 0.110 | 0.015 – 0.204 | **0.023** |
| Within-pair Father Lifespan | -0.045 | -0.132 – 0.041 | 0.303 |
| Extra-Group [yes] | 0.285 | -0.294 – 0.865 | 0.334 |
| Incubation Date | 3.347 | 2.857 – 3.837 | **<0.001** |
| Helpers [one] | 0.178 | -0.047 – 0.402 | 0.121 |
| Helpers [two+] | -0.071 | -0.366 – 0.224 | 0.639 |
| Weight (Residual) | 0.211 | 0.107 – 0.315 | **<0.001** |

Note: Sample size is 4361 individual chicks. The model includes random effects of mother ID (n = 534, τ = 0.86), social father ID (n = 484, τ = 0.81), genetic father ID (n = 565, τ = 0.15), and cohort (n = 30, τ = 0.10). The residual variance is 3.29. A value of 1 for the binomial variable ‘extra-group’ indicates the chick was sired by a male outside the natal territory, while a value of 0 indicates the sire was the within-pair social mate. All extra-pair father terms are interaction terms between the effect and the extra-pair binomial variable. Both within-pair father terms are also interaction terms, with each effect interacting with a second dummy variable for within-pair sire (0/1).

Male Recruitment

* The positive effect of maternal lifespan on survival during the fledgling stage carries over into survival to recruitment, and this effect is robust to adding helpers
* There is also a negative effect of mother age (so having a young, long-lived mother is best)
* the positive effect of within-pair father age does not remain past independence as it is marginally non-significant here
* having at least one helper at your natal territory positively influences recruitment probability

**Table x.** Effects of parental ages and parental lifespans on male recruitment probability (survival to the breeding season after their hatching) from a generalized linear mixed-model (Bernoulli distribution, logit-link function).

|  |  |  |  |
| --- | --- | --- | --- |
| Predictors | Log-Odds | CI | p |
| Intercept | -2.863 | -3.479 – -2.247 | **<0.001** |
| Mother Age | -0.100 | -0.195 – -0.005 | **0.039** |
| Mother Lifespan | 0.106 | 0.033 – 0.178 | **0.004** |
| Extra-pair Genetic Father Age | -0.028 | -0.128 – 0.072 | 0.583 |
| Extra-pair Genetic Father Lifespan | -0.021 | -0.108 – 0.066 | 0.630 |
| Cuckolded Social Father Age | -0.021 | -0.121 – 0.079 | 0.679 |
| Cuckolded Social Father Lifespan | -0.025 | -0.105 – 0.056 | 0.549 |
| Within-pair Father Age | 0.074 | -0.040 – 0.188 | 0.203 |
| Within-pair Father Lifespan | 0.042 | -0.055 – 0.139 | 0.394 |
| Extra-Group [yes] | 1.114 | 0.371 – 1.857 | **0.003** |
| Incubation Date | 1.930 | 1.294 – 2.567 | **<0.001** |

Note: Sample size is 2278 males. The model includes random effects of mother ID (n = 495, τ = 0.05), social father ID, nested within mother ID (n = 691, τ = 0.42), genetic father ID (n = 499, τ = 0.03), and cohort (n = 30, τ = 0.02). The residual variance is 3.29. A value of 1 for the binomial variable ‘extra-group’ indicates the chick was sired by a male outside the natal territory, while a value of 0 indicates the sire was the within-pair social mate. All extra-pair father terms are interaction terms between the effect and the extra-pair binomial variable. Both within-pair father terms are also interaction terms, with each effect interacting with a second dummy variable for within-pair sire (0/1).

**Table x.** Effects of parental ages, parental lifespans, and number of helpers residing on the natal territory on male recruitment probability (survival to the breeding season after their hatching) from a generalized linear mixed-model (Bernoulli distribution, logit-link function).

|  |  |  |  |
| --- | --- | --- | --- |
| Predictors | Log-Odds | CI | p |
| Intercept | -2.850 | -3.463 – -2.236 | **<0.001** |
| Mother Age | -0.103 | -0.198 – -0.008 | **0.033** |
| Mother Lifespan | 0.104 | 0.033 – 0.176 | **0.004** |
| Extra-pair Genetic Father Age | -0.030 | -0.129 – 0.070 | 0.560 |
| Extra-pair Genetic Father Lifespan | -0.020 | -0.107 – 0.066 | 0.644 |
| Cuckolded Social Father Age | -0.030 | -0.132 – 0.071 | 0.554 |
| Cuckolded Social Father Lifespan | -0.025 | -0.105 – 0.055 | 0.542 |
| Within-pair Father Age | 0.063 | -0.052 – 0.178 | 0.281 |
| Within-pair Father Lifespan | 0.041 | -0.055 – 0.137 | 0.403 |
| Extra-Group [yes] | 1.094 | 0.353 – 1.835 | **0.004** |
| Incubation Date | 1.930 | 1.296 – 2.564 | **<0.001** |
| Helpers [one] | 0.146 | -0.125 – 0.418 | 0.292 |
| Helpers [two+] | 0.128 | -0.208 – 0.463 | 0.455 |

Note: Sample size is 2278 males. The model includes random effects of mother ID (n = 495, τ = 0.04), social father ID, nested within mother ID (n = 691, τ = 0.41), genetic father ID (n = 499, τ = 0.03), and cohort (n = 30, τ = 0.02). The residual variance is 3.29. A value of 1 for the binomial variable ‘extra-group’ indicates the chick was sired by a male outside the natal territory, while a value of 0 indicates the sire was the within-pair social mate. All extra-pair father terms are interaction terms between the effect and the extra-pair binomial variable. Both within-pair father terms are also interaction terms, with each effect interacting with a second dummy variable for within-pair sire (0/1).

**Discussion**

WP father age positively influences early-life chick fitness as older fathers have heavier nestlings that are more likely to survive to independence.

* The lack of an effect of WP father lifespan suggests this positive effect of WP paternal age is due to improvements with age and not simply that longer-lived individuals are of higher quality (selective appearance) (van de Pol and Verhulst 2006)
* The WP father age effect is unlikely to be a consequence of germ-line level changes with age. Sperm DNA damage increases with paternal age (Velando et al. 2011; Johnson et al. 2015), and sperm age typically has either negative or null effects on offspring fitness (Johnson and Gemmell 2012; Lemaître and Gaillard 2017).
* More likely is that the effect of WP father age is related to changes in paternal care or environment. Since there was no effect of WP father age on male recruitment, the effects of WP father age appear to be isolated to the period where offspring are reliant on parental care.

Given that the WP father age effect is most likely environmental, it is not clear why EP social father age does not positively influence chick fitness as well.

* Differences between cuckolded males and successful males may explain these divergent patterns if these differences interact with male age.
* It’s possible that males reduce their parental care for clutches that contain more extra-pair chicks through signals of partner infidelity (e.g. Schroeder et al. 2016). If males become better parents with age, but the ability to discern partner fidelity also improves with age, we could expect to see the divergent pattern of father ages on offspring fitness where only the offspring sired WP enjoy the fitness benefit of older social fathers.
* Alternatively, It’s possible that the degree of cuckoldry a male experiences is correlated with his overall quality and quality of his offspring. This reduced siring success of cuckolded males could result from female choice and/or sperm competition (Pizzari et al. 2008; Fitzpatrick and Lüpold 2014; Vuarin et al. 2019). In post-hoc analysis we found that males that have more WP siring success have fitter offspring than males with less WP siring success (supplementary material).
  + So, dominants with siring success produce fitter chicks on average, and these are the chicks that make up the WP offspring category. What’s not clear is why these chicks improve with the male’s age. It’s possible that WP successful sires are improving their chick fitness with increased experience, whereas the cuckolded males do not improve with increased experience (because they’re just low quality, they’re already trying their very best).
  + Alternatively, if only high quality males are capable of achieving within-pair siring success at late ages, the apparent improvement of chick fitness with within pair sire age could be resultant of a selective disappearance of low quality males at these later ages, which is unrelated to lifespan.

As there were no effects of EP father age, there is no evidence of genetic deterioration with age.

* Although sperm typically deteriorates in quality with male age (Johnson et al. 2015; Lemaître and Gaillard 2017), the effects of senescent sperm carrying over to influence offspring fitness are contentious. Although some studies have found evidence of negative effects of male age on some measures of offspring fitness(Ducatez et al. 2012; Bouwhuis et al. 2015; Schroeder et al. 2015), many others have not found any such associations (Fox et al. 2003; Fricke and Maklakov 2007; Avent et al. 2008; Carnes et al. 2012). In natural conditions, if senescence rates vary amongst individuals, females may avoid senescent males or their sperm may lose to less senescent males (Vuarin et al. 2019), and so the sample of older males that are successful sires may be biased towards only high quality males(Pizzari et al. 2008; Fitzpatrick and Lüpold 2014).
* This may be the case in the fairy-wrens as we see no negative male age effects, even when controlling for longevity.
* This suggests that female preference for older males is neither adaptive nor maladaptive in the context of offspring early life fitness.

Maternal age effects

* The effects of maternal age and maternal lifespan on survival to independence and male recruitment are counteracting each other. This suggests that mothers that live longer are either inherently higher quality or reside on higher quality territories. The positive effect of lifespan appears considerably stronger than the senescence in offspring fitness with maternal age.

Helpers

* Partially explain the effect of WP fathers on weight, but do not contribute to that effect for survival to independence. Also does not contribute to maternal effects on independence and recruitment.
* Older dominant males and females are more likely to have more helpers, and having more helpers apparently improves chick weight, but not survival or recruitment. It’s not clear whether this is due to helpers *per se* increasing provisioning rates, or simply that increased number of helpers is associated with a better territory quality (i.e. better food availability) overall (Cockburn 2008).
  + Previous studies (Seychelles Warblers, Hammers 2019 & Alpine marmots, Berger 2018) show that helpers compensate for declines in offspring fitness with parental age, obscuring negative parental age effects. Our study demonstrates that an association between helper number and parental age can also create apparent positive associations between parental age and chick fitness.