The Age-old question: Evolutionary causes & ecological consequences of ageing in the wild

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Declaration

I, Eve Cooper, declare that this thesis, submitted to the degree of Doctor of Philosophy of The Australian National University, is my own original work unless otherwise referenced or acknowledged. All chapters are co-authored, as indicated. This document has not been submitted for consideration at any other academic institution.

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Abstract

Iteroparous animals often express dramatic variation in life-history traits over the course of a lifetime. Understanding the evolutionary causes and ecological consequences of the age-related improvements and declines (i.e. senescence) in fitness-related traits is central to life-history theory. However, the evolution of ageing is highly complex, and poorly understood. Why is there such dramatic variability in ageing patterns not only across species, but also within species across individuals, and even within individuals across traits? In this thesis I investigate the eco-evolutionary dynamics of ageing and senescence, first broadly using a cross-species meta-analysis, and then more narrowly, using 30 years of individual-level life-history data collected on a wild population of superb fairy-wrens (*Malurus cyaneus*).

Chapter 1 investigates the effect of early-life environmental quality on late-life rates of senescence via a meta-analysis of studies of 14 wild populations of birds and mammals. I found no effect of early-life environment on survival senescence, but reproductive output declined more rapidly when early environmental conditions were poor. This effect of early-life environment on reproductive senescence suggests a gene-by-past-environment interaction, and indicates one mechanism by which senescence rates will vary between individuals living within a single population. I discuss the consequences of this finding for our understanding of the evolution of senescence.

Chapter 2 investigates ageing in a suite of different traits using data from a long-term study of superb fairy-wrens. While I found that survival senescence begins at the age of maturity in both sexes, the onset of senescence in some reproductive traits is substantially later, and in other reproductive traits there is no senescence at all. I suggest eco-evolutionary pathways that may explain why these traits do not age in synchrony in the fairy-wrens. Most notably, I discuss the likely role of sexual selection in driving the evolution of ageing in some traits, and as a corollary, lead to variability in ageing between traits within an individual.

Chapter 3 investigates the age-specific genetic architecture and selection on males' date of moult into breeding plumage each year, a sexually selected trait in the fairy-wrens that apparently defies senescent declines. Female fairy-wrens strongly prefer males that moult earlier in a given year. Notably, moult date continuously improves with age, despite physiological (survival) senescence co-occurring within males. I used quantitative genetic and multivariate selection models to investigate the relative roles of

adaptation and constraint driving the evolution of the continuous age-related improvement in moult date.

Chapter 4 investigates broader ecological consequences of ageing, by examining if and how parental and helper ages impact the fitness of the next generation (transgenerational effects of age). I exploited the natural structure of the fairy-wren social system to disentangle germline from environmental effects of paternal age, by estimating paternal age effects in extra-pair sires that provide only genetic material, and cuckolded mates that provide only parental care. Additionally, because fairy-wrens are cooperative breeders, I tested if the ages of helpers also impact chick fitness. I show that ageing in both parents and helpers can influence the fitness of the next generation in complex ways, and that here too sexual selection likely plays a key role in driving some of the observed patterns.

My results highlight the complexity of processes shaping ageing and senescence. Through the lens of life-history theory, I illustrate here how longitudinal individual-based studies of wild animal populations are a uniquely powerful tool for investigating the evolution of ageing and senescence in the natural world.

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Introduction

Senescence can be defined as the progressive decline of organismal function and fertility that often characterizes late life (Kirkwood and Austad 2000). As a process that impairs both survival and fertility, senescence will, by definition, have a negative impact on an organism's evolutionary fitness. Why has natural selection therefore not eliminated senescence? Understanding the ultimate mechanisms driving the biological phenomenon of senescence is of great value to our understanding of how evolutionary processes are realized in the natural world. Classical theories on 'mutation accumulation' (Medawar 1952), 'antagonistic pleiotropy' (Williams 1957), and 'the disposable soma' (Kirkwood 1977) still form the basis of our understanding of the evolution of senescence, ultimately as a consequence of declining selection in late life.

The study of senescence was largely ignored by ecologists until the late 1990s (Nussey et al. 2008, 2013; Kowald and Kirkwood 2015; Gaillard and Lemaître 2020). In fact, it was often assumed that senescence would not be observable in natural populations of wild animals; due to high levels of mortality, it was expected that animals in the wild simply would not live long enough to grow 'old' (e.g. Rose 1991; Hayflick 2000; Kirkwood and Austad 2000). However, with growing data from long-term individual-based studies of wild animal populations, it became evident that senescence could be detected in wild populations with adequately large sampling effort (Nussey et al. 2008; Reichard 2016). Today, we now have compelling evidence that senescence is a widespread phenomenon in wild animal populations (Nussey et al. 2013; Lemaître and Gaillard 2017). This large body of evidence that senescence is pervasive in wild animals has however also highlighted how complex and poorly understood the processes underlying senescence are. The eco-evolutionary feedbacks driving senescence as well as its consequences on demography and life-history evolution are only now beginning to be recognized (Bonduriansky et al. 2008; Monaghan et al. 2008; Lemaître and Gaillard 2017; Gaillard and Lemaître 2020).

One notable finding from individual-based studies of phenotypic ageing is that the onset and rates of senescence often vary dramatically between individuals within a species, and even within a population (Nussey et al. 2013). Understanding how inter-individual variation in senescence rates is maintained, despite more rapid senescence generally relating to lower fitness, is key to understanding how senescence evolves and persists. In experimental studies, manipulation of extrinsic conditions, such as diet (Nakagawa et al. 2012; English and Uller 2016), stress (Crespi et al. 2013; Ridout et al. 2018), and

the social environment (Snyder-Mackler et al. 2020), can all have considerable effect on measures of senescence, indicating that senescence can be plastic. Clarifying how broadly and to what extent environmental plasticity (i.e. gene-by-environment interactions) modulates individual senescence rates in the wild is highly valuable in understanding the evolution of senescence (Wilson et al. 2008).

Not only does senescence vary between individuals, but it has also been found to vary between reproductive- and survival-related traits within an individual (Brunet-Rossinni and Austad 2006; Bouwhuis and Vedder 2017; Lemaître and Gaillard 2017). In it unclear why within a population some fitness-related traits will display early and rapid rates of senescence, while other traits continue to improve even into old ages (e.g. Nussey et al. 2009; Froy et al. 2013; Hayward et al. 2015). Our understanding of how a wide range of factors, including sexual selection (Bonduriansky et al. 2008), life-history trade-offs (Jones et al. 2008; Lemaître et al. 2015), and environmental factors (Adler et al. 2016; Hooper et al. 2018) all work to form the variable patterns observed in ageing across traits within an individual is still in its infancy. For example, in likely the most taxonomically extensive review to date, of 45 animal, plant, and algae species, Jones et al. (2014) were unable to identify any discernable association between the shape of a species' survival curve or rate of survival senescence and the shape or rate of reproductive ageing in that species. The lack of discernable patterns are reflective of the complexity of selection pressures and life-history characteristics that are likely acting simultaneously to produce the diversity of ageing patterns observed in natural populations. More empirical investigations into how different fitness-related traits change with age within species are needed given our poor understanding of how life-history modulates ageing, and vice-versa, how ageing modulates life-history.

Another key component to investigating the evolution and ecology of senescence is understanding how the genetic architecture and selection pressures have shaped the observed age-related changes in fitness-related traits. Quantitative genetic and selection modeling are powerful tools to investigate these questions (Moorad and Promislow 2009; Charmantier et al. 2014). Some work to date has been done on the age-related changes in the additive genetic and environmental sources of variances in traits that are senescing (Charmantier et al. 2006; Wilson et al. 2007; Chantepie et al. 2015). However, no equivalent study has been done on traits that appear to resist senescence, and continue to improve with age despite senescence in other fitness-related traits occurring simultaneously. Investigating the genetic architecture and selection pressures underlying these traits is needed in order to better understand the present and past evolutionary forces which can result in the senescence of some aspects of phenotype while other aspects continually improve.

Further investigation into variation in senescence between and within individuals in wild populations is valuable not only for our understanding of the evolutionary causes of senescence, but also its potential ecological consequences (Clutton-Brock and Sheldon 2010; Nussey et al. 2013; Gaillard and Lemaître 2020). For example, negative effects of ageing parents on offspring fitness has been recognized in humans for over 100 years (Bell 1918). This reflects an intergenerational effect of age, and there is considerable evidence from lab populations that these effects are common in other animal species besides humans (Monaghan et al. 2020). In the wild, parental age effects have the potential to influence demography and play an important role in eco-evolutionary feedback loops shaping life-history (Barks and Laird 2020). However, the extent to which parental age effects impact wild populations is unclear (Barks and Laird 2020; Monaghan et al. 2020).

Evidently, the processes shaping ageing are highly complex. It is not simply a declining force of natural selection with increasing age that molds senescence, but many other processes, including gene-by-environment interactions, sexual selection, and life-history trade-offs all play an equally, if not in some cases an even more predominant role in realized senescence onset and rates in the natural world (Bonduriansky et al. 2008; Monaghan et al. 2008; Gaillard and Lemaître 2020).

The central aim of this thesis is to empirically investigate potential evolutionary causes and ecological consequences of ageing and senescence, using the patterns of phenotypic age-related change observed in wild animal populations. The first chapter uses meta-analysis to investigate if the developmental environment is capable of influencing individual senescence rates. This chapter offers the first cross-species synthesis of the magnitude and significance of this possible gene-by-past-environment effect on senescence in the wild. The subsequent three chapters are dedicated to questions related to variation in ageing and senescence using the finer scope of a single population of superb fairy-wrens (Malurus cyaneus). The superb fairy-wren is a small passerine endemic to southeastern Australia. The cooperative breeding structure and high level of sexual dimorphism results in considerable variation in social environment, breeding dynamics, and life-history between individuals (Cockburn et al. 2016). These characteristics of the fairy-wren system provide an excellent basis for investigating within- and between-individual variation in ageing. Using 33 years (12 generations) of detailed data on the life-histories of thousands of individuals tracked from birth to death in a wild population of superb fairy-wrens, each of the three subsequent chapters will build on the last in an effort to investigate the multi-dimensional causes and consequences of ageing in the natural world. In Chapter 2, ageing across nine different fitness-related traits is quantified to better understand how trade-offs, sexual selection, and life-history

differences can all work to shape asynchronous ageing rates between different fitness components. Chapter 3 focuses on a single trait in male fairy-wrens, a sexually-selected trait driven by female choice that is notable for its lack of senescence. Using quantitative genetics and selection models this chapter investigates the age-specific genetic architecture and selective pressures that drive the age-related improvement, and resistance to senescence observed in this trait. This chapter provides the first empirical investigation into the age-dependent variation in additive genetic variance and selection on a trait which continually improves with age up to maximum ages. Lastly, Chapter 4 attempts to extend our understanding of ageing in the fairy-wren system by investigating how the ageing of one individual's phenotype might influence the fitness of other individuals' phenotypes, specifically through intergenerational effects. The unique social and mating structure of the fairy-wren system allows for novel avenues of investigation of intergenerational effects. The high level of promiscuity in the population allows for germline-level vs. environmental-level effects of father age on chick fitness to be effectively disentangled. Additionally, this chapter provides the first investigation into the effects of the ages of subordinate male 'helpers' at the nest on the fitness of the chicks.

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Chapter 1

Ageing with a silver-spoon: A meta-analysis of the effect of developmental environment on senescence

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Abstract

What determines variation between individuals in how they senesce, and are environmental conditions experienced during development relevant to late-life performance? We report a meta-analysis of studies of wild populations to determine how the quality of the environment experienced during development affects rates of survival and reproductive senescence. From studies of 14 bird or mammal species, we calculated effect sizes for the interaction between the effects of environmental quality during development and age in predicting survival (*N* = 18) or reproduction (*N* = 30) over time in late life. We found no evidence that developmental environment affected rates of survival senescence (β_{mean} = -1.2 x 10^{-4} ± 0.022SE). However, a better developmental environment was associated with slower rates of reproductive senescence in late life (β_{mean} = 0.062 ± 0.023SE), indicating a small, but significant, "silver-spoon" effect of early-life conditions that persisted through to late life. Our results illustrate how the effects of environmental conditions during development can persist throughout life, and indicate one possible cause of phenotypic plasticity in senescence.

Impact summary

Can the quality of the environment experienced during the first stage of life, the developmental period, have persistent effects that last into the final stage of life, affecting rates of senescence? Senescence, or the decline in reproduction or survival in old age, is common in both wild and domestic populations, but may also vary substantially between individuals in a population for different environmental and genetic reasons. Several recent analyses of data from detailed long-term studies of wild animal populations have now tested the effect of developmental environment on senescence, across a range of species. Here, we used a meta-analysis of these studies to summarise the effect of developmental environmental quality on senescence rates in the wild. Across 14 species of birds and mammals, there was no evidence that developmental environment influenced survival senescence, however there was a significant influence on reproductive senescence. Being exposed to a higher quality environment during the developmental period provided a “silver-spoon”, whereby individuals experienced slower declines in reproductive ability at the very last stage of their lives. Our results illustrate how the very first phase of life can shape the very last phase of life.

Introduction

Long-standing evolutionary questions surround the ultimate causes and consequences of senescence, defined as the decline in fitness-related traits with age (Medawar 1952; Williams 1957; Kirkwood and

Austad 2000). One aspect of an individual's senescence pattern, their rate of senescence, is measurable as the rate of age-related decline between the onset of senescence and death. Declines in survival probability and reproductive output (coined "survival" or actuarial, senescence, and "reproductive" senescence, respectively) are the most commonly used metrics of senescence rates (Lemaître and Gaillard 2017), likely because they correspond to direct components of individual fitness (Bouwhuis et al. 2012; Kowald and Kirkwood 2015). Increasing numbers of longitudinal wild animal studies have now demonstrated that variation in individual senescence rates even within populations is common in natural environments (Nussey et al. 2013). In determining the evolutionary causes and consequences of senescence, it is therefore important to understand what causes these inter-individual differences. In essence, what determines how fast, or how slow, an individual will senesce?

The extrinsic environment may play a key role in shaping rates of senescence in both reproduction and survival (Kawasaki et al. 2008; Nussey et al. 2013; Lemaître and Gaillard 2017). Populations of the same species exposed to different environments often experience widely different patterns of senescence (Austad 1993; Lemaitre et al. 2013; Douhard et al. 2016; Holand et al. 2016). On an evolutionary scale, differences in senescence patterns observed between populations could be caused by natural selection, whereby differences in extrinsic mortality risk cause differential selection pressures on senescence rates (Medawar 1952; Williams 1957). Conversely, the quality of the environment experienced in late-life has a direct effect on senescence, with better environmental conditions typically being associated with slower senescence (Reichert et al. 2010; Pardo et al. 2013; Oro et al. 2014; Arlet et al. 2015; Bleu et al. 2015; Chantepie et al. 2016). Additionally, senescence rates can theoretically be influenced by the extrinsic environment not only at the time when senescence occurs, but at any point in an individual's life-history. It is therefore conceivable that the earliest stage of life, the developmental period, could influence an individual's senescence rates.

The theory of developmental programming suggests that the period of development, which includes both gestation and juvenility up until the point of sexual maturity, is distinct in its role of modulating the adult phenotype (Lindström 1999; Hales and Barker 2001; Monaghan 2008). Developmental programming first gained prominence in a series of seminal studies on human health where it was found that an apparent poor developmental environment is strongly associated with increased risk of impaired glucose tolerance (Hales et al. 1991), type two diabetes (Hales and Barker 1992), and cardiovascular disease (Barker et al. 1993) later in life. Since then, it's been demonstrated that poor developmental conditions in humans is also associated with earlier menopause (Elias et al. 2003; Tom et al. 2010),

reduced longevity (Modin et al. 2009; Todd et al. 2017), and even behavioral shifts reflecting a faster pace-of-life (Mell et al. 2018).

Amongst non-human animals, exposure to good environmental conditions during development, such as high nutritional availability, can have generally positive impacts on a multitude of aspects of individual fitness beyond the developmental period itself (see Lindström 1999 for a review). Grafen (1988) referred to this phenomenon as the "silver-spoon" effect. Although there is now abundant evidence for the silver-spoon effect on performance in early and mid-life in wild animals, what is not yet established is what influence the environment experienced during the developmental period has on late life, and specifically on senescence (Nussey et al. 2013; Lemaître et al. 2015; Lemaître and Gaillard 2017). If silver-spoon effects do extend to the last stage of life, a better quality developmental environment is predicted to be associated with relatively slower senescence rates. This phenomenon has been demonstrated to occur in laboratory experiments on model species where a reduction in calories (Ozanne et al. 2004), or protein (Aihie Sayer et al. 2001; Langley-Evans and Sculley 2006), during development has negative effects on late-life fitness. However, Lemaître et al. (2015) reported that trade-offs between early-life reproduction and late-life reproduction are frequently observed in the wild across many taxa. If a high-quality environment during early life results in higher rates of early-life reproduction, then the existence of such trade-offs leads to a prediction that favourable early environmental conditions should be associated with faster rates of senescent decline in late life. This phenomenon of trade-offs has recently been demonstrated to occur in lab-based experiments of arthropods (Adler et al. 2016; Hooper et al. 2017).

Recent reviews have highlighted the uncertainty in our current understanding of the influence of the developmental environment on late-life fitness. For example, there is clear mechanistic evidence that female gonads are incredibly sensitive to nutritional perturbations during development (Gunn et al. 1995; Rae et al. 2001). However, despite this, there is no clear evidence that nutrition during development influences lifetime reproductive performance, or reproductive senescence onset in female domestic livestock (reviewed in Gardner et al. 2008). A cross-taxa meta-analysis found no evidence that experimentally restricted nutrition during development affects longevity overall, although the effect changes direction depending on taxonomic group, and whether nutrition limitation occurs pre- or post-natally (English and Uller 2016). A follow-up meta-analysis found that, in some circumstances, dietary restriction during development increases inter-individual variability in longevity, suggesting that nutritional stress during development might increase phenotypic variation, for example through

exposing cryptic epigenetic variation (Senior et al. 2017). These results highlight the complexity of the potential impact of the developmental period on late-life, and emphasize the need for a review to test whether silver-spoon effects persist to affect senescence rates in the last stage of life.

Here we provide, to our knowledge, the first quantitative review of the effect of environmental conditions experienced during the developmental period on senescence rates in wild populations. Using data from longitudinal studies of wild animal populations, we amalgamated effect sizes through meta-analyses to determine if there is a global mean effect of the quality of the developmental environment on rates of (i) survival senescence, and (ii) reproductive senescence. We then investigated the influence of study characteristics and biological factors in moderating the observed effects.

Methods

Literature search

We performed a broad search of the scientific literature in order to identify published tests of the effect of developmental environment on rates of senescence. For a detailed account of the parameters and methods involved in our literature search, see appendix S1. In brief, we first used *Web of Science* to identify relevant papers by conducting a key word search with the terms: (senescence OR ageing OR aging) *AND* ("early environment*" OR "early*life" OR "natal environment*" OR "silver*spoon" OR "predictive adaptive response" OR "cohort effect*"). We then conducted forward and backward searches on 6 classic senescence papers as well as 5 recently relevant and influential review papers (see appendix S1 for details). This resulted in a pool of 6766 unique papers. From this pool, we extracted all papers that met the criteria that the study should: (i) use individual-level data from a population of wild animals to investigate the effect of the environment experienced during development on senescence rates in either survival probability and/or reproductive output (ii) include one or more linear model(s) testing the effect of an interaction between age (measured as a value of discrete time periods, typically years) and a measure of developmental environmental quality, on senescence rates (measured in the same units of time as 'age'); (iii) use a metric of environmental quality that was extrinsic to the individual, but that could theoretically have a causal influence on the fitness of the individual. Examples of suitable metrics of environmental quality included weather variables, conspecific population density, food availability, or predation risk. As we were interested in the influence of environmental conditions experienced during development, we only included papers that quantified the metric of environmental conditions during the period of gestation or the period of juvenility (defined as before sexual maturity).

From the published material, we identified 10 papers which measured the effect of developmental environment on senescence rates as per our inclusion criteria. Several additional papers from our initial search pool indicated the availability of the data necessary to complete the required analysis, but did not present results in such a way that they met our criteria. In these cases, we contacted the authors to enquire about the relevant analyses for their study system. From this, we obtained additional results for four species that were previously unpublished.

Data synthesis

In total, we extracted 48 effect sizes from 14 independent studies of 6 bird and 8 mammal species (Table 1). For 7 species, 18 effect sizes predicted survival senescence rates, and for 13 species, 30 effect sizes predicted reproductive senescence rates. For the survival senescence models, the response variable was the probability of survival over a given period (typically a year) at a given age. For the reproductive senescence models, the predictor variable was either the probability of reproducing (yes/no), or the magnitude of reproductive output (number of offspring) over a given period (typically a year) for a given age, depending on the biological suitability of either of these measures for the specific study population.

Table 1. Summary of the 14 studies included in meta-analyses.

Species	Reference	*N**	Environmental metrics tested for influence on reproductive senescence	Environmental metrics tested for influence on survival senescence
Barn swallow, *Hirundo rustica*	Balbontín et al. (2015)	96 (female and male)	Population density, predation	-
White stork, *Ciconia ciconia*	Baos et al. (2012)	111 (female), 138 (male)	Acute pollution exposure	-
Great tit, *Parus major*	Bouwhuis et al. (2010)	488 (female)	Population density, birthdate, population average reproductive success, number of siblings, food availability, maternal age	-
Mauritius Kestrel, *Falco punctatus*	Cartwright et al. (2014)	52 (female)	Habitat quality	-
Tawny owl, *Strix aluco*	Millon et al. (2011)	40 (female), 88 (male)	Food availability	-
Seychelles Warbler, *Acrocephalus sechellensis*	Hammers et al. (2013)	270 (female and male)	-	Population average reproductive success, food availability, social group size
Svalbard Reindeer, *Rangifer tarandus*	Douhard et al. (2016)	157 (female)	Weather (precipitation)	Weather (precipitation)
Soay sheep, *Ovis aries*	Hayward and Pemberton (2015; unpublished)	447 (female), 130 (male)	Population density	Population density

Red deer, *Cervus elaphus*	Nussey et al. (2007)	214-253 (female)	Population density	Population density
Mountain goat, *Oreamnos americanus*	Panagakis, Hamel, and Côté (2017)	142 (female)	Population density	Population density
Bighorn sheep, *Ovis canadensis*	Pigeon and Festa-Bianchet (2017; unpublished)	140 (female)	Population density, weather (mean precipitation), weather (pacific decadal oscillation), weather (mean temperature)	Population density, weather (mean precipitation), weather (pacific decadal oscillation), weather (mean temperature)
Red squirrel, *Tamiasciurus hudsonicus*	Hämäläine, Haines and Boutin (2017; unpublished)	102 (female), 71 (male)	Food availability	-
Banded mongoose, *Mungos mungo*	Marshall and Cant (2017; unpublished)	13-53 (female), 11-34 (male)	Weather (variation in rainfall), weather (mean rainfall)	Weather (variation in rainfall), weather (mean rainfall)
Asian elephant, *Elephas maximus*	Mumby et al. (2015)	455 (female)	Weather (mean rainfall)	-

**N* = the number of individuals used in a study.

Since the biological relevance of different measures of environmental quality will vary across species, it is unsurprising that a broad variety of indices of environmental quality was used. Across studies, conspecific population density was the most widely-used metric (6 out of the 14 studies, generating 11 effect size), whereas across effect sizes, weather conditions were the most frequently used (21 out of the total 48 effect sizes). Direct measures of food availability accounted for the third most common metric (6 effect sizes from 4 studies). All the other metrics used to measure environmental quality, including acute pollution exposure, birth date, cohort birth-rate, social group size, anthropogenic habitat exposure, maternal age, sibling number, and predation risk, were each used in only 1 or 2 studies, generating 1 or 2 effect sizes each.

We followed the predictions of the authors in assigning the directionality of each measure of environment to represent either 'better' or 'worse' environmental quality. Increasing numerical values of some environmental measures were inferred to represent increasing environmental quality (e.g. food availability), whereas others implied decreasing environmental quality (e.g. population density). To standardize effect sizes, we corrected the direction of each effect size so that the increasing numerical value of the metric of environmental quality reflected increasing environmental quality.

We further categorized effect sizes by several study-design and biological factors: *type of environmental measure*, *class*, *time of environmental measure* and *sex*. Due to the large variety of measures of environmental quality, we did not have appropriate statistical power to determine the independent influence of all 11 measures. Instead, we categorized *type of environmental measure* into three groups: measures of population density (*density*), measures related to weather variables (*weather*), and all other measures (*other*; see above). *Class* was categorized as either *mammal* (32 effect sizes from 8 species), or *bird* (16 effect sizes from 6 species). We categorized *time of environmental measure*, as *gestation* if the measure of the environment was taken before birth (8 effect sizes from 3 species), or *juvenility* if the measure of the environment was taken within or at the first year of life (40 effect sizes from 12 species)*Sex* had three categories (*female*, 35 effect sizes from 13 species; *male*, 8 effect sizes from 5 species, or *both*, 5 effect sizes from 2 species). We later reduced analysis of *sex* to only two categories (*female* and *male*; see methods – meta-analysis below).

Following the formulae listed in Koricheva et al. (2013), we transformed each interaction term between developmental environment and age (i.e. the effect size which predicted senescent decline) into a correlation coefficient (*r*) using the relevant inference (test) statistic (i.e. X^2, *F*, *T*, or *z*). Then, to avoid a

skewed distribution near $r = \pm 1$, we transformed correlation coefficients with Fisher's z-transformation (Koricheva et al. 2013). We used these z-transformed correlation coefficients (Z_r) as standardized effect sizes in all meta-analyses (see below). We calculated the variance for each effect as $1/(N - 3)$, where N is the number of individuals in the analysis (Koricheva et al. 2013).

When inference statistics were not adequately reported in published materials, we contacted authors to request these metrics. In the single case in which an author was unable to reproduce the necessary statistics, we conservatively estimated the non-significant effect size as 0 (following Nakagawa & Santos, 2012).

Multi-level meta-analyses

We conducted two separate random effects multi-level meta-analyses (Konstantopoulos 2011; Koricheva et al. 2013) to determine the mean effect (β_{mean}) of developmental environment on both reproductive senescence rates and survival senescence rates. We used a multi-level structure to control for potential non-independence of effect sizes. Studies contributed multiple effect sizes to either models of reproductive or survival senescence if they contained analyses of both males and females, modeled more than one measure of environmental quality, or modeled environmental quality over each of the time periods (*gestation* and *juvenility*). Within studies, a single model also contributed multiple effect sizes if different measures of environmental quality were analyzed within the same model. To control for potential non-independence within studies and within models, we ran three-level meta-analyses with a random effect of *model*, nested within a random effect of *study* (Konstantopoulos 2011; Nakagawa and Santos 2012; Koricheva et al. 2013). By controlling for study, we did not need to control for non-independence within species as each species was represented by only one study. To estimate residual variance, we added an observation-level random effect (Harrison 2014).

After determining the global mean effect on rates of survival senescence and reproductive senescence, we used meta-regressions to investigate if the mean effect sizes varied dependent on each biological or study-design factor (see "Data synthesis"). Due to the relatively small sample of effect sizes, each categorizing factor (i.e. *type of environmental measure*, *class*, *time of environmental measure*, and *sex*) was investigated independently as the single categorical moderator term in a meta-regression. For the meta-regression with moderator *sex*, we removed effect sizes which considered both sexes due to small sample size (3 effect sizes for survival senescence, and 2 effect sizes for reproductive senescence). For each meta-regression, we determined if the mean effects of categories were different from each other

using a Wald-type chi-square test (Q_m). We also determined the mean effect for each category (β) and tested if the effect within each category was different than 0 using a z-test.

Heterogeneity and Sensitivity Analyses

To quantify heterogeneity between effect sizes caused by differences between studies and differences within studies, we calculated I^2 statistics. I^2 is the ratio of true heterogeneity to the total variance (true heterogeneity plus sampling error) amongst effect sizes (Koricheva et al. 2013). Due to the codependence in our meta-analysis models, and resulting use of *study* and *model* as nested random effects, along with an observation-level random effect, it was necessary to use a modified I^2 statistic appropriate for multi-level models (Nakagawa and Santos, 2012). For each of the two meta-analyses, we calculated three I^2 values, one attributable to the proportion of true variation between studies, the second attributable to the proportion of true variation within studies (i.e. between models within the same study), and the third attributable to residual variation. The sum of the percentages of total variation due to these three sources equals the traditional I^2 of Higgins et al. (2003). By convention, benchmarks for I^2 of 25, 50, and 75% are used to indicate low, moderate and high values of inconsistency between studies (Higgins et al. 2003).

Biases within a dataset used for meta-analyses can result from a multitude of factors, including unequal research attention across taxonomy, publication biases towards significant results or predicted outcomes, or limitations in the comprehensiveness of literature searches methods (Koricheva et al. 2013). To investigate the potential for biases in our dataset we first created funnel plots and performed Egger's regressions on residuals of the individual effect sizes for each meta-analysis (Egger et al. 1997; Nakagawa and Santos 2012). Second, we added year of publication as a moderator variable to analyses to determine if there had been a shift in the mean reported effect with time ($\beta_{\text{publication year}}$). Since our dataset included both published and unpublished effect size estimates, we also conducted bias detection tests on the subset of effect sizes from published materials to differentiate apparent publication bias from other causes of bias.

Although it was unavoidable in the context of this study and the available published data, deriving effect sizes from inference statistics rather than bivariate correlations has the potential to introduce inaccuracies in estimates of Z_r (Aloe 2015). The problem may be further amplified by increasing covariance with covariates in the model from which the focal effect test statistic is derived (Aloe 2015; Noble et al. 2017). To investigate the possibility of covariates altering the magnitude or direction of

effect sizes, we added the number of covariates as a moderator variable to analyses. For detailed discussion on the potential shortcomings associated with using partial correlation coefficients in lieu of bivariate correlations, readers are encouraged to see Aloe 2015 and Noble et al. 2017.

As an additional potential shortcoming of using partial effect sizes to derive estimates of Z_r, non-independence between measurements may also influence the estimate of effect (Nakagawa and Cuthill 2007; Noble et al. 2017). Sources of non-independence can be controlled for by adding random effects into the model from which an effect size is derived (Nakagawa and Santos 2012). Since individuals are measured repeatedly (i.e. annually), having *individual ID* as a random effect in the models from which we derived effect sizes is important to control for this source of non-independence between measurements. For 44 of the 48 derived effect sizes, the original model included *individual ID* as a random effect. We re-ran our models excluding the four studies which did not include *individual ID* and this did not change any interpretations of results. Other sources of non-independence, including similarities in space (e.g. region), time (e.g. cohort, current year), and social structure (e.g. group ID) were also sometimes, but not always, controlled for in the primary source models as random effects (appendix S2). Since the importance of these various factors as sources of non-independence will depend on the specifics of each individual study system, we did not make any assumptions about whether or not the inclusion of these random effects was necessary to each individual study. It is important to note however, that if sources of non-independence were not adequately accounted for in the models from which effect sizes were extracted, then this could influence our results by falsely inflating *N* and reducing the variance as it was calculated.

The full dataset is provided in appendix S2. All statistical analyses were conducted in R (R Core Development Team, 2017, version 3.3.3) with the *metafor* package (Viechtbauer, 2010).

Results

We found no effect of developmental environment on survival senescence [$\beta_{mean} = -1.2 \times 10^{-4}$, 95% confidence interval (CI) = -0.043 to 0.043, table 2, figure 1A]. However, there was a positive effect on late-life reproductive success ($\beta_{mean} = 0.062$, 95% CI = 0.016 to 0.107, table 3, figure 1B), indicating that, on average, a better quality developmental environment was associated with slower rates of reproductive senescence.

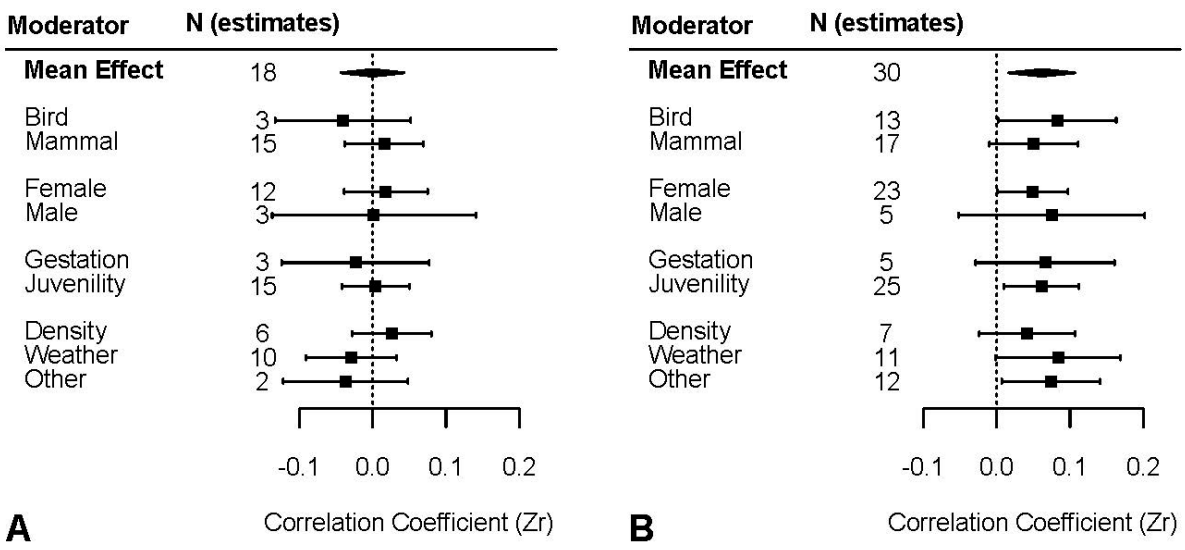

Figure 1. The overall mean effect and the marginal mean effects (with associated 95% CIs) for each of the meta-regressions categories (*class, sex, time of environmental measure, and type of environmental measure*) for (A) survival senescence rates and (B) reproductive senescence rates.

Table 2. Results from the random-effects meta-analyses on rate of survival senescence. No effects are significant.

Moderator	Q_m*	*d.f.* (Q_m)	*P* (Q_m)	Category	$m^{\dagger}$	$k^{\ddagger}$	Mean (Z_r)	Standard error	Lower CI (2.5%)	Upper CI (97.5%)	*z* value	*P (z)*
Mean effect	-	-	-	-	7	18	-0.0001	0.022	-0.043	0.043	-0.005	0.996
Type of environmental measure	2.377	2	0.305	Density	5	6	0.026	0.028	-0.028	0.080	0.939	0.348
				Weather	3	10	-0.029	0.032	-0.091	0.033	-0.919	0.358
				Other	1	2	-0.037	0.043	-0.122	0.048	-0.851	0.395
Class	1.070	1	0.301	Bird	1	3	-0.041	0.048	-0.135	0.052	-0.865	0.387
				Mammal	7	15	0.016	0.027	-0.038	0.069	0.571	0.568
Time of environmental measure	0.250	1	0.617	Gestation	2	3	-0.023	0.051	-0.124	0.077	-0.455	0.649
				Juvenility	6	15	0.004	0.004	-0.042	0.050	0.171	0.864
Sex	0.045	1	0.831	Female	6	12	0.018	0.029	-0.039	0.075	0.620	0.535
				Male	2	3	0.001	0.077	-0.149	0.151	0.016	0.988

*Q_m = Wald-type chi-square distribution test statistic for the effect sizes between categories (95% CI), $^{\dagger}m$ = number of species, $^{\ddagger}k$ = number of effect sizes.

Table 3. Results from the random-effects meta-analyses on rate of reproductive senescence. Significant mean effects are bold.

Moderator	Q_m*	*d.f.* (Q_m)	*P* (Q_m)	Category	$m^{\dagger}$	$k^{\ddagger}$	Mean (Z_r)	Standard error	Lower CI (2.5%)	Upper CI (97.5%)	*z* value	*P (z)*
Mean effect	**-**	**-**	**-**	**-**	**13**	**30**	**0.062**	**0.023**	**0.016**	**0.107**	**2.666**	**0.008**
Type of environmental measure	1.288	2	0.525	Density	6	7	0.042	0.033	-0.024	0.107	1.251	0.211
				Weather	4	11	0.084	0.043	-0.001	0.169	1.936	0.053
				Other	**6**	**12**	**0.074**	**0.034**	**0.007**	**0.141**	**2.175**	**0.030**
Class	0.397	1	0.529	**Bird**	**5**	**13**	**0.083**	**0.041**	**0.002**	**0.163**	**2.018**	**0.044**
				Mammal	8	17	0.050	0.031	-0.010	0.111	1.623	0.105
Time of environmental measure	0.009	1	0.924	Gestation	3	5	0.066	0.049	-0.029	0.161	1.362	0.173
				Juvenility	**11**	**25**	**0.061**	**0.026**	**0.010**	**0.112**	**2.357**	**0.018**
Sex	0.151	1	0.698	**Female**	**12**	**23**	**0.049**	**0.025**	**0.001**	**0.097**	**1.982**	**0.048**
				Male	4	5	0.075	0.065	-0.052	0.202	1.161	0.246

*Q_m = Wald-type chi-square distribution test statistic for the effect sizes between categories (95% CI), $^{\dagger}m$ = number of species, $^{\ddagger}k$ = number of effect sizes.

Heterogeneity between effect sizes not accounted for by sampling error, I^2, was low for reproductive senescence (I^2_{total} = 37%, $I^2_{between\text{-}studies}$ = 25%, $I^2_{within\text{-}studies}$ = 12%, residual variance < 0.1%) and negligible for survival senescence (I^2_{total} < 0.1%).

The results of the two meta-analyses were robust to Egger's regression bias detection tests. We detected a significant bias towards positive results from the subset of effect sizes extracted from published material (t_{21} =2.19, P = 0.04, table S1, figure S2), but not in models of the complete dataset, in which there was no significant asymmetry in funnel plots for survival senescence (t_{16} =0.58, P = 0.57, table S1, figure S2A), or reproductive senescence (t_{18} =1.31, P = 0.20, table S1, figure S2B). There was no effect of adding the year of publication of the study (or time of acquirement for unpublished data) as a moderator variable in meta-regressions in either the model of survival ($\beta_{\text{publication year}}$ = 0.008, 95% CI = -0.032 to 0.047) or reproductive senescence ($\beta_{\text{publication year}}$ = -0.006, 95% CI = -0.045 to 0.032), indicating no discernable shift in results with time.

The number of covariates in the model from which each effect size was derived had no effect on Z_r for either estimates predicting reproductive senescence rates ($\beta_{\text{number of covariates}}$ = 6.1 x 10^{-5}, 95% CI = -0.009 to 0.009), or survival senescence rates ($\beta_{\text{number of covariates}}$ = -0.005, 95% CI = -0.016 to 0.007).In the meta-regressions of both the effect on survival and reproductive senescence rates, there were no significant differences in mean effect between categories (P(Q_m) values, table 1, table 2).

Discussion

Our meta-analysis of 8 mammal and 6 bird species indicated that variation in early environmental conditions affected rates of reproductive senescence, but found no evidence of effects of early environment on survival senescence.

Reproductive senescence

The significant mean effect of developmental environment on reproductive senescence indicates that the environmental conditions during development have far-reaching effects all the way into the last stage of life. The positive direction of this effect implies that good developmental conditions provide a silver-spoon, whereby the rates of decline of reproductive output in late life are slower when the quality of the environment experienced during development is better.

Although the effect of developmental environment on reproductive senescence was significant, the effect size was relatively small, as per the conventional benchmarks proposed by Cohen (1988). This is

not entirely surprising given the multitude of complex and potentially interacting environmental and genetic factors that are expected to influence senescence.

In an evolutionary context we assume that senescence is partially genetically determined (Kirkwood and Austad 2000; Patridge and Gems 2007; Nussey et al. 2013; Kowald and Kirkwood 2015; Reichard 2016), and several studies have confirmed the heritability of individual senescence patterns (Arking 1987; Charmantier et al. 2006; Wilson et al. 2007; Wang et al. 2014). The effect of developmental environment on reproductive senescence may therefore represent a 'genotype by past-environment' interaction. This plasticity in senescence rate will weaken the ability of the trait to respond to selection, and thus might even act as a mechanism by which senescence persists in the wild.

Although in some study systems an individual's developmental environment may be correlated with their late-life environment, we believe it is unlikely that the effect of development environment on reproductive senescence is driven by such a correlation. For four of the species in our dataset (great tit, Svalbard reindeer, red deer, and Soay sheep) current environmental conditions were controlled for in the regression models from which effect size was extracted. For Mauritius kestrels, the environmental measure used had no association with fitness when measured in adults (Cartwright et al. 2014), indicating that the measure of environmental quality used only had biological relevance during the highly sensitive developmental life stage. For other species, the environmental measures selected cycled from low to high multiple times within the lifespans of individuals that reached the age where senescence became apparent (prey density in tawny owls; Lambin et al. 2000, and monsoon season in Asian elephants; Mumby et al. 2015), were highly stochastic within the study system they were used (spruce masting in red squirrels; Boutin et al. 2006), or necessarily changed between development and adulthood due to breeding dispersal (territory quality in Seychelles warblers; Hammers et al. 2013). In these cases, a correlation between developmental and late-life environment is unlikely. It is also worth noting that since trade-offs within individuals between early-adult and late-life reproduction have been demonstrated to occur commonly in wild populations (Lemaître et al. 2015), our tests may actually be conservative. This is because if good environmental conditions during the first part of life (i.e. during development and early-reproductive life) result in higher reproductive investment in early adult life, investment in late-life reproduction should be reduced, potentially increasing the rates of reproductive senescence. In addition, if a correlation between developmental and late-life environment was what was driving the effect of developmental environment on senescence, we would expect to see a

significant effect in survival senescence rates (Reichert et al. 2010; Bleu et al. 2015; Le Coeur et al. 2016; Péron et al. 2016), in addition to the observed effect on reproductive senescence rates.

The low level of heterogeneity amongst effect sizes ($I^2_{Reproduction\ (total)}$ = 37%) indicates that there may be some differences in the true effect of developmental environment on reproductive senescence rates based on biological or study design factors. Although none of the meta-regressions found significant differences between the categories within moderator terms (*type of environmental measure*, *class*, *time of environmental measure*, and *sex*), there were some suggestive trends. The reproductive senescence of birds, but not mammals, was significantly affected by developmental environment. Additionally, environmental measures of weather had roughly twice the magnitude of effect in predicting reproductive senescence rates as compared to environmental measures of population density. Although there were apparent differences between categories in *sex* (significant effect in females, but not males) and *time of environmental measure* (significant effect of measures taken during juvenility, but not gestation), in these two cases the differences were likely driven by the relatively small sample sizes available for the non-significant categories.

Survival senescence

The lack of a perceptible effect of developmental environment on survival senescence was uniform across studies and meta-regression categories. This uniformity in non-significance was likely a driver of the negligible heterogeneity seen between effect sizes ($I^2_{Survival\ (total)}$ < 0.1%). However, the I^2 statistic is known to have low power when sample sizes are small (Huedo-Medina et al. 2006; Nakagawa and Santos 2012; Koricheva et al. 2013), and so the relatively small sample size may be contributing to the low I^2 value.

It is possible that a silver-spoon effect does actually slow survival senescence, but that the effect is masked by selective disappearance, directly caused by differences in environmental quality experienced during development (often referred to as the "selection hypothesis"; Nol and Smith 1987; Dugdale et al. 2011). Ability to survive into old age may be causally linked with ability to withstand harsh extrinsic conditions. Thus, stronger selection pressure for survival-related traits could result in a greater average ability to resist senescence amongst those that developed under poor conditions, concealing any silver-spoon effect on survival senescence rates.

Alternatively, the lack of effect on survival senescence rates may result from adaptive physiological mechanisms present during development that primarily conserve survival-enhancing traits at the cost of

reproduction-enhancing traits. When available energy is limited by poor environmental conditions, trade-offs that favor early-life survival over early-life reproductive development may be expected to occur (Kirkwood and Rose 1991). Thus, there is a carry-over cost of reduced reproductive ability when developmental environment is poor, but a relatively lower carry-over cost to survival ability. As a result, survival senescence may be less plastic than reproductive senescence, being less influenced by variability in the extrinsic environment during development.

Future directions

Several questions remain regarding the influence of developmental environment on senescence rates. Most notably, we found no relevant studies of amphibians, reptiles, fishes, or any invertebrates, which is presumably reflective of the relative lack of long-term individual-based studies of these species in the wild (Clutton-Brock and Sheldon 2010). Given the inherent physiological differences (Wang et al. 2006), commonality of indeterminate growth (Kozlowski 1996), and slower or absent senescence patterns (Flouris and Piantoni 2015) in ectotherms, it is plausible the environment experienced during development may have vastly different mechanistic influences on senescence (Hooper et al. 2017, 2018) However, due to the lack of long-term studies, effects remain principally unknown. Additionally, our results are suggestive of differences between birds and mammals, as well as between different measures of developmental environment (categorized as *density*, *weather*, or *other*) on the magnitude of effect observed in reproductive senescence rates. A greater number of studies would allow for a more robust test of the biological significance of these trends.

Through our literature search, we identified several studies with data that would have been adequate to investigate this question, but the specific analyses had not been done. In the hope of encouraging a more extensive synthesis in the future, we have formulated some suggestions for specific analytical methods to investigate the influence of developmental environment on senescence in wild populations. We recommend that: *(i)* To differentiate the influence of environmental quality during development and during other periods of life-history, the measure of environmental quality should be contained to only some pre-defined period, ending before sexual maturity. *(ii)* The effect should be analyzed using a model predicting either survival probability or reproductive output within a discreet time sequence (i.e. annually). As a predictor term, age should interact with the specific measure of developmental quality in order to specifically determine an effect on senescence rates. *(iii)* These models must only include individuals above the age of the onset of senescence (i.e. once reproductive or survival probability begins to decline), otherwise, any perceivable effect may be reliant on altering survival or reproductive

rates in another period of life. *(iv)* Models with repeat measures of individuals should include individual ID as a random effect. An effort to control for other expected co-dependences through time and space should be made where ever possible (e.g. a random effect of year if fitness varies considerably with year). *(v)* Metrics of environmental quality should be chosen with care, and ideally, a metric should be demonstrated to scale linearly with fitness before being used.

We hope that publication of additional studies, and in particular, across other taxa, will further clarify our understanding of the long-term effects of developmental environment on late-life performance.

Author contributions

E.C. designed the study, conducted the literature search, performed statistical analyses, and wrote the first draft of the manuscript. L.K. conceived of the project and contributed in revising the manuscript.

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Chapter 1 Supplementary Material

Online supplementary files available at: https://doi.org/10.1002/evl3.79

Table S1: Eggers regression models for the subset of effect estimates from the survival senescence model, the reproductive senescence model, and all published effect sizes (reproductive and survival) combined.

Effect estimates	*K*	*t*	*d.f.*	*p*
Survival	18	0.579	16	0.571
Reproductive	30	1.311	28	0.200
Published	**23**	**2.187**	**21**	**0.040**

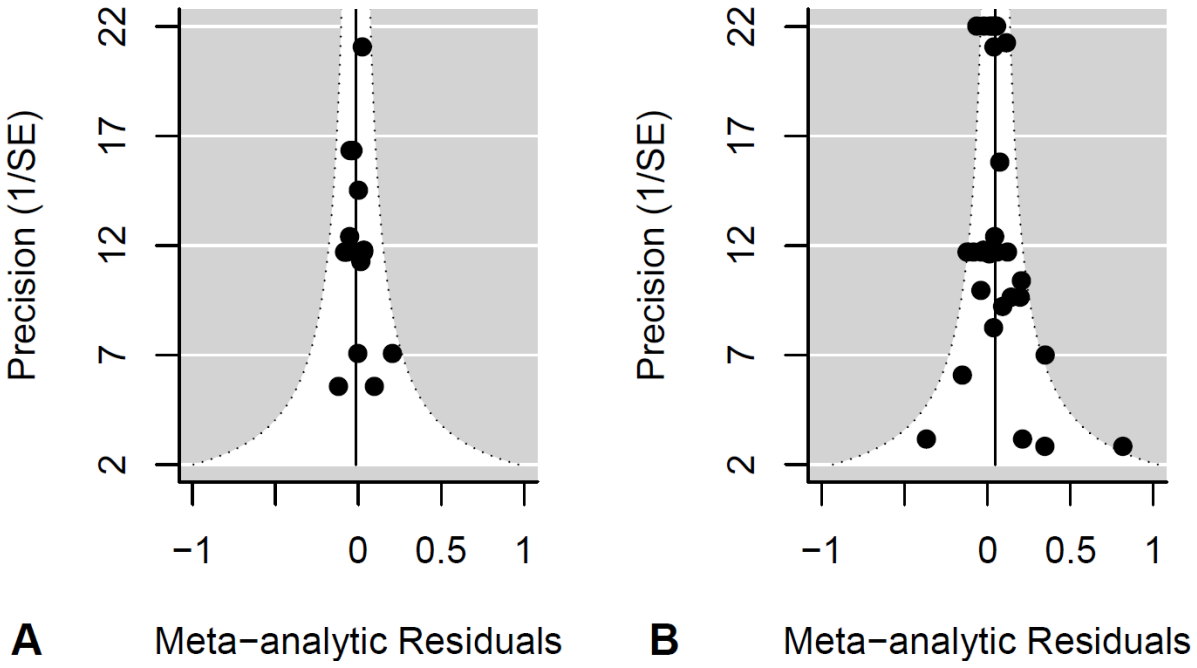

Figure S1. Meta-analytic residuals (A) and meta-analytic residuals (B).

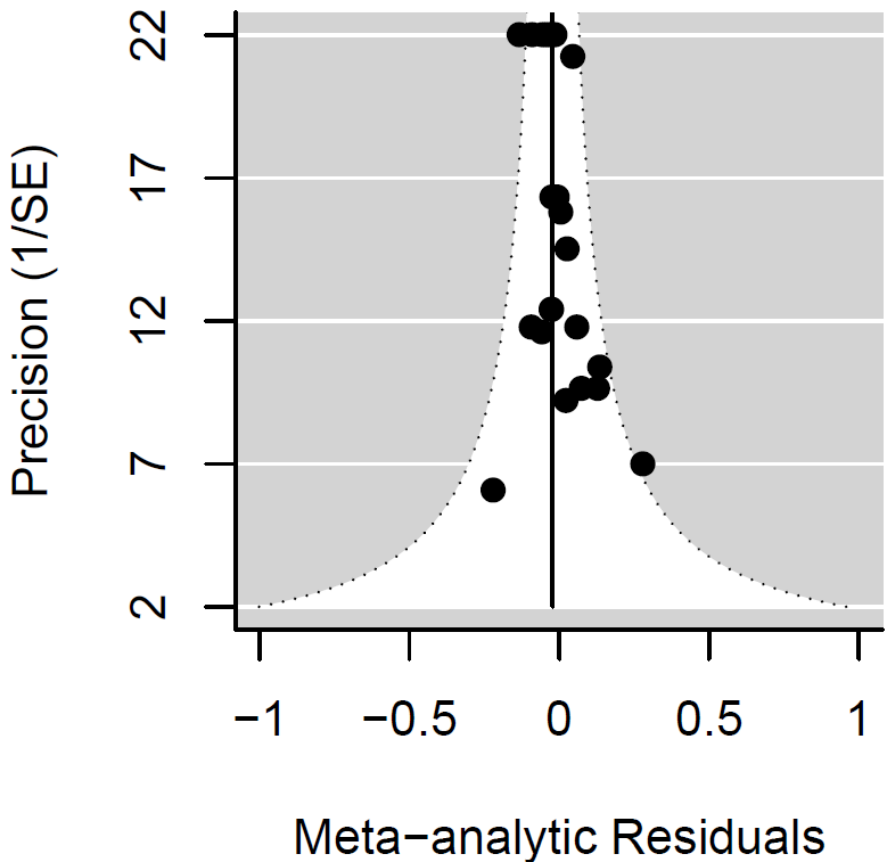

Figure S2. Meta-analytic residuals

Appendix S1

Literature Search

All parts of the literature search were conducted using *Web of Science* between May and September 2017. We included results from all years to present, and included the databases: Science Citation Index Expanded (SCI-EXPANDED; 1945-present), Social Sciences Citation Index (SSCI; 1956-present), Arts & Humanities Citation Index (A&HCI; 1975-present), Conference Proceedings Citation Index- Science (CPCI-S; 1990-present), Conference Proceedings Citation Index- Social Science & Humanities (CPCI-SSH; 1990-present), Current Chemical Reactions (CCR-EXPANDED; 1985-present, *Includes Institut National de la Propriete Industrielle structure data back to 1840*), and Index Chemicus (IC; 1993-present).

With these databases, we first conducted a key-word search with the terms: Topic = (senescence OR ageing OR aging) *AND* Topic ("early environment*" OR "early*life" OR "natal environment*" OR "silver

spoon" OR "predictive adaptive response" OR "cohort effect*"). Following the search protocol of the senescence review paper by Lemaître et al. (2015), we then conducted forward searches on six 'classic' papers on the evolution of ageing:

Hamilton, W.D. (1966). The moulding of senescence by natural selection. *J. Theor. Biol.,* 12, 12–45.

Kirkwood, T.B. (1977). Evolution of ageing. *Nature,* 270, 301–304.

Kirkwood, T.B & Holliday, R. (1979). The evolution of ageing and longevity. *Proc. R. Soc. Lond. B.,* 205, 531–546.

Kirkwood, T.B. & Rose, M.R. (1991). Evolution of senescence: late survival sacrificed for reproduction. *Phil. Trans. R. Soc. Lond. B.,* 332, 15–24.

Kirkwood, T.B. & Austad, S.N. (2000). Why do we age? *Nature,* 408, 233–238.

Williams, G.C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution,* 11, 398–411.

We also conducted backwards and forwards searches on the following five recent or influential review papers related to senescence or the influence of early life on subsequent life-history:

Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends Ecol. Evol.,* 14, 343–348.

Lemaître, J-F., Berger, V., Bonenfant, C., Douhard, M., Gamelon, M., Plard, F., Gaillard, J-M. (2015). Early-late life trade-offs and the evolution of ageing in the wild. *Proc. R. Soc. B.,* 282: 20150209.

Lemaître J-F. & Gaillard, J-M. (2017). Reproductive senescence: new perspectives in the wild. *Biol. Rev.,* 92, 2182-2199.

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Phil. Trans. R. Soc. B.,* 363, 1635-1645.

Nussey, D.H., Froy, H., Lemaître, J-F., Gaillard, J-M., Austad, S.N. (2013). Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. *Ageing Res. Rev.,* 12, 214-225.

In total, the above searches yielded 6766 unique results (see endnote library). Titles, abstracts, and, where necessary, paper bodies were reviewed by E.C. to identify relevant papers under the inclusion criteria (see below). By this method, 10 papers containing the required analysis were found. Two of these papers did not report the necessary statistics on some non-significant results, which were needed to calculate correlation coefficients. In both these cases, we contacted authors directly and they were

able to provide us the neccessary information. Through the aide of Raquel Baos, the unpublished statistics required for white storks (*Ciconia ciconia*) was acquired. Dan Nussey provided statistics necessary to calculate the effect of density on survival senescence rates in red deer (*Cervus elaphus*).

For additional papers we found that demonstrated the availability of data necessary to complete the required analysis, but did not present results in such a way that they met our parameters, we contacted the primary authors to enquire about the relevant analyses for their study system. From this, we obtained additional analyses that were previously unpublished. Analyses were provided by Alexandre Millon (tawny owls; *Strix aluco*), Harry Marshall (banded mongooses; *Mungos mungo*), Gabriel Pigeon (bighorn sheep; *Ovis canadensis*), Anni Hämäläinen and Jessica Haines (red squirrels; *Tamiasciurus hudsonicus*). Additionally, Adam Hayward provided the raw data used in analysis from a 2015 paper on Soay sheep (*Ovis aries*; Hayward *et al.*, 2015, *Exp. Gerontol.,* 71, 56-68). From this raw data, E.C. calculated the 2 survival and 1 reproductive senescence rate effect sizes included for this species.

Chapter 2

Ageing and senescence across reproductive traits and survival in superb fairy-wrens (*Malurus cyaneus*)

Cooper, E.B., Bonnet, T., Osmond, H., Cockburn, A. & L.E.B. Kruuk. *American Naturalist* (in press).

Abstract

Why do senescence rates of fitness-related traits often vary dramatically? By considering the full ageing trajectories of multiple traits we can better understand how a species' life-history shapes the evolution of senescence within a population. Here, we examined age-related changes in sex-specific survival, reproduction, and several components of reproduction using a long-term study of a cooperatively-breeding songbird, the superb fairy-wren (*Malurus cyaneus*). We compared ageing patterns between traits by estimating standardized rates of maturation, the age of onset of senescence, and rates of senescence, while controlling for confounding factors reflecting individual variability in life-history. We found striking differences in ageing and senescence patterns between survival and reproduction, as well as between reproductive traits. In both sexes, survival started to decline from maturity onwards. In contrast, all reproductive traits showed improvements into early adulthood, and many showed little or no evidence of senescence. In females, despite senescence in clutch size, number of offspring surviving to independence did not decline in late life, possibly due to improvements in maternal care with age. Superb fairy-wrens have exceptionally high levels of extra-group paternity, and while male within-group reproductive success did not change with age, extra-group reproductive success showed a dramatic increase in early ages, followed by a senescent decline, suggesting that male reproductive ageing is driven by sexual selection. We discuss how the superb fairy-wrens' complex life history may contribute to the disparate ageing patterns across different traits.

Introduction

The ageing pattern of any fitness-related trait is often characterized by both a period of early-adulthood improvement (maturation), and a period of later-life deterioration (senescence) (Baudisch 2011; Jones et al. 2014). The diversity of ageing patterns observed between species and between individuals within species has motivated a wealth of theoretical work describing how ageing should evolve under the framework of life-history theory (see Shefferson et al. 2017; Gaillard and Lemaître 2020 for recent reivews). There is now increasing evidence that reproductive or survival-related traits do not age synchronously even within an individual (Froy et al. 2013; Hayward et al. 2013, 2015; Zhang et al. 2015; Piper et al. 2017). Despite great progress in the evolutionary understanding of ageing, in practice it is still not possible to accurately predict how specific traits will age relative to other traits in any given species (Brunet-Rossinni and Austad 2006; Jones et al. 2014; Bouwhuis and Vedder 2017; Lemaître and Gaillard 2017). For example, in likely the most taxonomically rigorous study to date, which included 45 animal, plant, and algae species, Jones et al. (2014) were unable to identify any discernable association between the shape of a species' survival curve or rate of survival senescence and the shape or rate of reproductive ageing in that species. The lack of discernable patterns reflects the complexity of selection pressures and life-history characteristics that are likely acting simultaneously to produce the diversity of ageing patterns observed in natural populations.

In general, traits with a greater contribution to fitness should be expected to senesce slower or later than traits with lower implications for survival or reproduction (Lemaître and Gaillard 2017; Cohen et al. 2020). However, empirical evidence suggests that relative fitness costs are not able to fully explain differences in onset and rate of senescence (Brunet-Rossinni and Austad 2006; Bouwhuis and Vedder 2017). Age dependent trade-offs (Lemaître and Gaillard 2017), constraints (Cohen et al. 2020), indirect genetic effects (Moorad and Nussey 2016), as well as the relative strength of sexual selection in a population (Bonduriansky et al. 2008; Hooper et al. 2018), may all explain some of these inconsistencies and differences in trait-specific ageing. However, to date, there is little information about how these different factors work together to produce asynchronous ageing of fitness-related traits in ecologically realistic conditions. Useful insights can be provided by studies of ageing in long-term monitoring of wild populations and by focusing on how a suite of fitness-related traits change with age, instead of only describing changes in reproduction or survival (Brunet-Rossinni and Austad 2006; Lemaître and Gaillard 2017; Gaillard and Lemaître 2020). Investigating how a suite of traits, rather than only reproduction or

survival, change with age can thus help to elucidate how life-history modulates patterns of ageing (Nussey et al. 2009; Hayward et al. 2013, 2015).

Theoretical models predict that physiological fertility and other reproductive traits, such as maternal care, are expected to age in varied asynchronous ways, depending on life-history characteristics (Pavard et al. 2007; Gillespie et al. 2013; Moorad and Nussey 2016; Barks and Laird 2020). For instance, if the ability to provide maternal care improves with experience, resulting in improved offspring survival with maternal age, the rate of fertility senescence should be reduced through natural selection as there is greater benefit to having offspring later in life when more experience has been accrued (Gillespie et al. 2013; Barks and Laird 2020). By quantifying ageing in both female prenatal traits that are indicative of fertility (e.g. clutch size), and postnatal traits that are indicative of offspring quality (e.g. offspring survival to independence), the relative roles of physiological ageing versus the ageing of maternal care in driving overall reproductive maturation and senescence rates can be empirically tested (Karniski et al. 2018).

Measuring multiple components of reproductive ageing can also clarify the role of sexual selection in shaping overall ageing patterns. In males, ageing in reproductive success could be a result of changes in investment in secondary sexual traits, with implications for female mate choice (Cleasby and Nakagawa 2012), or of changes in paternal effects via parental care (Lemaître and Gaillard 2017; Barks and Laird 2020). Age-related improvements and/or subsequent senescence in male reproductive success may be driven by female choice if females prefer particular male age classes (Kokko 1997; Promislow 2003; Bonduriansky et al. 2008). For example, female preference for older males may be adaptive if age is an honest signal of male quality, or if intersexually selected traits are age-dependent (Akçay and Roughgarden 2007; Hsu et al. 2015). Alternatively, male reproductive ageing could be driven by paternal effects, such as age-related associations with territory quality (Lemaître and Gaillard 2017). In socially monogamous but promiscuous species, in which sometimes males provide only sperm, but other times also share a common territory with young, the relative roles of female choice and paternal effects can be disentangled. We are not aware of any study to date that has explicitly separated age-related changes in extra-pair and within-pair reproductive success in measuring male reproductive ageing.

Measuring sex-specific survival rates can also be insightful in determining the impact of life-history on ageing, especially when the sexes experience considerably different sexual selection pressures (Bonduriansky et al. 2008). Increased promiscuity and more pronounced secondary sexual traits have both been linked with earlier onset or faster senescence in males relative to females across species

(Clutton-Brock and Isvaran 2007; Tidière et al. 2015) . Although note that Lemaitre and Gaillard (2013) did not find any evidence that variation in male allocation to copulatory traits explained variation in male senescence rates in herbivore species. To date, most of the research on the role of sexual selection in sex-biased ageing has focused on mammals (see Tidière et al. 2015 for a review), and only one cross-species review to date has included birds (Clutton-Brock and Isvaran 2007). Further, the bird studies included in this review are of species that are predominantly monogamous and without male-biased investment in dramatic secondary sexual traits. As a result, little inference can be made about the general effect of sexual selection on male senescence in birds. The paucity of empirical data on ageing via sexual selection in birds (relative to mammals) is especially important because, in mammals, much of the sexual selection is mediated via intrasexual competition, favoring evolution of secondary sexual traits used in duels between males (Cassini 2020). Thus, it is not clear if the negative effects of sexual selection on male senescence observed in mammals are broadly applicable to instances of intersexual competition, where selection on male secondary sexual traits is driven by female choice rather than male-male competition.

Since ageing trajectories in a species will depend on the ecological context, an understanding of the ultimate and proximate mechanisms of ageing requires analyses of wild populations. There are several practical constraints that make this sort of analysis challenging. It requires that individuals are accurately aged, and (ideally) tracked until their death. In order to adequately investigate patterns occurring in the oldest age classes, where sample sizes will be heavily restricted by mortality, the overall sample size must be substantial, and may take decades to collect (Nussey et al. 2008). Additionally, factors influencing the expression of each trait which are not a direct consequence of ageing, including heterogeneity between individuals, in the environment, and in individual life-history should be controlled for in order to accurately assess the specific effect of age on trait expression (van de Pol and Verhulst 2006).

In addition to the practical constraints, quantifying ageing also raises several statistical and philosophical challenges. Ageing trajectories may not follow simple parametric functions and so ideally need to be modelled without constricting the effect of age to a particular functional shape. Further, in order to make quantitative comparisons of ageing between traits, it is necessary to define which features of the ageing function to quantify. In this study we therefore took a two-stage approach of (a) modelling the ageing trajectory across the lifespan of each trait non-parametrically using generalized additive mixed-models (GAMMs) (Wood 2017) and then (b) using standardized estimates from these non-parametric

models, we ran a break-point analysis to generate measures of (i) rates of early-adulthood improvement ('maturation'), (ii) age of onset of declines in late life and (iii) rates of these declines ('senescence') for each trait. This two-step approach to investigating ageing has the potential to be applied across other traits and species. Standardized methods for investigating trait-specific ageing across species are key to improving our understanding of how ageing patterns evolve. For this reason, we provide an annotated code detailing our methodology in the hope of encouraging a standardized approach to ageing research across other wild populations (appendix 1).

In this study, we investigate trait-specific ageing in a wild population of superb fairy-wrens (*Malurus cyaneus;* hereafter 'fairy-wren'*)*, a small passerine species endemic to south-eastern Australia. The fairy-wren offers an excellent system to investigate how sexual selection and life-history may impact survival and reproductive ageing. A 30-year longitudinal study of a wild population of fairy-wrens provides a large sample of individuals with extremely detailed data on their reproductive histories and timing of death (Cockburn et al. 2016). With this dataset, we were able to quantify survival, as well as a suite of inter-related components of reproduction, and overall reproductive success, separately for each sex. By measuring age-related changes in survival in males and females separately, we sought to quantify how differences in the sexual selection pressures each sex experiences impacts their ageing. Fairy-wrens are highly sexually dimorphic: males display molt into bright blue breeding plumage in advance of each breeding season, while females remain brown throughout the year. Plumage brightness heightens predation risk across a range of bird species (Huhta et al. 2003). In the fairy-wrens specifically, males increase their vigilance behavior when in blue plumage, which suggests they may be under greater predation risk (McQueen et al. 2017). Additionally, the increased testosterone associated with molting is immunosuppressive in fairy-wrens, possibly further impacting their survival (Peters 2000). The blue molt is driven by intersexual selection, with females strongly preferring males that molt earlier in the year (Dunn and Cockburn 1999). If the pattern observed across mammals – that intrasexual selection results in more rapid survival senescence in males – is broadly applicable to intersexual selection as well, then we predict more rapid or an earlier onset of survival senescence in male fairy-wrens, relative to females. In order to investigate whether female reproductive ageing in fairy-wrens is driven by shifts in the quantity of offspring produced (i.e. prenatal traits), or changes in the 'quality' (i.e. postnatal traits), we measured ageing in clutch size and breeding start date (prenatal traits), in addition to measuring number of offspring that survive to independence (encompassing the effects of both prenatal traits, and postnatal maternal care-related traits). The fairy-wren breeding season can last up to six months, but

the date of starting breeding can vary widely between females in a given season (Cockburn et al. 2016; Lv et al. 2019). Females can successfully raise up to three clutches in a breeding season, and the number of clutches they do raise will be impacted by how early they begin their season: earlier start dates are associated with higher numbers of young produced in a season (Lv et al. 2019). Thus, both clutch size and breeding start date are prenatal traits that will impact the quantity of reproductive output for females. If female reproductive ageing is driven by changes in the quantity of offspring, we expect to see the rates of age-related changes in breeding start date and clutch size to match the rates of overall reproductive ageing (measured through offspring surviving to independence) in females. Conversely, if overall reproductive ageing is asynchronous with these traits, this would suggest that age-related changes in maternal effects related to 'quality' (notably, maternal care) rather than quantity of offspring are driving reproductive ageing (Moorad and Nussey 2016; Barks and Laird 2020).

In males, we measured overall reproductive ageing using the total number of nestlings produced annually. In addition, we analyzed ageing of extra-group and within-group reproductive success separately, and age-related changes in the dates on which males molted into their blue breeding plumage each year. There are two different pathways to reproductive success for males in this socially monogamous, group living social structure - either through within-group or extra-group mating (Hajduk et al. 2018). While within-group reproductive success is impacted by paternal effects (e.g. shared environment), extra-group reproductive success is driven by female choice (Double and Cockburn 2000). If female mate choice is strongly determined by a male's age, we expect to see the age-related changes in overall male reproductive success match that of extra-group success. Conversely, if other paternal effects change with age more strongly, we would expect to see the age-related changes in overall reproductive success match that of the within-group success. Lastly, measuring ageing of the sexually-selected trait of molt date will demonstrate if and how sexual selection is age-dependent in this population. The level of similarity between ageing patterns in extra-group success and in molt date will indicate the extent to which female choice is based on molt date.

Methods

Species and Study Site

The population of superb fairy-wrens located in and around the Australian National Botanic Gardens, Canberra, Australia (35°16 S, 149°06 E) has been intensively monitored since 1988 (Cockburn et al.

2003). The study site, approximately 60 ha in area, contains 40-90 territories encompassing between 120-230 year-round resident adults. In this study, we used data from the years 1988-2017.

Fairy-wrens are cooperative breeders. Year-round territories are occupied by a single breeding female, a dominant male with which she maintains a social bond, and between zero and five subordinate males. These subordinate males, hereafter referred to as 'helpers', aid the breeding female and dominant male in provisioning of young and in defence of the territory (Dunn and Cockburn 1996). About half of the helpers (47%) are the sons of the female on the territory (Hajduk et al. 2018). The other half of helpers are unrelated to the female on the territory, typically as a result of their mother having died and a new female moving into the territory where they reside (Cockburn et al. 2003). Male dominance ranking on a territory is determined entirely by age. When the dominant male on a territory dies, the oldest helper male residing on the territory takes the dominant position (Cockburn et al. 2008b).

Although the female on a territory is pair-bonded to the dominant male, there is an extremely high rate of extra-pair mating. Only 39% of offspring are sired by the dominant male, with the rest being sired either by the helper males on the same territory or, much more commonly (95% of the time), by males on other territories (Mulder et al. 1994; Hajduk et al. 2018).

Females typically breed every year starting at the age of one, and both helper and dominant males can sire offspring from age one as well (Cockburn et al. 2003). The breeding season typically lasts from September to February. Throughout a breeding season, a female can successfully raise up to three broods, with broods containing an average of three young (Cockburn et al. 2016). However, due to high rates of nest predation, as many as eight clutches may be initiated in a season (Cockburn et al. 2016).

Shortly after hatching (or, for immigrants into the study area, shortly after their arrival), individuals are colour-banded, and a blood sample taken to assign parentage using SNP genotyping (van de Pol et al. 2020). Many components of life-history are continuously tracked for each individual. Nestling trajectories, such as the day of hatching and fledging are usually known within one day. Dates of mortality, immigration, emigration, changes in group composition or territory borders, and male molt from the brown winter plumage to blue breeding plumage are known within an accuracy of one week or shorter. Emigration from the study area typically only occurs when juvenile females disperse from their natal territories. Males are extremely philopatric, with 72% of adult males remaining on their natal territory their entire life (Cockburn et al. 2008*b*). Males that do disperse move to an immediately neighbouring territory 95% of the time (Cockburn et al. 2008*b*), so any male dispersal is easily tracked.

Females disperse from their natal territory and must establish themselves on a new territory as the dominant female for their first breeding season at the age of one. Females remain on their first breeding territory for their entire lives 80% of the time; in the rare cases that they do move subsequently, it is most common for them to move directly to an adjacent territory (Cockburn et al. 2003; Double et al. 2005). Therefore, disappearance of any individual from the study area (failure to be seen in our year-round weekly censuses) can reasonably be assumed to be a death, except in the case of juvenile (aged <1 year) females, which were excluded from the analyses presented here.

The aim of this study was to estimate patterns of ageing across a broad variety of traits in the population. We only included individuals of known birth year and death year in analyses. This excluded any individuals that had not died yet, or that were born outside the study area and had immigrated into the study area at an unknown age in adulthood. The cohort (and hence age) of many immigrant females is known precisely, as immigration in the first year is confined to two narrow periods of the year. The effect of age on each trait was modeled using annual measures taken on individuals from the age of one year old (the age of reproductive maturity), until their death. We analyzed a total of nine traits (survival and reproduction in each sex, as well as 2 additional components of reproduction in females and 3 additional components of reproduction in males). These are outlined below, and sample sizes for all traits are given in table 1.

Survival (Both Sexes)

We analyzed male and female survival separately. Annual survival for each individual was considered from September 1st (the start of the breeding season) to August 31st of the subsequent year. We treated individuals in their first year of breeding as one year olds, although in actuality first year breeders will vary in age from six months to one year due to variation in birthdate. For results to be comparable with the results for reproductive traits, and since death cannot be differentiated from emigration in juvenile (<1 year old) females, survival was only measured from the age of one onwards. For each individual, annual survival was recorded as a binary variable.

Female Reproductive Traits

- *Female independent offspring*. We calculated the annual fecundity of a female as the number of her offspring that reached independence in a given year, calculated for every year that a female was alive (from age one onwards). Across the entire study period, the earliest a chick has been known to reach independence and disperse from their natal territory is five weeks after fledging and so we used the cut-

off of four weeks after fledging as a measure of offspring survival to potential independence. If females died before the end of the breeding season, their incomplete record for that season was not included in the analysis.

- *Breeding start date*. For every year that a female was alive, the Julian day on which she began incubating her first clutch of the breeding season was determined (number of days counted from January 1st). In order to control for effects of annual weather and food conditions (Lv et al. 2019), the median population-level breeding start date for that given year was subtracted from each breeding start date. Thus, breeding start date was a relative value that compared each breeding female to the population median in that year.

- *Clutch size*. The number of eggs a female produced in a clutch had a mode of 3, and a range from 1 to 5. Within our dataset, females produced between 1 and 6 clutches within a breeding season. Females start incubation on the day they lay the last egg of their clutches. All clutches were included in analyses except those that were abandoned or depredated before incubation started.

Male Reproductive Traits

- *Total offspring* was measured as the total number of offspring a male sired each year. Since paternity could only be determined in chicks that reached the age at which a blood sample was taken (Cockburn *et al.*, 2020), paternity of chicks that did not survive until bleeding is unknown (23% of chicks, almost entirely due to predation; Cockburn unpublished data). Individual partial breeding seasons, where the male died at any point before the end of the season were excluded from the analysis. Total offspring is the sum of within-group and extra-group success (see below). Note that our count of male reproductive success does not include the component of survival to independence as for females, because males provide no parental care to the majority of their offspring (i.e. those sired extra-group).

- W*ithin-group offspring* was measured as the number of offspring a male sired each year via the female with whom he shared a territory (i.e. via his social partner for dominant males, via the dominant female on the territory for helpers).

- *Extra-group offspring* was measured as the number of offspring a male sired via females residing outside of his own territory.

- *Molt Date* refers to the day on which a male has completely shed his brown plumage and grown the nuptial blue plumage. There is great variation between individuals in the timing of this molt, from as

early as eight months before to as late as three months after the start of the breeding season (Cockburn et al. 2008*a*). Males that molt earlier have higher siring success in that year's breeding season (Dunn and Cockburn 1999; Cockburn et al. 2008*a*). The date that each male completes molt is tracked via regular censuses of the population, to an accuracy within a week. We quantified molt date as the number of days after the median breeding start date of the females in the given year, so that negative values indicate molting before the start of the breeding season and signify a higher 'quality' of the individual.

Statistical Analysis

We had two aims. First, for each of the above described traits, we modelled the patterns of age-related change while controlling for individual and annual heterogeneity, possible selective disappearance, differences in social status, and other extrinsic factors that may influence the effect of age on each trait. Second, using standardized trait measures, we sought to compare rates of maturation (age-related increases), onset of senescence, and rates of senescence (age-related declines) amongst traits. In the appendix, we provide code which can be used to conduct this two-step analysis, which we hope will be broadly applicable to measuring ageing in any phenotypic trait in a wild population of animals (appendix 1). Additionally, we provide lifetables detailing basic metrics of reproduction and survival at each age for males and females as a point of comparison to our modelled results (supplementary section S1).

Characterizing Trait-specific Ageing: Generalized Additive Mixed Models

Ageing trajectories may not follow simple parametric functions and so ideally need to be modelled without constricting the effect of age to a particular functional shape. For this reason, we used nonparametric smoothing functions, implemented through generalized additive mixed models (GAMMs), to describe patterns of ageing in survival and in each of the reproductive traits (Wood 2017). GAMMs were fit using the *mgcv* package in R version 3.5.0 (R Core Team 2018; Wood 2017). GAMMs are an extension of generalized linear mixed models (GLMMs), which, in addition to the basic parametric terms of GLMMs, can also include nonparametric terms that are not forced to conform to any particular parametric function (Wood 2017). In our GAMMs, we modeled age, and any other relevant nonparametric terms, using penalized thin-plate regression splines. These splines estimate the relationship between the term and the response variable with a number of penalized additive smoothing functions, determined by REML. The penalized additive value of the number of smoothing functions is the effective number of degrees of freedom for the term. When the effective degrees of

freedom is 1, the relationship is effectively linear (Wood 2017). Thus, using GAMMs allowed us to describe ageing patterns across traits without making any *a priori* assumptions about the overall shape of the trajectory, and in particular about the existence and onset of trait maturation or senescence (Jones et al. 2008; Nussey et al. 2009).

We report a p-value for each non-parametric term from a Wald test, which estimates the probability that the additive value of all the smoothing functions in the spline is 0 (Amodio and Ambrosio 2014). We also report p-values for parametric values in our GAMMs. Due to the high number of terms we tested in this study, we chose the p-value of 0.01 as a conservative threshold against which to assess the strength of evidence in all models.

We ran each GAMM using the appropriate error distribution for the response trait. Female and male survival each followed a Bernoulli distribution (fitted with a logit link function). Female breeding start date was measured as the Julian date of first egg laid, centered by the population-level average Julian date for first egg laid for the given year, resulting in a normal distribution. Clutch size was modeled using a quasi-Poisson distribution (with a log link function) which differs from a Poisson distribution model only in that the dispersion parameter is not fixed at 1, and so a dispersion parameter below 1 is used to model under-dispersed count data. Female independent offspring, male total offspring, male within-pair offspring, and male extra-pair offspring were each modeled using a Poisson distribution (with a log link function). The data in these models were over-dispersed, and so we included an observation-level random effect to model the extra-Poisson variation present in the data (Harrison 2014). In order to include an observation-level random effect in these models, we used gamm4, an extension of the mgcv package which has improved performance when fitting GAMMs with a large number of random effect levels (Wood 2017). The data in each model were also zero-inflated. In order to confirm that these four Poisson models reasonably fit our data, despite the zero-inflation, we ran 1000 simulations of each GAMM model and plotted the predicted variance against the raw data variance for each age (supplementary section S2). In addition, we also modelled female independent offspring and male total offspring using Poisson zero-inflated model distributions, which allowed further partitioning of reproductive output into two components: i) probability of reproduction (vs not), ii) amount of reproductive success, for each sex (described in further detail in supplementary S3). In brief, zero-inflated models are principally used in the case where there are two 'types' of zero values present in the dataset; in the fairy-wrens this could reflect either a complete failure to reproduce (a definite zero), or, reproducing with a failure of offspring surviving to fledgling (a zero resulting from extrinsic

environmental stochasticity). Male molt date was measured as the number of days after the median population-level breeding start date for the given year that a male entirely completed his molt into blue plumage. This resulted in a normal distribution for molt date. In 7% of cases (152/2116), males did not fully complete their molt into blue plumage, and so molt date was censored at 10 days past the latest date of molt completion on record. In another 1% of cases (24/2116), males kept their blue plumage from the previous season, without ever returning to brown plumage, and we censored these males to a value 10 days earlier than the earliest molt date on record.

All models included individual identity (ID) and the breeding year of measurement (running from September 1st – August 31st; year) as multi-level random effects. These effects controlled for the non-independence of repeated measures on the same individual across years, and for annual variation in the trait mean associated with differences in environmental conditions, respectively.

In the modelling of ageing of reproductive traits, the problem of 'selective disappearance' can arise if covariance exists between individual reproductive fitness and lifespan or age at maturity (van de Pol and Verhulst 2006). For example, if individuals that have higher reproductive performance tend to live longer on average, then in later age classes, as poorer-quality individuals die out, the population-level reproductive performance could appear to be increasing, even if at a within-individual level reproduction does decline at these ages (van de Pol and Verhulst 2006). Fairy-wrens reach sexual maturity and are capable of reproducing in their first breeding season (age one-year-old), but they do vary considerably in lifespan. Previous studies have found evidence for non-linear relationships between lifespan and reproductive traits (Reid et al. 2003; Nussey et al. 2009), and so lifespan was initially added as a nonparametric spline to each model. In these models lifespan generally had an effective degrees of freedom value close to 1 (supplementary section S4). Since it did not alter our results, we ultimately fitted lifespan as a (linear) parametric effect as this allowed for clear interpretation of the magnitude of the effect.

In an individual's final year of life, their reproductive investment may differ sharply from their reproduction in previous years. In some species, the final year of life results in a sharp increase in reproductive performance (referred to as 'terminal investment'; (Weladji et al. 2010; Froy et al. 2013)), while in others, the final year of life is associated with decreased reproduction (Coulson and Fairweather 2001; Reed et al. 2008). We controlled for any terminal effects in all our models of reproductive traits by adding a two-level fixed effect denoting if it was the individual's last year of life.

Clutch size in fairy-wrens tends to vary throughout the breeding season, with smaller clutches at the beginning and end, and the largest clutches in the middle of the breeding season. To control for this temporal variation we added a spline term of Julian date of clutch incubation to the model of clutch size, which took a roughly quadratic form.

All models of male traits included both dominant and helper males. The dominance status of an individual is typically stable within a year but can be transient between years, with helpers moving to dominant breeding positions. In order to compare reproductive ageing and survival between dominants and helpers, these models included a two-level fixed effect denoting an individual's social status (dominant or helper) in the year of observation.

Standardized rates of maturation and senescence

In a second stage of analysis, we used the estimates produced from the GAMM modelling of each trait to assess standardized rates of maturation, onset of senescence, and rates of senescence for each trait using simple linear regression. For each GAMM, we extracted the predicted mean estimate and standard error of the age term for each age. For zero-inflated models of reproductive success in either sex, this resulted in two sets of age effect estimates and standard errors, one for the probability of producing any offspring, and the other for the number of offspring. We standardized each estimate in two steps. First, we subtracted the mean predicted value of the effect of age from the estimate, so that all trait values were centered to zero. Second, we divided the centered estimate by the range of values predicted by all terms together in the GAMM to control for the variance explained by the age term relative to other variables in the GAMM. Estimates were increased by one order of magnitude for ease of interpretability. For molt date and breeding start date, the signs of the values were reversed so that an increasing value denoted trait improvement across all traits. This process of standardization allows for direct comparison of ageing patterns between different traits (Schielzeth 2010).

We then ran a simple linear regression for each trait as a function of age, using only these standardized estimates of age. In the regressions, we weighted each data point by the inverse of the standard error for that age predicted by the GAMM. Weighting the data points ensured that estimate precision was accounted for, while at the same time not allowing the much larger sample size at lower ages in the GAMMs to entirely drive the regression prediction.

Since ageing is commonly characterized by early-life trait increases (maturation) and late-life trait declines (senescence) (Jones et al. 2008, 2014), a single linear regression may not be adequate in describing the ageing patterns of most traits. We used the 'Segmented' package in R to assess if more than one regression line better described each trait as a function of age, and to then model any segment(s) of the regression (Muggeo 2008). For each regression, we used a Davies test to assess if the trait would be better described by two linear regressions of age, rather than one (Davies 1987, 2002; Cheng 2017). The null hypothesis of the Davies test is that there is no breakpoint at any age in the regression where the slopes delineated by that breakpoint would be significantly different from each other (Davies 2002). Thus, a significant Davies test result indicates that the relationship between age and the trait is better represented by two linear regressions rather than one. As with all previous analyses in this study, we used a p-value threshold of 0.01 to assess significance. For models that did not have a significant breakpoint according to the Davies test, we used the original simple linear regression to describe the ageing pattern of that trait. For models with a significant breakpoint, we used the 'segmented' function in R to create two regressions of the trait of interest as a function of age. This method implements a bootstrap restarting algorithm described in Wood (2001) to assess the breakpoint of the model and then estimates the two resulting linear regressions. Since there can be a period of plateau between trait maturity and trait senescence, we also tested for a second break point in segmented models. In models with a second significant Davies test, two breakpoints were modeled. As a result, traits were represented by either a single continuous linear regression with one slope representing the entirety of adult life, a segmented linear regression with two slopes representing early-adulthood and late-life, or a segmented linear regression with three slopes representing early-adulthood, mid-life and late-life.

Results

Mean adult lifespan (excluding individuals that died before maturity) was 3.44 years (± 2.22 SD, 979 individuals) for males and 2.97 years (± 1.73 SD, 751 individuals) for females. Maximum adult lifespan observed in the study was 12 years in males and 10 years in females, although only 19% of males and 13% of females survived to age 5 (sample sizes for each age in figure 1A,B). There was substantial variation between traits in their changes across the lifespan, described further below. Detailed results for each GAMM, including R^2 values, relevant test statistics, and the variance explained by each random effect is available in the supplementary material (section S5).

Table 1 Spline and parametric effects describing sex-specific ageing in survival and reproduction-related traits in superb fairy-wrens using generalized additive mixed-models (GAMMs). Terms where $p < 0.01$ are in bold. Random effects of individual and year were included in each model. An observation-level random effect was included in female fecundity and male success models to account for over dispersion. Full details of each model, including the relevant test statistics, the reference degrees of freedom for each spline term, the deviance and adjusted R^2 values, and the variance and number of levels for each random effect, are given in supplementary material S4.

Trait	Model Struc.	Sample size	Spline terms EDF		Parametric terms (SE)			
			Age	Incubation	Intercept	Longevity	Status [dom]	Terminal effect
Female survival	Bernoulli	1896 (751)	**1.000**	-	**59.870 (12.960)**	-	-	-
Male survival	Bernoulli	2747 (979)	**1.001**	-	**45.983 (12.419)**	-	-0.191 (0.103)	-
Female breeding start	Gaussian	1089 (678)	**5.555**	-	**12.152 (1.414)**	-0.477 (0.353)	-	-2.714 (1.367)
Female clutch size	Quasi-Poisson	3865 (771)	**7.828**	**5.336**	**1.148 (0.014)**	0.003 (0.003)	-	0.005 (0.007)
Female independent offspring	Poisson	1372 (606)	**4.540**	-	**0.374 (0.085)**	0.026 (0.016)	-	-0.129 (0.188)
Male molt date	Gaussian	2116 (1024)	**5.709**	-	**-42.588 (3.222)**	0.777 (0.569)	**-4.193 (1.588)**	-0.856 (1.934)
Male extra-group offspring	Poisson	2310 (780)	**3.731**	-	**-6.303 (0.926)**	0.061 (0.051)	0.025 (0.170)	0.191 (0.619)
Male within-group offspring	Poisson	2310 (780)	2.019	-	**-6.281 (0.770)**	0.052 (0.042)	**2.017 (0.182)**	-0.480 (0.414)
Male total offspring	Poisson	2310 (780)	**3.567**	-	**-5.037 (0.801)**	0.057 (0.034)	**0.861 (0.115)**	-0.110 (0.274)

Note: Sample size is the number of data points included in the model, followed by the number of individuals in brackets. Sample sizes for females are lower than for males due to the biased adult sex-ratio, and sample sizes for some traits (e.g. female breeding start date) are lower than others due to exclusion of missed or uncertain records of specific life-history events. EDF is the effective degrees of freedom for the spline term. Longevity is the number of years an individual lived, and provides a test of selective disappearance. Status is a binary term specifying

whether a male held a dominant [dom] breeding position or a helper position on his home territory in a given year. Terminal effect is a binary term denoting whether or not it was the individual's final breeding season. Note that parameter estimates for Bernoulli models are on a logit scale and for Poisson and Quasi-Poisson models are on a log scale.

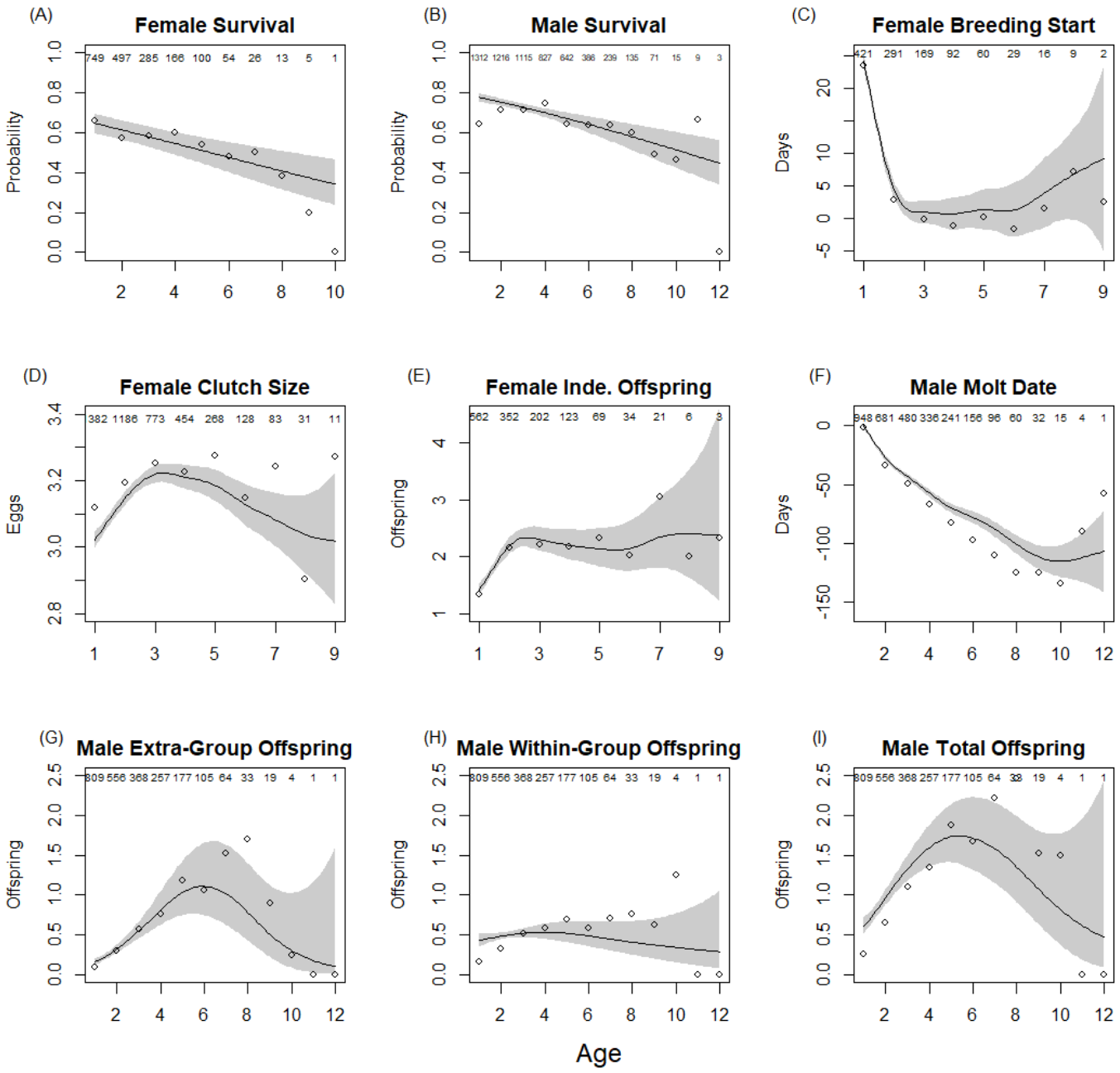

Figure 1 Superb fairy-wren ageing patterns as predicted by GAMMS for: (A) female survival, (B) male survival, (C) breeding start date measured as the day females lay their first clutch relative to the population average date, (D) female clutch size, (E) the number of independent offspring reared for females, (F) molt date, measured as the number of days relative to the median start of female breeding that males complete their molt into breeding plumage, (G) offspring sired outside the territory (extra-group) for males, (H) offspring sired within the territory (within-group) for males, and (I) total offspring sired for males. Shaded area represents ±2 standard errors from the estimate, including the uncertainty

about the overall mean. Number of data points are denoted for each age at the top of each graph. Circles represent the means of raw data values.

Table 2 The estimates for the effect of age on each trait in a linear regression, or a segmented linear regression (when significant breakpoint(s) were found). The data values analyzed in these models were the mean values for each age as predicted by the respective GAMM model (table 2), centered on the mean trait value and divided by the range of trait values (see Methods). Each data point was weighted by the inverse of its standard error. Terms where $p < 0.01$ are in bold

Trait	Across-life slope (99% CI)	Early-adulthood slope (99% CI)	First Breakpoint age (99% CI)	Mid-life slope (99% CI)	Second Breakpoint age (99% CI)	Late-life slope (99% CI)
Female survival	**-0.560 (-0.560, -0.560)**	-	-	-	-	-
Male survival	**-0.511 (-0.511, -0.511)**	-	-	-	-	-
Female breeding start date	-	**2.921 (2.132, 3.710)**	2.2 (2.1, 2.5)	-	-	-0.160 (-0.336, 0.015)
Female clutch size	-	**0.803 (0.513, 1.093)**	3.2 (2.9, 3.6)	-	-	**-0.344 (-0.497, -0.191)**
Female independent offspring	-	**5.046 (2.675, 7.418)**	2.0 (1.7, 2.3)	-	-	0.081 (-0.380, 0.542)
Male molt date	**-**	**1.395 (1.033, 1.756)**	2.4 (2.0, 2.8)	**0.567 (0.504, 0.630)**	9.5 (8.9, 10.1)	-0.208 (-0.651, 0.235)
Male extra-group offspring	-	**1.760 (1.072, 2.447)**	5.7 (5.0, 6.3)	-	-	**-1.501 (-1.967, -1.035)**
Male within-group offspring	-	**0.214 (0.140, 0.288)**	4.3 (4.0, 4.7)	-	-	**-0.259 (-0.287, -0.232)**
Male total offspring	-	**0.981 (0.552, 1.410)**	5.4 (4.7, 6.1)	-	-	**-0.831 (-1.109, -0.554)**

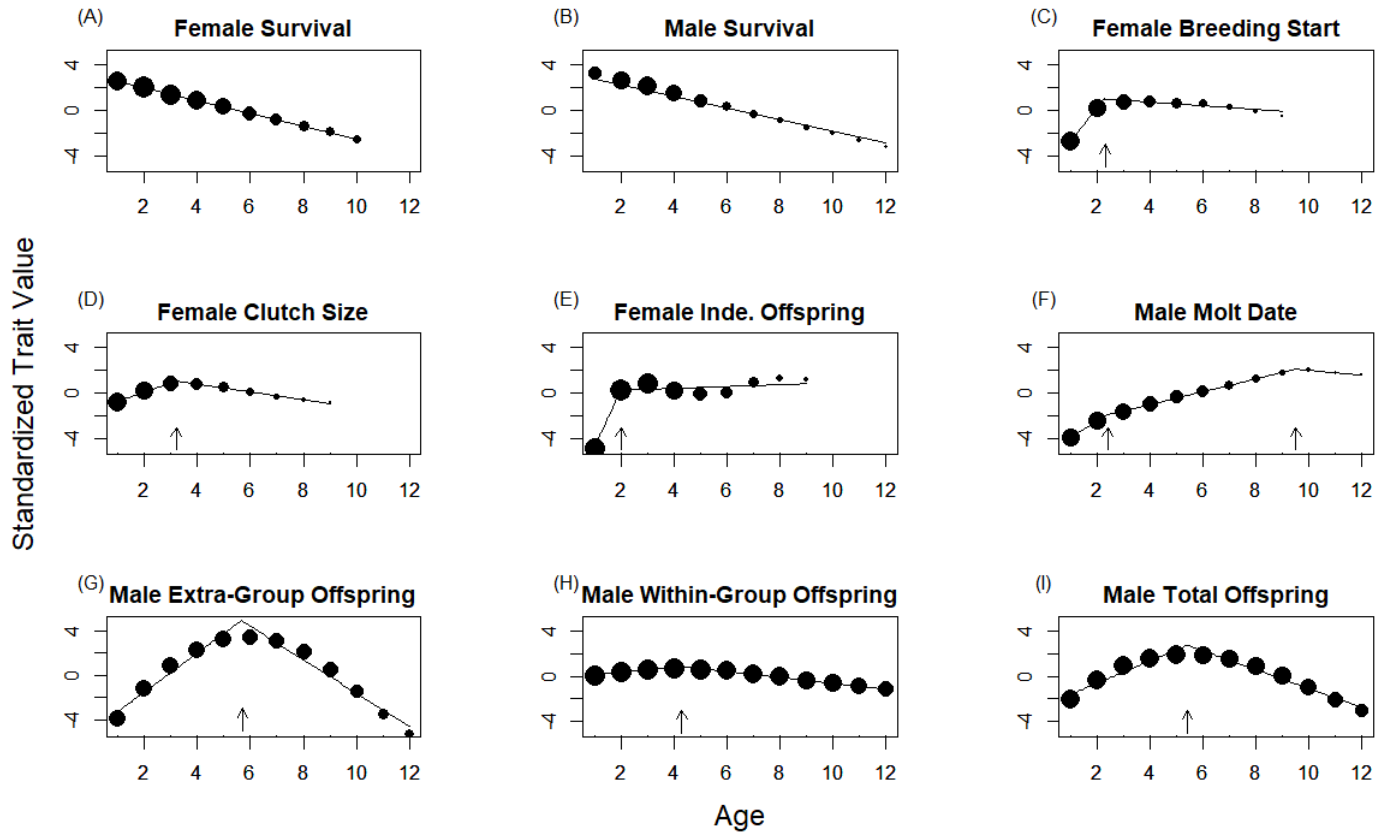

Figure 2 Standardized ageing patterns, with segmented or continuous linear regressions estimating the effect of age on: (A) female survival, (B) male survival, (C) breeding start date measured as the day females lay their first clutch relative to the population average date, (D) female clutch size, (E) the number of independent offspring reared for females, (F) molt date, measured as the number of days relative to the median start of female breeding that males complete their molt into breeding plumage, (G) offspring sired outside the territory (extra-group) for males, (H) offspring sired within the territory (within-group) for males, and (I) total offspring sired for males. Filled circles represent the raw data, which are the estimates for each age predicted from the corresponding GAMM and z-transformed to allow direct comparisons between traits. The size of the circle represents the inverse of the standard error associated with the GAMM estimate. Regressions are weighted by these inverse standard errors. Regressions which are continuous did not have a significant break point (A and B) and the regression with two break points (C) had a second significant break point ($p < 0.01$). Arrows denote locations of break points. Note that female breeding start date and male molt date have been multiplied by -1 for consistency with the other traits, so that higher values indicate earlier dates.

Survival

Both female and male survival appeared to decline linearly in the GAMMs (figure 1A,B), and as such there was no support for any breakpoint in the models of female or male survival, with survival declining across adulthood in both sexes (figure 2A,B; table 2). The rate of decline, based on the linear models, was similar in males and females (male slope: -0.51, female slope: -0.56; table 2). However, the male survival GAMM had a greater intercept than that of the female model (male intercept: 0.73 ± 0.10; female intercept: 0.60 ± 0.10) and in a *post-hoc* analysis, a GLMM of the effect of sex on annual survival probability (sample size 4792 individual years, random effects of year and individual) indicated that, overall, males had a higher chance of survival than females (log-odds ratio = 0.29 ± 0.06), despite the similar rates of decline. There was a non-significant negative effect of being a dominant male (compared to a helper) on male annual survival (table 1; p = 0.06).

Female Reproductive Traits

The three female reproductive traits – independent offspring, breeding start date, and clutch size – all had periods of trait maturation that ended around the age of two or three years old (table 2). Of the three female reproductive traits, only one, clutch size, showed evidence of senescence after the initial period of trait improvement (table 2).

In the zero-inflated model of independent offspring (supplementary section S3), while the probability of any independent offspring showed a non-significant positive trend across the total female lifespan (slope: 0.320; CI: -0.040, 0.681), the (non-zero-inflated) total number of offspring showed early-life improvements (slope: 1.370; CI: 1.061, 1.678), and late-life senescence beyond age 6.6 (slope: -0.523; CI: -0.885, -0.161).

In each GAMM of the reproductive traits, we included longevity to test for any selective disappearance and a two-level factor of final year of breeding to test for any terminal effects. There was no support for either an effect of longevity or terminal effects in any model of female reproductive performance (table 1).

Male Reproductive Traits

Male reproductive traits exhibited periods of trait maturation, but the duration of maturation varied between traits. We found evidence of senescence for all male traits with the exception of molt date (table 2). Extra-group success reached its peak about 1.5 years later than within-group success (table 2; breakpoint of 4.3 vs. 5.7). Extra-group success demonstrated a rate of maturation approximately 8 times greater, and a rate of senescence approximately 6 times greater, than the age-related changes for

within-group success (table 2). As expected, male total success had a breakpoint and ageing rates that roughly averaged the values of the within-group and extra-group estimates, with a slight bias towards the estimates of the extra-group (figure 2), since 61% of paternity is achieved extra-group (Hajduk et al. 2018). Molt date was the only trait to have two breakpoints, with a steep rate of maturation up until age 2.4, followed by a relatively shallower rate of improvement to age 9.5, after which there was no change with age (table 2; figure 2).

There was no effect of social status (dominant or helper position) on extra-group success, but dominant males did have substantially higher within-group success, which resulted in a positive effect of dominance on total offspring (table 1). Dominant males also had an earlier molt date (table 1). Similarly to females, there was no support for either an effect of longevity or terminal effects in any model of reproductive performance for males (table 1).

Discussion

We investigated fairy-wren ageing patterns by measuring annual survival and reproductive success in each sex, as well as two components of reproductive success for females (clutch size and breeding start date), and three for males (extra-group offspring, within-group offspring, and molt date). The ageing of these traits was highly asynchronous, with variable rates and durations of both early-adulthood improvements and late-life declines, some traits not showing any period of maturation in early adulthood, and others not showing any evidence of senescence. Our results add to the growing body of empirical evidence that traits do not senescence synchronously within an individual (Nussey et al. 2009; Froy et al. 2013; Hayward et al. 2013, 2015; Zhang et al. 2015; Piper et al. 2017; Tompkins and Anderson 2018). They also show that senescence is not ubiquitous across all traits. Below we discuss the insights that our results provide for understanding ageing in wild animal populations, and consider how the distinctive ecological and evolutionary forces faced by this species may be driving these patterns.

Survival

Annual survival probability declined from their first year of adult life at similar rates in males and females (table 2). Similar to other small passerines, the days immediately after fledging are the single most dangerous time for both sexes (Cockburn, *unpublished data*). Survival is unlikely to peak until after the attainment of a breeder or helper position at age one, so this age is likely to be an accurate estimate of senescence onset.

Although male display a costly secondary sexual trait (Peters 2000; McQueen et al. 2017), this did not appear to cause a faster rate of survival senescence in males relative to females, and a post-hoc analysis indicated that females had lower annual survival overall compared to males. Our results indicate that the association between strong intrasexual selection and more rapid male senescence in mammals may not be broadly applicable to intersexual selection in birds in relation to dramatic plumage coloration. The life-history factors which mold senescence are complex and can be interacting. Although the annual molt into nuptial plumage may increase mortality risk in males (Peters 2000; McQueen et al. 2017), other aspects of male life-history in fairy-wrens may decrease their mortality, such as cooperative breeding delaying peak reproductive performance (Promislow 2003).

The onset of survival senescence in the first year of breeding is aligned with the classic evolutionary ageing theory prediction that selection on somatic maintenance should decline from the age of sexual maturity (Williams 1957; Hamilton 1966). However, it is contrary to most empirical evidence of survival senescence in iteroparous birds and mammals, which shows that survival senescence is most commonly delayed until some years after the age of sexual maturity (Jones et al. 2008; Péron et al. 2010; Gaillard and Lemaître 2017). One rationale explaining the delayed survival senescence in these other species is that, when the actual possibility of reproductive success occurs some time after the physiological possibility of reproductive success (sexual maturity), there could be strong selective pressure to continue to invest heavily in somatic maintenance beyond sexual maturity, delaying the onset of survival senescence (McElligott et al. 2002; Brunet-Rossinni and Austad 2006). In female fairy-wrens, reproductive success is relatively likely from age one (supplementary table S1), and so the onset of survival senescence at this age makes intuitive sense. In contrast, in male fairy-wrens, young adults are more likely to be helpers as they queue for dominance on their territory by age, which substantially lowers their within-group reproductive success at age one. However, by controlling for dominance status in our male survival model, we have modelled the effect of age on survival independent of any delayed social maturity caused by being a helper. Thus our results represent the effect of age on survival, while controlling for plasticity in senescence that is driven by how social circumstances lead to changes in life history with age. This may be why our results align more closely with the classic ageing theories, which did not take into account life-history trade-offs. It is important to note however that survival rates measured in the wild are a consequence of both extrinsic and intrinsic sources of mortality. As a result, the observed age-related changed in survival may also be influenced by changes in life-history or behaviour which impact extrinsic mortality risk, rather than intrinsic senescence *per se*.

Reproduction

The effects of age on female and male reproductive traits were considerably different from those on survival. Unlike survival, all reproductive traits experienced some period of early-adulthood improvement, and not all traits demonstrated senescence. There were also some notable differences in ageing patterns amongst the reproductive traits.

For females, the number of independent offspring increased dramatically from age one to age two, after which point there was no change with age. Although there was no change in reproductive fitness contribution for females at these later ages, the zero-inflated model revealed that this lack of overall change was a consequence of counteracting changes in the probability and the (non-zero-inflated) total number of independent offspring produced. The probability of producing any independent offspring slowly increased across all ages ($p = 0.017$), whilst the (non-zero-inflated) number of independent offspring increased in early adulthood, before plateauing and demonstrating senescence at late ages (supplementary S3). This decline in total number of independent offspring may be driven in part by the senescence of clutch size, but not breeding start date (table 2; figure 2). The improvement in probability of independent offspring may indicate an improvement in maternal care abilities with age. Disentangling different components of reproduction in female fairy-wrens has illustrated that, underlying a lack of overall reproductive ageing, there can be dynamic and counteracting shifts in traits related to physiological fertility, and post-natal maternal effects.

The early-adulthood improvement and subsequent late-life declines in male total reproductive success appeared to be entirely driven by the dramatic age-related changes in extra-group success (Figure 1G-I, Figure 2G-I). The effects of age on within-group offspring in both the GAMM and standardized regression were much weaker, or non-existent. These results suggest that sexual selection plays an important role in reproductive ageing, specifically intersexual selection, as male extra-group success in fairy-wrens is primarily driven by female mate choice (Double and Cockburn 2000). We believe that it is likely that female precopulatory mate choice specifically, and not other avenues of intersexual selection such as sperm competition or cryptic (postcopulatory) female choice, are driving male reproductive ageing. If these other modes of sexual selection did play a role, we would expect to see similar age-related changes in within-group success. Thus, it appears female preference for middle-aged males is driving both male reproductive improvement in early adulthood, and male reproductive senescence in late life.

In addition to the larger change with age in extra-group reproductive success (compared to within-group success) in males, the extra-group success also reached its peak at later ages when compared with both male within-group success and female independent offspring (1.4 and 3.7 years later respectively; table 2). Only 5% of adult males survive to age six, the point at which they would have reached peak extra-group reproductive success. Selection for an earlier reproductive peak in males is expected to break down when reproductive success is reliant on an age-dependent sexually-selected trait (Bonduriansky et al. 2008), which may explain the peak of male extra-group success relatively later in life seen here. Earlier molt date is a signal of high male quality and a strong predictor of male reproductive success (Dunn and Cockburn 1999; Cockburn et al. 2008*a*). Since molt date steadily improved by shifting earlier with age until very old ages (table 1, figure 1F), this pattern is likely a major contributor to the observed pattern of increase in male extra-group success. To investigate this prediction further, we added molt date as an additional explanatory variable to the GAMM of extra-pair success and compared the rates of maturation in the subsequent segmented regressions to the rates estimated by our model which did not control for molt date. We found that there was still early-life improvement in extra-pair success when controlling for molt, but the rate of maturation was reduced by approximately half (supplementary section S6). This indicates that the improvement in molt date with age does indeed play a considerable role in driving early-life improvements in extra-group success.

That molt date continuously shifts to earlier dates and shows no real evidence of senescence may seem counterintuitive given the evidence in this study of significant survival senescence from the onset of adulthood. Given that the production of costly secondary sexual traits is generally expected to be condition-dependent (Zahavi 1975; Andersson 1986), one might predict that these traits deteriorate as a part of the general somatic deterioration associated with survival senescence. It may be possible to make sense of this phenomenon by viewing it through the lens of life-history theory. With increasing age, expected future fitness (residual reproductive value) declines, resulting in increases in the relative value of current reproduction (Kirkwood and Rose 1991). Thus, increased investment in secondary sexual traits which improve reproductive success within the current breeding season should be increasingly favored over investment in somatic maintenance and future reproduction. Game theory models have predicted that progressive increases in costly secondary sexual trait expression with age are evolutionarily stable in iteroparous organisms (Kokko 1997). Additionally, this theory is supported by empirical evidence as continued improvement in ornamental traits into old age classes, despite senescence in both reproductive success and survival, appears to be a common finding in longitudinal animal studies (Nussey et al. 2009; Evans et al. 2011; Preston et al. 2011; Potti et al. 2013). Similar to

these studies, in the fairy-wrens, extra-pair mating success displays senescence at ages where molt date continues to improve, suggesting that other factors, unrelated to molt date, are also influencing extra-pair success in late-life. Since sperm quality is known to decline with age (Hansen and Price 1995; Radwan 2003; Preston et al. 2011; Velando et al. 2011; Cornwallis et al. 2014), this could be contributing to the expedited senescence of extra-group success in old males.

The considerable variation in reproductive ageing seen in both females and males illustrates the complexity of reproductive ageing, where different reproductive traits can be driven by differing ecological and evolutionary forces resulting in a variety of different ageing patterns.

Individual Variability in Ageing

We controlled for any potential correlation between lifespan and performance by adding a fixed effect of longevity to all of the models of reproductive traits. Longevity was not significant in any model. Thus, there is no evidence that longer-lived individuals have inherently different reproductive investment strategies or face different reproductive constraints. In addition, for most of the fairy-wren traits the random effect of individual identity explained a very small amount of the variance in comparison with, for example, the random effect of year, which in most models explained several orders of magnitude more variance (see details of models in supplementary section S5). This suggests that variation in performance in the population is more driven by extrinsic conditions rather than variation in intrinsic quality. The one exception to this was molt date where the variance explained by individual identity was relatively large. To further explore this, we fit an additional model for molt date with the inclusion of a random effect of identity as an interaction term with age, to test for between-individual variation in ageing rates of molt date. The variation explained by the ID x age term in this additional model was significant in a Wald test ($p < 0.001$), which suggests that males vary in the shapes of their molt ageing patterns. We plan to further investigate how both genes and the environment contribute to this individual-level variation in molt ageing in future work (Charmantier et al. 2014).

Conclusions

Within our study population of superb fairy-wrens, we found marked differences between ageing trajectories of survival and reproductive traits, and between individual aspects of reproduction within the sexes. Despite strong declines in survival from age one in both sexes, senescence was only detected in four of the seven models of reproductive traits, and for males only at later ages to which only 13% of individuals survive. It is likely that these instances of reproductive senescence could only be identified

due to the magnitude of our sample sizes and longevity of the study. This finding highlights the difficulty in confidently assessing late-life ageing patterns in wild animal populations. Continuing long-term data collection from individual based studies is thus especially valuable for investigating senescence patterns in the wild (Charmantier et al. 2014).

Our study adds to the increasing body of literature that demonstrates that traits do not age in unison, and suggests that the pattern of ageing in each trait will be molded by unique selection pressures. The exact nature of these selection pressures may be difficult to infer due to the complexity of potential evolutionary and ecological forces acting on any one trait. Key to making progress in this field is the ability to compare ageing across traits, populations, and studies. We have presented here a comprehensive analytical approach to quantifying ageing patterns in traits that follow very different ageing trajectories, and to assessing differences in the key parameters of early-adulthood rates of maturation, onset of senescence and later-life rates of senescence. This approach could be used for multi-trait comparisons within other populations, and also for comparisons across populations. To facilitate this, we have provided annotated code of our methods (appendix 1), in the hope of increasing the number of future studies that use standardized ageing analyses.

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Author Contributions

Data collection: HLO and AC. Planning of analysis: LEBK and EBC. Data analysis and drafting the paper: EBC. Revising of the paper: EBC, LEBK, AC, TB,. Approval of final manuscript: all.

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Chapter 2 Supplementary material

S1. Life Tables

S1a. Methods

Using all individuals of known age and known death date, we summarized age-specific measures of survival and reproduction. For each age we tallied the number of males and females in our dataset, and the proportion of these individuals that survived until the next age class. Average lifespan of both females and males that survived until at least adulthood was also calculated. For female reproduction, we tallied the number of individuals we had full breeding records for each age, and calculated the average probability they would produce any independent offspring, and the average number of independent offspring they produced and the standard deviation. We summarized male reproduction similarly, except that we used the measure of offspring sired (rather than raised to independence), and we also separated within-group success from extra-group success. We measured number of offspring sired rather than offspring raised to independence because parental care is irrelevant for male extra-group success, and we wanted to make the measures of within-group and extra-group male reproduction comparable. Generation time, measured as the average age of reproduction, was calculated for the female and two male measures of reproduction.

S1b. Results

Table S1 Summary statistics for survival and reproduction. For both female and male fairy-wrens, the number of individuals alive at the beginning of each age (No. Living), the proportion that survived to the subsequent year (Prop. Survived), the number of individuals breeding for each age (No. Reproductive) , and their probability (Prob.) and number (No.) of offspring with the standard deviation (SD). For females, only offspring that survived to independence (approx. 41 days from hatching) are included in reproductive success and for males all offspring that survived to blood sampling age (approx. 7 days from hatching) are included. Male reproductive success is separated into offspring sired by the female within his territory (within-group), and by females outside his territory (extra-group).

	Females					***Males***						
Age	*No. Living*	*Prop. Survived*	*No. Reproductive*	*Prob. Independent Offspring*	*No. Independent Offspring (SD)*	*No. Living*	*Prop. Survived*	*No. Reproductive*	*Prob. Within-Group Offspring*	*No. Within-Group Offspring (SD)*	*Prob. Extra-Group Offspring*	*No. Extra-Group Offspring (SD)*
1	749	0.66	627	0.53	1.23 (1.39)	1046	0.65	898	0.07	0.14 (0.62)	0.06	0.09 (0.45)
2	497	0.57	399	0.73	2.01 (1.80)	634	0.70	586	0.15	0.32 (0.94)	0.12	0.30 (1.03)
3	285	0.58	225	0.73	2.08 (1.80)	437	0.70	404	0.20	0.48 (1.24)	0.2	0.54 (1.37)
4	166	0.60	141	0.68	2.05 (1.89)	295	0.70	271	0.20	0.56 (1.39)	0.2	0.73 (1.98)
5	100	0.54	85	0.69	2.08 (1.95)	203	0.63	188	0.25	0.65 (1.43)	0.26	1.12 (2.40)

6	54	0.48	43	0.70	2.14 (2.02)	126	0.61	113	0.18	0.55 (1.40)	0.31	1.10 (2.24)
7	26	0.50	23	0.87	3.00 (2.30)	75	0.63	69	0.29	0.65 (1.21)	0.33	1.52 (2.93)
8	13	0.38	11	0.91	2.09 (1.04)	46	0.54	36	0.28	0.69 (1.58)	0.33	1.75 (3.21)
9	5	0.20	4	0.75	1.75 (1.71)	22	0.36	21	0.29	0.71 (1.38)	0.29	0.81 (1.86)
10	1	0	-	-	-	8	0.38	6	0.33	0.83 (1.33)	0.17	0.17 (0.41)
11	-	-	-	-	-	3	0.33	2	0	0 (0)	0	0 (0)
12	-	-	-	-	-	1	0	1	0	0 (-)	0	0 (-)

Age classes are one year in length and commence September 1st of the calendar year. Number of reproductive individuals is smaller than number of living individuals for each age class because those that died after September 1st but before they could have possibly accumulated any reproductive success (due to dying too early in the breeding season) were included as a living individual but excluded as a reproductive individual.

Age-specific survival probabilities in the fairy-wrens showed only slight differences between the sexes. Maximum lifespans were ten and twelve years for females and males respectively (table 1). However, the smaller sample size of females may have contributed to our lack of observation of females at extreme ages. Mean adult lifespan, measured as age of death among individuals that survived to the age of 1 year, was longer for males: 3.44 years (± 2.22 SD) than females 2.97 years (± 1.73 SD) (Wilcoxon rank sum test p-value < 0.001, n = 751 females, 979 males). In females, survival was highest at age one, stabilized through age four, and then declined from age five onwards (table 1). In males, age one survival was similar to that of females but then improved at age two, remaining stable through age four, after which survival began to similarly decline each year (table 1).

In contrast to survival, female reproduction improved substantially after the first breeding season, and then remained relatively stable up to late ages, where there was another increase at age seven and eight, but with small sample sizes (table 1). Generation time, the average age of reproduction in females (*sensu* Leslie 1966), was 2.59 (n = 6905 offspring from 837 mothers).

For males, within-group reproductive success showed improvements from age one through age five, likely due to a larger proportion of males gaining dominant social status on their territory in these years, as well as a smaller proportion of males living on a territory with their mother, which both improve their siring success within the territory (table 1). Beyond this age, when virtually all males in the sample are dominant, within-group reproductive success stopped improving. Extra-group reproductive success increased with age up through age eight, after which point there was a decline (table 1). Within-group generation time was 3.41 years, and extra-group generation time was 3.69 (n = 6209 offspring from 910 biological fathers).

S1c. Discussion

The raw survival data (table S1) indicates that males have higher survival than females from age two to five, and have slightly longer average lifespans. These lifetable results differ from the survival GAMM predictions which indicate comparable levels of survival in both males and females and senescence onset at age one. These differences are likely due to the male survival GAMM controlling for the effect of dominant social status. The GAMM suggests that helpers tend to have higher survival than dominants (table 2, p=0.06), which is why higher male survival

is observed at early ages when not taking this variable into account. Further investigation is needed in order to determine how and to what extent dominance status influences male survival, but that is beyond the scope of this paper.

Reproduction results do not differ substantially in the lifetables in comparison to the results of the main text.

S2. GAMM simulations

Female fecundity and male reproductive success data followed a roughly Poisson distribution except that they contained an excess of zeros and were over dispersed. We ran simulations of the Poisson GAMM models for female fecundity and male reproductive success (total, within-pair, and extra-pair) checked against raw data means and variance for the effect of age. Here we verify that the true values fall within the variability of model predictions, indicating this modeling technique is appropriate for our data distribution.

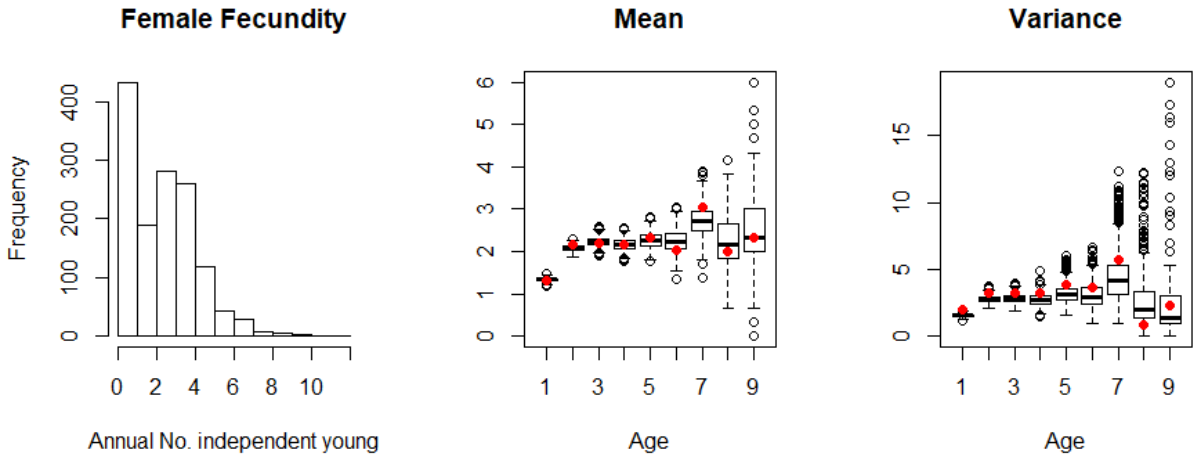


Figure S1a. Raw data distribution, and boxplots of predicted means and variance of female fecundity in 1000 simulations of the GAMM model. Red dots represent the raw means and variance for every age class.

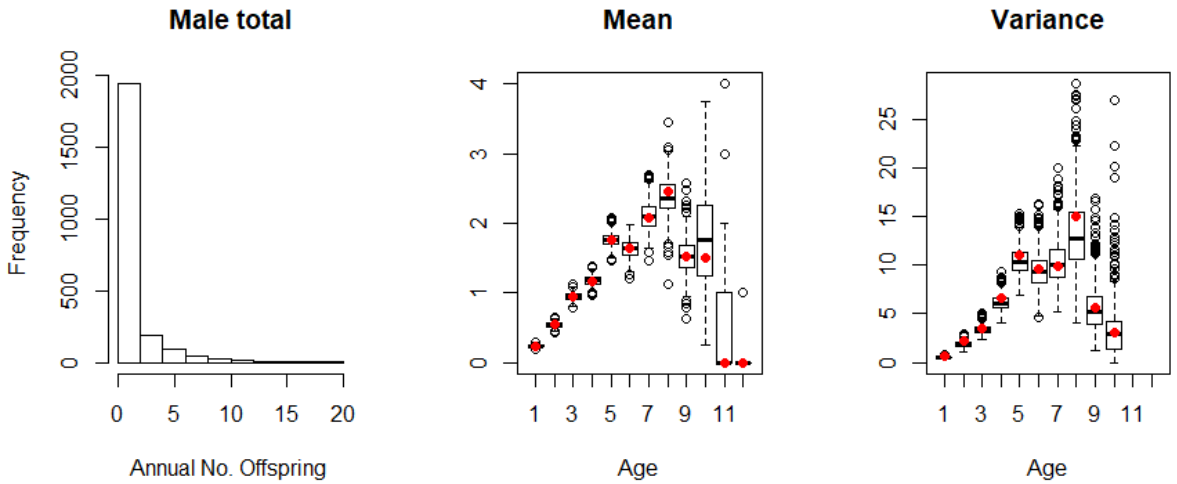


Figure S1b. Raw data distribution, and boxplots of predicted means and variance of total mating success in males in 1000 simulations of the GAMM model. Red dots represent the raw means and variance for every age class.

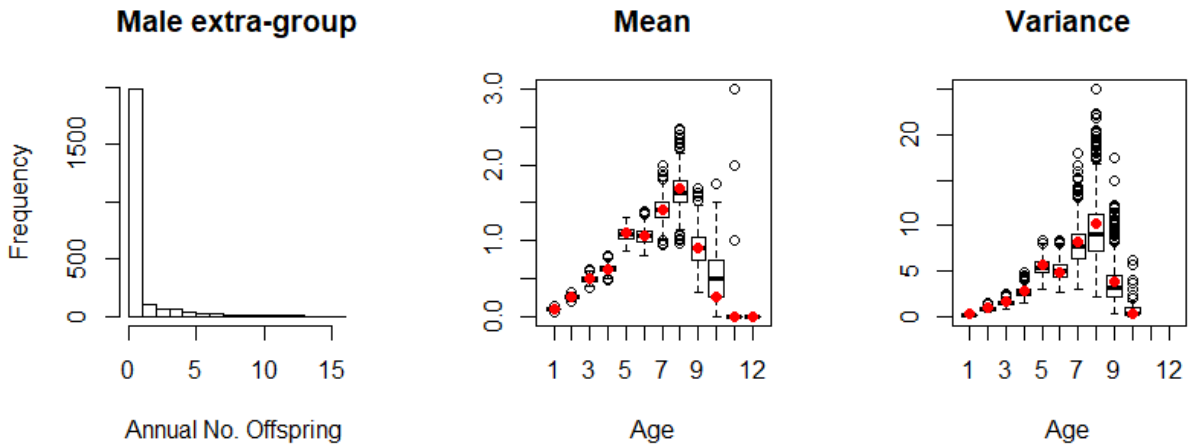


Figure S1c. Raw data distribution, and boxplots of predicted means and variance of extra-group mating success in males in 1000 simulations of the GAMM model. Red dots represent the raw means and variance for every age class.

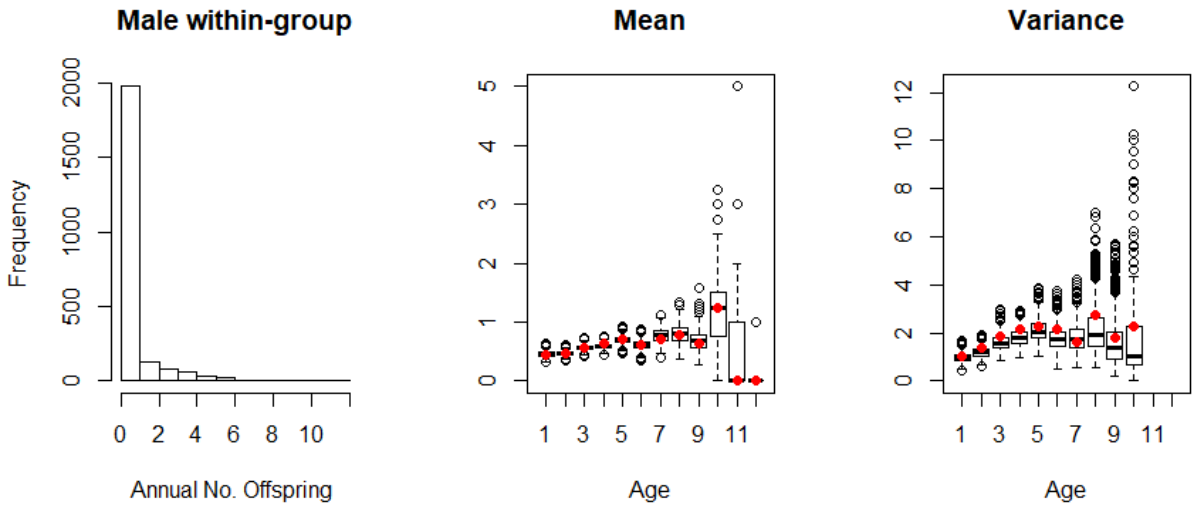


Figure S1d. Raw data distribution, and boxplots of predicted means and variance of within-group mating success in males in 1000 simulations of the GAMM model. Red dots represent the raw means and variance for every age class.

S3. Zero-inflated reproductive models

S3a. Rationale

In both sexes in the fairy-wren population, many individuals will gain no reproductive success in a given year (measured as offspring reaching independence for females, and total banded offspring for males; see *methods* in main text for details). This results in these traits following a zero-inflated count data structure. Models with Poisson error distribution did fit the data well when an observation-level random effect was included to account for over-dispersion (see supplementary S2), and these models succinctly summarize total annual reproductive fitness contributions. Thus, we used these models in the main text. Zero-inflated Poisson models provide another means of modelling over-dispersed count data with an 'excess' of zeros (Greene 1994; Wood et al. 2016). In principal, zero-inflated models should be used when there are two 'types' of zero values present in a dataset and the probability of a non-zero value is determined by one of two different processes, depending on the individual data point. In the case of the fairy-wren data, this distinction is likely a reasonable assumption. Some individuals have zero reproductive success in a given year as a result of never mating (for males), or being infertile or, perhaps absolutely incapable of raising offspring (for females). For other individuals in the dataset, having zero reproductive success in a given year may be at least partially a consequence of extrinsic stochasticity, rather than entirely due to intrinsic conditions (e.g. especially high predation in a given time and location, the fertility of an individual's mate, etc.). Thus, the two types of zeros in the fairy-wren data could reflect either a complete failure to reproduce, or, reproducing with a failure of offspring surviving to fledging. The zero-inflated model fits two response variables. One response fits the total number of offspring produced, excluding some zeros in order to meet the assumptions of a Poisson count data distribution (fitted with a log link function). The other response variable fits the remaining excess of zeros as a Bernoulli response (fitted with a logit link function). Thus, these models effectively allow for age-related changes in the probability of offspring to be disentangled from age-related changes in the absolute

number of offspring, which may be driven by different processes. For this reason, we have chosen to fit zero-inflation models as a complement to the primary analyses of the paper, in order to investigate if there are differences in the age-related changes in probability and quantity of reproductive output for each sex.

For female independent offspring, and male total offspring, we fitted zero-inflated Poisson GAMM models. We followed the same methodology for each portion (probability and number) of each model as is described in the main text, resulting in two GAMMs (with two separate responses for the binary and count portions) and four segmented regressions for each trait. The predicted values for the count portion of each GAMM are higher than that of the predicted values in the main text results because the Poisson models here only include a small portion of the zeros in the overall dataset (in order to remove the zero-inflation). Multiplying the probability (binomial) and the count (Poisson) portions of the zero-inflated model results in comparable predictions to those Poisson models illustrated in figure 1 of the main text.

S3b. Results

Table S5.1 Spline and parametric effects describing ageing in eight traits in superb fairy-wrens using generalized additive mixed-models (GAMMs). Terms where $p < 0.01$ are in bold. Random effects of individual and year were included in each model.

Trait	Distribution	Sample size	EDF	Parametric terms (SE)			
			Age	Intercept	Longevity	Status [dom]	Terminal effect
Female independent offspring		1558 (678)					
Probability	- Bernoulli		**4.417**	0.063 (0.110)	0.035 (0.024)	-	-0.246 (0.299)
Number	- Poisson		**3.325**	**0.793 (0.077)**	0.017 (0.015)	-	-0.006 (0.184)
Male total offspring		2310 (780)					

Probability	- Bernoulli	**3.332**	**-3.996 (0.776)**	0.0212 (0.038)	**0.976 (0.141)**	-0.024 (0.339)
Number	- Poisson	**3.716**	**0.906 (0.159)**	-0.019 (0.027)	0.089 (0.102)	-0.247 (0.246)

Note: Sample size is the number of data points included in the model, followed by the number of individuals in brackets. EDF is the effective degrees of freedom for the spline term. Longevity is the number of years an individual lived, and provides a test of selective disappearance. Status is a binary term specifying whether a male held a dominant [dom] breeding position or a helper position on his home territory in a given year. Final breed is a binary term denoting whether or not it was the individual's final breeding season. Note that parameter estimates for the Bernoulli distribution are on a logit scale and those for the Poisson distribution are on a log scale.

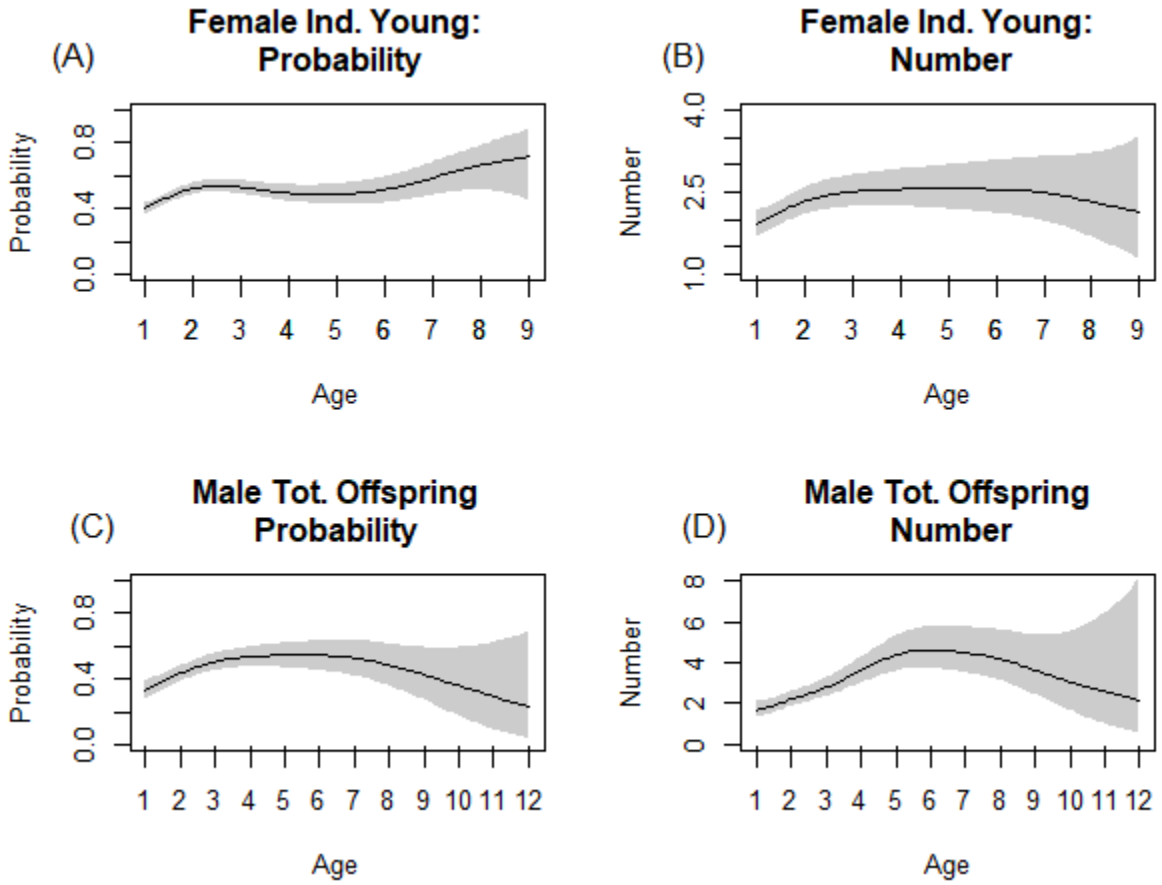


Figure S3.1 Age-specific probabilities and (non-zero-inflated) numbers of offspring reaching independence for females (A & B), and offspring sired for males (C & D) modeled using zero-inflated Poisson GAMMs.

Table s3.2 The estimates for the effect of age on both probability and number of male and female reproductive output (see main *methods*) in segmented linear regressions. The data values analyzed in these models were the mean values for each age as predicted by the respective GAMM model (table 2), centered on the mean trait value and divided by the range of trait values (see main *methods*). Each data point was weighted by the inverse of its standard error. Terms where $p < 0.01$ are in bold.

Trait	Across-life slope (99% CI)	Early-adulthood slope (99% CI)	First Breakpoint age (99% CI)	Mid-life slope (99% CI)	Second Breakpoint age (99% CI)	Late-life slope (99% CI)
Female independent offspring						
- *probability*	0.320 (-0.040, 0.681)	-	-	-	-	-
- *number*	-	**1.370 (1.061, 1.678)**	2.4 (2.2, 2.5)	0.048 (-0.085, 0.181)	6.6 (6.1, 7.1)	**-0.523 (-0.885, -0.161)**
Male total offspring						
- *probability*	-	**0.281 (0.136, 0.425)**	4.6 (3.8, 5.3)	-	-	**-0.172 (-0.267, -0.077)**
- *number*	-	**0.864 (0.725, 1.004)**	5.6 (5.2, 5.9)	-	-	**-0.409 (-0.565, -0.253)**

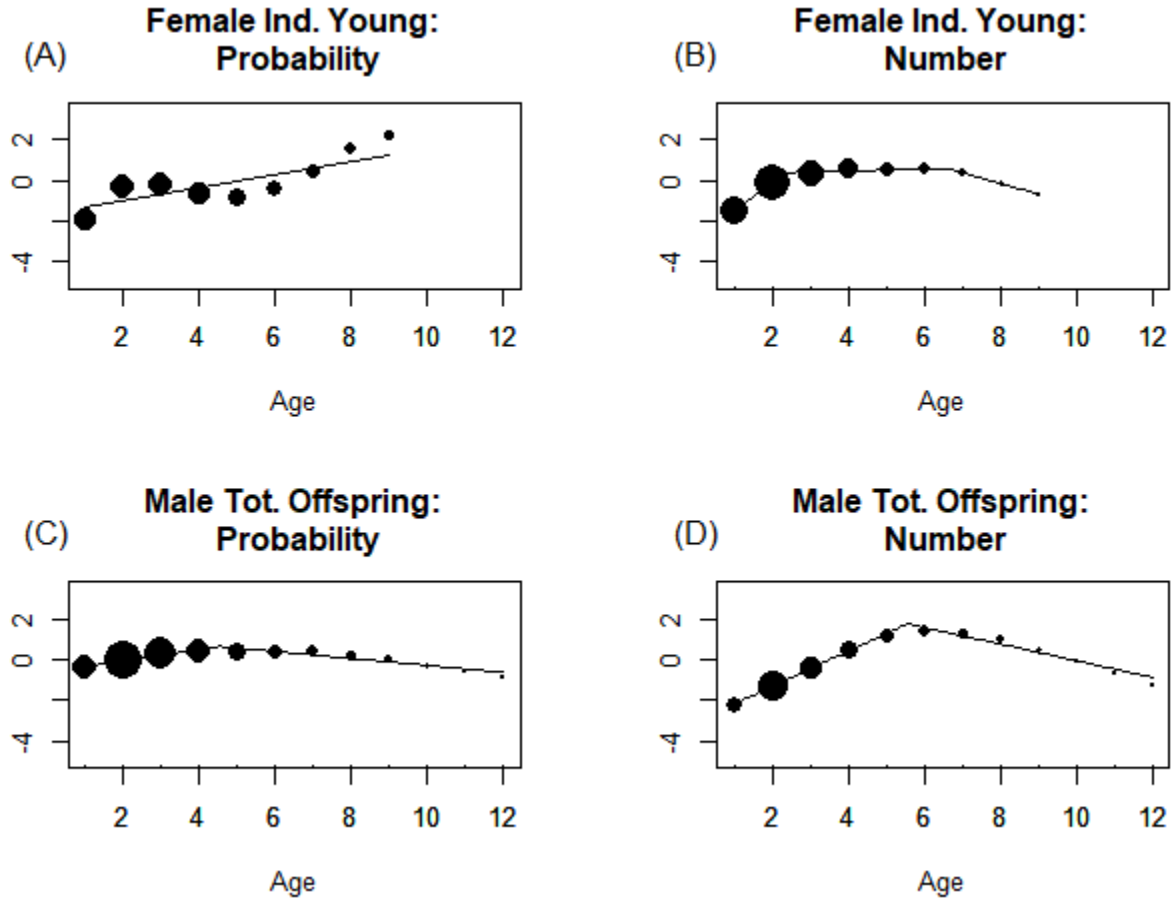


Figure S5.2 Segmented or continuous linear regressions estimating the effect of age on probabilities and (non-zero-inflated) numbers of offspring reaching independence for females (A & B), and offspring sired for males (C & D). Filled circles represent the raw data, which are the estimates for each age predicted from the corresponding GAMM and z-transformed to allow direct comparisons between traits. The size of the circle represents the inverse of the standard error associated with the GAMM estimate. Regressions are weighted by these inverse standard errors. Regressions which are continuous did not have a significant break point.

S4. Longevity Spline Results

Trait	Lifespan Spline EDF	P-value
Female breeding start date	1.015	0.43
Female clutch size	1.000	0.19

Female fecundity		
Probability	2.036	0.14
Number	1.366	0.38
Male moult date	1.011	0.40
Male within-group success		
Probability	2.025	0.40
Number	1.001	0.41
Male extra-group success		
Probability	1.000	0.50
Number	1.003	0.25

Table S2. Effective degrees of freedom (EDF) and p-value associated with the spline effect of lifespan in the GAMM for each trait. Additional spline and parametric terms in each model are denoted in figures S4-S12 and the estimates and statistics for these covariates are not notably different than those reported there.

S5. GAMM Detailed Results

Table S4 Results from a Poisson GAMM predicting annual number of offspring reaching independence for females. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**Chi squared**	**P-value**
Age	4.540	4.540	64.290	< 0.001
Parametric Terms	**Estimate**	**Standard Error**	**z-value**	**P-value**
Intercept	0.374	0.085	4.390	< 0.001
Longevity	0.026	0.017	1.556	0.120
Final breed season	-0.129	0.192	-0.670	0.503

Note: The model R^2 (adjusted) is 0.06 and the sample size is 1372. The model includes random effects of observation (n=1372, variance = 0.133), year (n =29, variance = 0.0.057), and individual (n = 606, variance = 0.057).

Table S5 Results from a Poisson GAMM predicting annual total number of offspring for males. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**Chi squared**	**P-value**
Age	3.567	3.567	46.100	< 0.001
Parametric Terms	**Estimate**	**Standard Error**	**z-value**	**P-value**
Intercept	-5.038	0.801	-6.285	< 0.001
Longevity	0.057	0.034	1.664	0.096
Status [dominant]	0.861	0.115	7.460	< 0.001
Final breed season	-0.110	0.275	-0.401	0.689

Note: The model R^2 (adjusted) is 0.01 and the sample size is 2310. The model includes random effects of observation (n = 2310), year (n =29, variance = 15.085), and individual (n = 780, variance = 0.475).

Table S6 Results from a Poisson GAMM predicting annual number of extra-group offspring for males. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**Chi squared**	**P-value**
Age	0.373	3.731	55.57	< 0.001
Parametric Terms	**Estimate**	**Standard Error**	**z-value**	**P-value**
Intercept	-6.303	0.926	-6.809	< 0.001
Longevity	0.061	0.051	1.214	0.225
Status [dominant]	0.025	0.170	0.150	0.881
Final breed season	0.191	0.384	0.497	0.619

Note: The model R^2 (adjusted) is 0.01 and the sample size is 2310. The model includes random effects of observation (n = 2310, variance = 0.994), year (n =29, variance = 17.029), and individual (n = 780, variance = 0.853).

Table S7 Results from a Poisson GAMM predicting annual number of within-group offspring for males. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**Chi squared**	**P-value**
Age	2.019	2.019	3.706	0.174
Parametric Terms	**Estimate**	**Standard Error**	**z-value**	**P-value**
Intercept	-6.281	0.770	-8.155	< 0.001
Longevity	0.052	0.042	1.246	0.213
Status [dominant]	2.017	0.182	11.062	< 0.001
Final breed season	-0.480	0.415	-1.159	0.247

Note: The model R^2 (adjusted) is 0.01 and the sample size is 2310. The model includes random effects of observation (n = 2310, variance = 0.740), year (n =29, variance = 12.570), and individual (n = 780, variance = 0.401).

Table S8 Results from GAMM predicting female clutch size. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**F-value**	**P-value**
Age	7.828	7.983	8.513	< 0.001
Incubation date	5.336	6.413	68.560	< 0.001
Parametric Terms	**Estimate**	**Standard Error**	**t-value**	**P-value**
Intercept	1.148	0.014	79.470	< 0.001
Longevity	0.003	0.003	1.010	0.312
Final breed season	-0.005	0.007	-0.714	0.475

Note: The model R^2 (adjusted) is 0.23 and sample size is 3865. The model includes random effects of year (n =28, variance = 1.24×10^{-6}), and individual (n = 979, variance = $5.63\ 10^{-2}$).

Table S9 Results from GAMM predicting female breeding start date. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**F-value**	**P-value**
Age	5.555	6.229	74.943	< 0.001
Parametric Terms	**Estimate**	**Standard Error**	**t-value**	**P-value**
Intercept	12.152	1.414	8.596	< 0.001
Longevity	-0.477	0.353	-1.350	0.178
Final breed season	-2.714	1.367	-1.987	0.047

Note: The model R^2 (adjusted) is 0.56 and sample size is 1089. The model includes random effects of year (n =28, variance = 1.87 x 10^{-4}), and individual (n = 678, variance = 9.05).

Table S10 Results from GAMM predicting male moult date. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**F-value**	**P-value**
Age	5.709	6.683	171.140	< 0.001
Parametric Terms	**Estimate**	**Standard Error**	**t-value**	**P-value**
Intercept	-42.588	3.222	-13.217	< 0.001
Longevity	0.777	0.569	1.366	0.172
Status [dominant]	-4.193	1.588	-2.640	0.008
Final breed season	-0.856	1.934	-0.442	0.658

Note: The model R^2 (adjusted) is 0.59 and sample size is 2116. The model includes random effects of year (n =28, variance = 3.23 x 10^{-4}), and individual (n = 1024, variance = 14.31).

Table S11 Results from GAMM predicting annual female survival probability. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**Chi squared**	**P-value**
Age	1.000	1.000	15.969	< 0.001
Parametric Terms	**Estimate**	**Standard Error**	**Z-value**	**P-value**
Intercept	59.870	12.960	4.620	< 0.001

Note: The model R^2 (adjusted) is 0.05 and sample size is 1896. The model includes random effects of year (n =29, variance = 0.22), and individual (n = 979, variance = 1 x 10^{-7}).

Table S12 Results from GAMM predicting annual male survival probability. EDF is the effective degrees of freedom and Ref. DF is the reference degrees of freedom for the relevant spline term.

Spline Terms	**EDF**	**Ref. DF**	**Chi squared**	**P-value**
Age	1.001	1.002	29.15	< 0.001
Parametric Terms	**Estimate**	**Standard Error**	**Z-value**	**P-value**
Intercept	45.983	12.419	3.703	< 0.001
Status [dominant]	-0.191	0.103	-1.853	0.064

Note: The model R^2 (adjusted) is 0.056 and sample size is 2747. The model includes random effects of year (n =29, variance = 0.21), and individual (n = 979, variance = 3.01 x 10^{-4}).

S6. The effect of molt on male extra-group success

In order to test if the early-life improvement of male extra-group siring success is driven by the improvement in their molt (a secondary sexual trait), we re-ran the GAMM and subsequent broken stick model for extra-group success, controlling for molt date as an additional explanatory variable in the GAMM. As expected, molt date had a highly significant effect on extra-group success in the GAMM (coefficient of -7.4 x 10^{-3} ± 1.6 x 10^{-3}).

The subsequent broken stick model had the same breakpoint as the models not controlling for molt (figure S2). Early-life rates of improvement remained significant in the model controlling for molt, but the slope was lessened by over half (figure S2; coefficient of 1.76 ± 0.69 in the original model, coefficient of 0.82 ± 0.41 in the model controlling for molt). These results indicate that molt date is partially responsible for driving early-life improvements in extra-group success. However, since there still remains some significant improvement after controlling for molt date, this suggests that some other characteristics associated with male age play a role in the early-life rate of improvement.

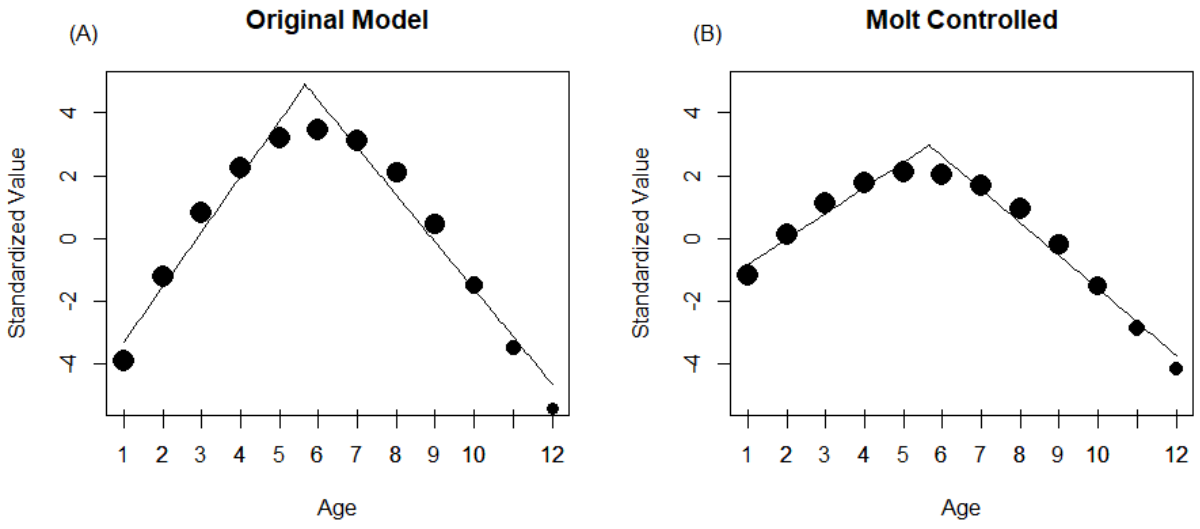


Figure S2 Broken-stick model results for the effect of age on the number of extra-group offspring produced by males in (A) the original model, and (B) when molt date has been added as a fixed effect to the model.

References

Greene, J. 1994. Accounting for Excess Zeros and Sample Selection in Poisson and Negative Binomial Regression Models. NYU Working Paper No. EC-94-10 9:265–265.

Leslie, P. H. 1966. The Intrinsic Rate of Increase and the Overlap of Successive Generations in a Population of Guillemots (Uria aalge Pont.). Journal of Animal Ecology 35:291–301.

Wood, S. N., N. Pya, and B. Säfken. 2016. Smoothing Parameter and Model Selection for General Smooth Models. Journal of the American Statistical Association 111:1548–1563.

Chapter 2 Appendix 1

Ageing of clutch size

Purpose

This guide demonstrates a method for investigating ageing patterns in a population using longitudinal data collected from individuals of known age. Ageing trajectories may not follow simple parametric functions and so need to be modelled using methodology that does not constrict their functional shape. However, in order to make quantitative comparisons of ageing between traits,populations, or species it is necessary to define which features of ageing to quantify and do so using standardized units of scale. We therefore take a two-step approach:

1. Model the effect of age on the trait of interest non-parametrically using generalized additive mixed-models (GAMMs).
2. Using standardized estimates for each age from the GAMM, run a break-point analysis and quantify rates and durations of early-life improvements and late-life declines.

We illustrate how to control for the effects of selective (dis)appearance, variability between individuals, variability in environment, and other factors that may confound the effects of age in a wild population.

This guide uses the example of clutch size (a proxy for reproductive investment) in a wild population of Superb fairy-wrens as the trait of interest which we want to investigate ageing in.

Packages

Load the neccessary packages and their dependencies. ‘mgcv’ is used to model GAMMs. ‘segmented’ is used for broken stick regressions.

```
library(mgcv)
library(segmented)
library(dplyr)
```

Dataset

We will use data collected between 1987 - 2016 on a wild population of fairy-wrens living in the Australian National Botanic Gardens. Each ID refers to a unique female. Females attempt to breed each year from age 1 and can have multiple clutches in a year. Each row in the dataframe refers to a single clutch.

```
clutch.dat <- read.table("data/clutchSize.txt",header=TRUE)
```

Generalized additive mixed model

Using the package mgcv, we will run a GAMM of clutch size (number of eggs) in response to the non-parametric effect of age. s() denotes non-parametric terms (splines), while terms without this notation are parametric (analogous to as they'd be fitted in a (g)lmm). Because we have prior knowledge that the fairy-wrens tend to lay larger clutches in the middle of the breeding season, we include a spline of the date when clutch was laid (julianDate) to control for this. Lifespan is fitted as a parametric effect in order to control for selective disappearance. Individual ID and year are fitted as random effects in order to control for non-indpendence between datapoints (multiple datapoints per ID), and environmental variability between years. Estimation method is set to REML. Family is set to quasipoisson to account for under dispersion in clutch size data (clutch size ranges from 2-4). K values are described further below.

Note that for a given population effects of selective (dis)appearance should not be assumed to be linear. Fitting lifespan as a spline to check the shape of the relationship was previously done. Since EDF of the lifespan spline was very close to one, a linear relationship was adequate in this case to control for potential selective disappearance in the population.

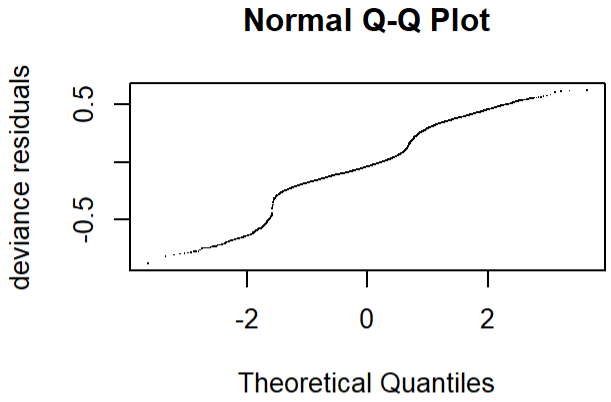
```
#model takes about 10 - 20 minutes to run
#instead of running, just load the previously run model below
#clutch.gam<-
gam(eggs~s(age,k=9)+s(julianDate)+lifespan+s(ID,bs="re")+s(year,bs="re"),data=clutch.dat,method="REML",family
=quasipoisson(link="log"))
#saveRDS(clutch.gam,"clutch.gam.rds"

#alternatively, load the previously run model
clutch.gam <- readRDS("clutch.gam.rds")
```

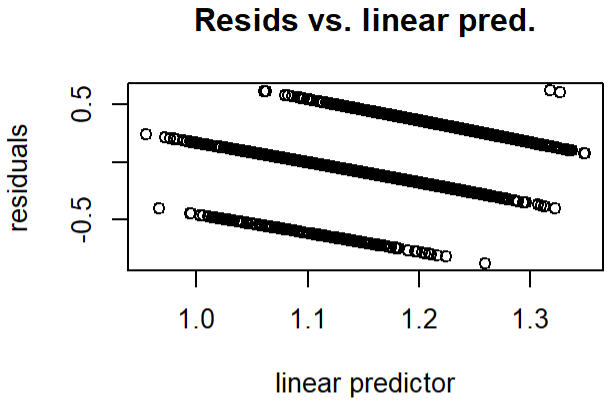
GAMM model review

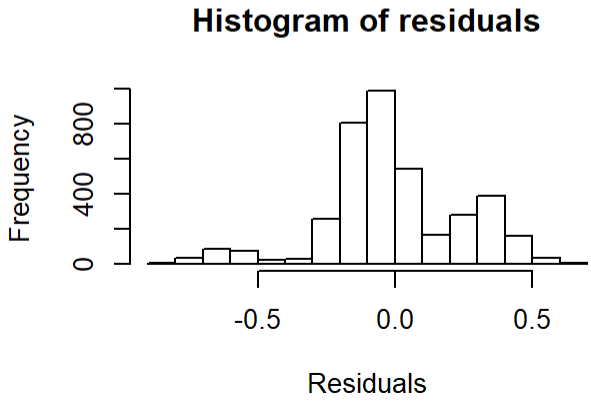
gam.check()

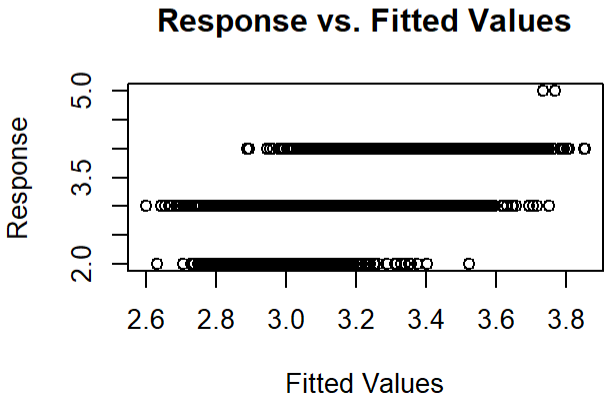
This gives the model convergence and some diagnostic plots. Basis dimension checking results for each spline are also given. In this specific case, significant p-values for the term s(age) are not of concern since the maximum k specified for age is also the maximum degrees of freedom (9). For other spline term(s) with a k-index close to 1, models should be rerun with higher k values specified for those splines. If the residuals in the updated model are different, it's preferable to keep the more complex model. In this specific case, higher k values did not change the model so the default k values were kept. See '?choose.k' for more information.

```
#check convergence of the model, and check that basis dimension (k) is adequate
gam.check(clutch.gam)
```










```
##
## Method: REML   Optimizer: outer newton
## full convergence after 8 iterations.
## Gradient range [-5.528896e-07,3.81231e-07]
## (score -3090.176 & scale 0.06517356).
## eigenvalue range [-2.447954e-07,1940.214].
## Model rank =  670 / 670
##
## Basis dimension (k) checking results. Low p-value (k-index<1) may
## indicate that k is too low, especially if edf is close to k'.
##
##                  k'      edf k-index p-value
## s(age)       8.00e+00 4.29e+00    0.94  <2e-16 ***
## s(julianDate) 9.00e+00 6.12e+00    0.96   0.005 **
## s(ID)        6.50e+02 2.70e+02      NA      NA
## s(year)      1.00e+00 5.38e-06    0.93  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

concurvity()

This is the GAMM equivelent to checking for colinearity in a GLMM. We can see that concurvities between fixed effects here are very small, and so nothing to worry about.

```
concurvity(clutch.gam,full=FALSE)
## $worst
##                  para      s(age) s(julianDate)     s(ID)
## para         1.000000e+00 6.940030e-26  2.118496e-22 1.0000000
## s(age)       4.930032e-26 1.000000e+00  5.563194e-02 0.4890978
## s(julianDate) 2.118401e-22 5.563194e-02  1.000000e+00 0.1713809
## s(ID)        1.000000e+00 4.890978e-01  1.713809e-01 1.0000000
## s(year)      9.999876e-01 1.145418e-07  2.937450e-08 0.9999997
##                 s(year)
## para         9.999876e-01
## s(age)       1.145418e-07
## s(julianDate) 2.937450e-08
## s(ID)        9.999997e-01
## s(year)      1.000000e+00
##
## $observed
##                  para      s(age) s(julianDate)      s(ID)
## para         1.000000e+00 3.499555e-29  1.226708e-26 0.006579043
## s(age)       4.930032e-26 1.000000e+00  4.400031e-02 0.003984690
## s(julianDate) 2.118401e-22 4.632261e-02  1.000000e+00 0.010150582
## s(ID)        1.000000e+00 3.817687e-01  1.139947e-01 1.000000000
## s(year)      9.999876e-01 1.456379e-08  1.405679e-08 0.006594320
##                 s(year)
## para         9.999876e-01
## s(age)       1.145418e-07
## s(julianDate) 2.937450e-08
## s(ID)        9.999997e-01
## s(year)      1.000000e+00
##
## $estimate
##                  para      s(age) s(julianDate)      s(ID)
## para         1.000000e+00 7.516471e-31  8.648451e-25 0.002657946
## s(age)       4.930032e-26 1.000000e+00  2.079127e-02 0.004229922
## s(julianDate) 2.118401e-22 2.873079e-02  1.000000e+00 0.001938552
## s(ID)        1.000000e+00 4.466261e-01  1.209394e-01 1.000000000
## s(year)      9.999876e-01 7.319916e-08  5.862414e-09 0.002657034
##                 s(year)
## para         9.999876e-01
## s(age)       1.145418e-07
## s(julianDate) 2.937450e-08
## s(ID)        9.999997e-01
## s(year)      1.000000e+00
```

model results

Finally, we can take a look at results of our model. We can see that both age and Julian date of incubation have significant, non-linear effects on clutch size. Lifespan does not have a significant effect suggesting lifespan is not correlated with larger or smaller clutches overall.

```
summary(clutch.gam)
##
## Family: quasipoisson
## Link function: log
##
## Formula:
## eggs ~ s(age, k = 9) + s(julianDate) + lifespan + s(ID, bs = "re") +
##     s(year, bs = "re")
##
## Parametric coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 1.140227   0.009379 121.568   <2e-16 ***
## lifespan    0.003273   0.002138   1.531    0.126
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Approximate significance of smooth terms:
##                  edf  Ref.df      F p-value
## s(age)       4.295e+00   5.193 16.744  <2e-16 ***
## s(julianDate) 6.121e+00   7.185 64.126  <2e-16 ***
## s(ID)        2.704e+02 648.000  0.928  <2e-16 ***
## s(year)      5.377e-06   1.000  0.000   0.291
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## R-sq.(adj) =   0.22   Deviance explained = 27.3%
## -REML = -3090.2  Scale est. = 0.065174  n = 3865
```

gam.vcomp()

This function gives the variance (standard deviation) for each term. For the random effects, the std.dev suggests how much of the residual variance they explain. We can see here that differences between individuals (ID) explains a larger amount of variation in clutch size than environmental differences between years (year).

```
gam.vcomp(clutch.gam)
##
## Standard deviations and 0.95 confidence intervals:
##
##                 std.dev       lower       upper
## s(age)       2.178484e-02 8.734350e-03 5.433479e-02
## s(julianDate) 5.234713e-04 2.788835e-04 9.825687e-04
## s(ID)        5.829804e-02 5.134252e-02 6.619584e-02
## s(year)      1.138819e-06 1.111537e-06 1.166771e-06
## scale        2.572022e-01 2.510406e-01 2.635150e-01
##
## Rank: 4/5
```

plot results

We can plot the results of our GAMM to visualize the ageing tragectory of clutch size. Specifying 'seWithMean=2' includes uncertainty about the overall mean from other variables in the model, excluding random effects.

```
plot(clutch.gam,select=1,seWithMean=2,shade=TRUE,trans=exp,shift=coef(clutch.gam)[1],main="Female Clutch Size",
    ylab="Eggs",xlab="Age",ylim=c(2.8,3.4))
```

Quantify ageing trajectories

We can see from the GAMM graph that it appears that clutch size increases in early ages, plateaus, and then declines at late ages. However, if we want to quantify the rates of these increases and decreases, a second step is neccessary. Now we will:

- extract age-specific estimates of clutch size for each age from the GAMM
- standardize these estimates by z-transforming them. This allows direct comparison of ageing between traits, populations, or species
- run a simple linear model using these standardized estimates

- use the package '?segmented' to test for a breakpoint in the linear model

This process allows for us to identify and quantify the rate and significance any early-life improvement (maturation) as well as late-life decline (senescence). If late-life declines are significant, breakpoint analysis indicates the age of onset of senescence.

First we extract the estimates from the GAMM and standardize them.

```
#pull estimates
pred.clutch<-data.frame(x=predict.gam(clutch.gam,type="iterms",se.fit=TRUE))
#create a new clutch data table excluding individuals without lifespan (since they're excluded from model)
clutch.datS<-clutch.dat %>%
  filter(!is.na(lifespan))
#add age column
pred.clutch$age <- clutch.datS$age
#summarize data for each age class
sum.clutch <-pred.clutch %>%
  group_by(age) %>%
  summarize(x=mean(x.fit.s.age.),se=mean(x.se.fit.s.age.))

#z-transform
#extract the GAMM model estimates again, this time specifying type='link' so that the linear predictor
incorporating all terms in the model is returned
pred.link<-data.frame(x=predict.gam(clutch.gam,type="link",se.fit=TRUE))
#now center each prediction on the mean prediction for the effect of age, divided by the range of predictions in
the overall model
clutch.reg<-sum.clutch %>%
  mutate(xz=(x-mean(sum.clutch$x))/(max(pred.link$x.fit)-min(pred.link$x.fit)))%>%
  mutate(xz=xz*10) #we multiplied our predictions by 10 for ease on interpretability, this step is entirely optional
```

Run a linear model using these standardized estimates. Weight the model by the inverse of the standard error. This ensures that estimate precision is accounted for (while at the same time not allowing the much larger sample size at lower ages in the GAMMs entirely drive the regression prediction).

```
summary(lm.clutch<-lm(xz~age,weights = 1/se, data=clutch.reg))
##
## Call:
## lm(formula = xz ~ age, data = clutch.reg, weights = 1/se)
##
## Weighted Residuals:
##     Min      1Q  Median      3Q     Max
## -12.4118  -4.9977  -0.3778   4.4660   8.5452
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  0.195576   0.451649   0.433    0.678
## age         -0.009472   0.101576  -0.093    0.928
##
## Residual standard error: 7.234 on 7 degrees of freedom
```

```
## Multiple R-squared:  0.001241,   Adjusted R-squared:  -0.1414
## F-statistic: 0.008696 on 1 and 7 DF,  p-value: 0.9283
```

From the linear model results, we can see that there is no effect of age on clutch size when we constrain the relationship to a single linear slope.

Now, we can test if there is a significant breakpoint in the model using a Davies test. This tests for a non-zero difference in slopes if the linear model were broken into two linear models, at multiple points in the model. Essentially it is testing if the relationship between age and clutch size might be better explained by two segmented/broken linear models, rather than one. We set k (the number of points where the test should be evaluated) to n-2 so that it's only evaluating breakpoints at integers. See '?davies.test' for details.

```
davies.test(lm.clutch,seg.Z =~age, k=7)
##
##  Davies' test for a change in the slope
##
## data:  formula = xz ~ age ,   method = lm
## model = gaussian , link = identity
## segmented variable = age
## 'best' at = 3, n.points = 7, p-value = 0.0003363
## alternative hypothesis: two.sided
```

The small p-value of the davies test indicates a high probability that there is a difference between slopes of a segmented model. In other words, it indicates that two seperate linear models might better describe this ageing pattern than one.

The function segmented() implements a bootstrap restarting algorithm to find the breakpoint in the linear model that with result in the largest difference in the slopes of the resulting two models. See '?segmented' for details.

```
summary(seg.clutch<-segmented(lm.clutch))
##
##  ***Regression Model with Segmented Relationship(s)***
##
## Call:
## segmented.lm(obj = lm.clutch)
##
## Estimated Break-Point(s):
##          Est. St.Err
## psi1.age 3.21  0.133
##
## Meaningful coefficients of the linear terms:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) -1.50038    0.15527  -9.663 0.000201 ***
## age          0.80276    0.07188  11.169 0.000100 ***
## U1.age      -1.14671    0.08128 -14.107       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 1.311 on 5 degrees of freedom
## Multiple R-Squared: 0.9766,  Adjusted R-squared: 0.9625
##
## Convergence attained in 2 iter. (rel. change 2.0425e-16)
```

The summary function tells us the breakpoint estimated by the function (3.21). Note that the estimate of 'U1.age' is not the slope of age in later life, it's the difference in the slope of age between early and late life.

To find the slopes of the two regressions we use slope().

```
slope(seg.clutch)
## $age
##          Est.  St.Err. t value CI(95%).l CI(95%).u
## slope1  0.80276 0.071877 11.1690   0.61799   0.98752
## slope2 -0.34396 0.037959 -9.0613  -0.44153  -0.24638
```

'slope1' refers to the slope of the regression from ages 1 - 3.21. 'slope2' refers to the slope of the regression in ages beyond 3.21.

Our results demonstrate a significant trend of improvement (increased clutch size) in early life up until age 3.21, followed by significant senescent declines in clutch size after that age.

A graph will help to illustrate what this pattern looks like:

```
plot.segmented(seg.clutch,xlab="Age",ylab="Eggs")
title(main="Female Clutch Size")
#optionally, we can also add the data points used in the model. I've sized them here relative to their weight in the
model.
points(xz~age,data=clutch.reg,pch=16,cex=1/clutch.reg$se*0.01) #se is multiplied by 0.01 simply to achieve an
attractive point size for the graph
```


Female Clutch Size
Eggs
1.0
0.5
0.0
-0.5
2
4
6
8
Age

Chapter 3

Why does a sexually-selected trait improve with age?

Cooper, E.B., Bonnet, T., Osmond, H., Cockburn, A. & L.E.B. Kruuk. *Manuscript in prep.*

Abstract

Sexually-selected traits often show improvement with age. What selection pressures and genetic architecture generate this continual improvement? It is possible that the age-related pattern of trait expression is a consequence of an absolute genetic constraint preventing young individuals from expressing high values of the sexually-selected trait. Alternatively, age-related improvement in the trait could be a consequence of adaptive plasticity in trait expression, for example if there is a greater fitness payoff to displaying the trait at higher levels at older ages. We investigate these hypotheses using age-specific quantitative genetic and selection models of the sexually-selected trait of males' dates of annual moult into breeding plumage in a wild population of superb fairy-wrens (*Malurus cyaneus*). Using 3254 moult records from 1110 males over 27 years, we found that there was significant additive genetic variance in the trait in every age class. Both genetic and non-genetic sources of phenotypic variance in the trait were greatest in the oldest age class. Selection differentials indicated positive selection on the trait across age classes, with some evidence that selection increased with age. However multivariate selection gradients, which controlled for covariance in trait expression between the age classes, showed that there was no evidence of direct selection on the trait in any age class, except for marginally significant positive selection in the oldest age class. These findings provide the first empirical illustration of how genetic variance and selection varies with age in expression of a sexually selected trait that shows phenotypic improvement with age.

Introduction

Senescence, characterized by a decline in expression of fitness-related traits during late life, is understood to evolve as a consequence of a declining force of selection with increasing age (Medawar 1952; Hamilton 1966; Kirkwood and Austad 2000). However, phenotypic senescence is often observed in some fitness-related traits, but not others (Brunet-Rossinni and Austad 2006; Bouwhuis and Vedder 2017; Cohen et al. 2020). In a review of trait-specific ageing across wild bird populations, Bouwhuis and Vedder (2017) found that while 89% of physiological traits showed evidence of senescence, only 50% of sexually selected traits did. Paradoxically, precopulatory sexually selected traits (SSTs) will sometimes even continue to improve with age, despite evidence of a generalized deterioration in reproductive and somatic functioning co-occurring at old ages (e.g. Galvan and Møller 2009; Nussey et al. 2009; Potti et al. 2013). These traits range in form from bright coloration (Candolin 2000; Miller and Brooks 2005; Galvan and Møller 2009; Potti et al. 2013) to courtship behaviour (Mountjoy and Lemon 1996; Forstmeier et al. 2006), to weapons used in intrasexual competition for mates (Nussey et al. 2009). Expression of these traits is typically assumed to be condition-dependent, and also to be associated with somatic costs. These costs may be realized immediately, such as via increased predation risk (Huhta et al. 2003), or, be more sustained across an individual's life, such as via increased oxidative stress (Garratt and Brooks 2012) or more rapid senescence (Adler et al. 2016).

A positive association between SST expression and age has been previously been assumed to be a consequence of selective disappearance of low quality individuals at older ages (Trivers 1972; Hansen and Price 1995). However, more recently, longitudinal individual-based studies of wild animal populations have provided evidence that often selective disappearance does not play a significant role in driving the positive association between age and SST expression at the population level, but rather within-individual increases in trait expression with increasing age are occurring (Nussey et al. 2009; Potti et al. 2013; Cooper et al. 2020).

How can individuals express continual improvement in SSTs with age, despite senescence simultaneously occurring in other fitness-related traits? Further, what prevents younger individuals from expressing high levels of the trait if they are capable of doing so at older ages? That old individuals express the SST at high levels while young individuals express the SST at low levels could be a consequence of either (1) genetic constraint, or (2) adaptive trait plasticity. First, it is possible that the age-related improvement in SST expression is a consequence of genetic constraints at young ages, whereby the genetic architecture of the trait prohibits high-levels of trait expression in early life.

Alternatively, adaptive plasticity in SST expression could result in age-related improvement in the trait if the cost-benefit ratio of increased trait expression shifts with age, resulting in trade-offs between the SST and other fitness-related traits (Kirkwood and Rose 1991). For example, if individuals can only improve other courtship behaviors with experience (and thus age), there will be greater benefit of high expression of the SST at later ages once they have improved efficacy in courting behaviors. Additionally, as a consequence of somatic senescence, future reproductive potential may decline with increasing age, resulting in an increase in the relative value of current reproduction (Kirkwood and Rose 1991; Kokko 1998). Investment in a somatically-costly SST may therefore be more profitable late in life, when the expected reproduction foregone by the increased risk of mortality will be less than in early life (Kokko 1998). This is possibly especially true in a species where, on average, males can rely on at least some reproductive success every year with their social partner (Cooper et al. 2020), independent of investment in the SST. In either of these cases, individuals increase their lifetime fitness payoff by investing more in SST expression with increasing age. Game theory models suggest that age-dependent sexual advertisement can be adaptive, as it is an evolutionary stable strategy under a variety of theoretical conditions (Lucas and Howard 1995; Lucas et al. 1996; Kokko 1997).

Despite the wealth of theoretical work on the topic, there has been relatively little empirical investigation into the evolution of SSTs that improve with age, especially in natural populations. Measuring how SST genetic architecture and selective forces change with age can offer valuable insights into the evolution of age-dependency in these traits. The evolutionary theory that senescence is a consequence of declining selection with increasing age has received empirical support from quantitative genetic analyses of senescent traits in wild populations (Charmantier et al. 2006; Wilson et al. 2007; Chantepie et al. 2015) . The age-related increases in additive genetic variance found in senescent traits is typically assumed a characteristic of senescence, resulting from declining strength of selection with increasing age (Charlesworth 1990; Moorad and Promislow 2009). However, to our knowledge, no study to date has attempted to investigate the age-specific genetic architecture and selection on a trait which shows continual age-related improvements, and thus no senescence.

The scarcity of empirical investigation into the age-dependent genetic architecture and selection on SSTs in natural conditions is likely related to logistical difficulties, namely the intensive sampling of a wild population that is required for such analyses. First, the expression of the SST needs to have been tracked across the lifespans of individuals in a wild population (to adequately measure age-related change). Second, the fitness of individuals needs to be determined, which, for males, typically requires

genotyping of all individuals in the population (to estimate lifetime reproductive success). Third, these sampling efforts should ideally continue across several generations of the population, so that potential associations between trait expression or reproductive fitness and time period can be adequately controlled for (Bennington and Thayne 1994).

The superb fairy-wren (*Malurus cyaneus*; hereafter 'fairy-wren'), a sexually dichromatic passerine endemic to southeastern Australia, is an excellent species in which to investigate the genetic architecture of an age-dependent SST. Male fairy-wrens moult from brown plumage into blue nuptial plumage each breeding season. Although virtually all males complete an annual moult, there is large variation in the time at which they complete their moult, from as early as March (six months before the breeding season) to as late as November (one month after the start of the breeding season) each year (Cockburn et al. 2008a). Female fairy-wrens have a strong preference for early-moulting males, with the date that a male completes his moult correlating strongly with his reproductive success in that year (Dunn and Cockburn 1999; Cockburn et al. 2008a). Additionally, moult date is also strongly correlated with age, demonstrating a steady improvement in the trait expression (i.e. earlier moult) with increasing age up to the maximum observed lifespan of male fairy-wrens (Figure 1, Cooper et al. 2020). Why males display age-related improvement is unclear. Survival senescence begins at age 1, and reproductive success begins to decline after age 6 (Cooper et al. 2020), and yet males continue to increase display of a costly trait as their phenotype is apparently deteriorating in other ways. Male moult date is condition-dependent, with earlier annual averages being associated with favorable weather conditions (specifically, higher rainfall) in a given year (van de Pol and Cockburn, 2011).

In this study, we investigate the age-specific genetic variances and strength of selection on male fairy-wren moult date in order to better understand the relative roles of genetic constraint and adaptive plasticity in shaping age-related improvement in a SST. Using life-history and pedigree data from a long-term study of a wild population of fairy-wrens, we use an linear mixed model with a random effect constrained by a pairwise relatedness matrix (i.e. an 'animal model'; Kruuk 2004) to quantify components of additive genetic and environmental variance underlying the trait within each age class, and to quantify the genetic and environmental correlations between age classes. We then use bivariate generalized linear models of moult date and lifetime reproductive success to quantify the strength of selection on trait expression at different ages across the lifespan of male fairy-wrens.

Methods

Study population

The population of fairy-wrens located in and around the Australian National Botanic Gardens, Canberra, Australia (35°16 S, 149°06 E) has been intensively monitored since 1988 (Cockburn et al. 2003). The study site is approximately 60 ha in area and contains 40-90 territories, encompassing between 120-230 year-round resident adults. All individuals within the study are colour-banded shortly after hatching (or, for immigrants into the study area, shortly after their arrival). Many components of life-history are tracked for each individual in the population through continual observation, and the date of each adult male's moult from the brown winter plumage to blue breeding plumage is known within an accuracy of one week or shorter. Fairy-wrens reach sexual maturity during their first year of life, and virtually all male fairy-wrens moult into breeding plumage before their first breeding season (i.e. within one year of hatching), and continue to moult annually thereafter (Cockburn 2008a, Cooper et al. 2020). Male fairy-wrens within the study site are typically tracked for the entirety of their life since males rarely disperse further than one territory away from their natal territory (Cockburn et al. 2016). Fairy-wren territories are relatively stable, with each group composed of a dominant female, a dominant male, and between zero and four (or, extremely rarely, five) subordinate 'helper' males who are typically living on their natal territory (Hajduk et al. 2020). These 'helper' males are sexually mature and can gain paternity both on their own territory (if the dominant female is not their mother), or through extra-group matings with females from other territories (Cockburn 2016; Hajduk et al. 2020). Extra-pair mating is common in the fairy-wrens, with 61% of offspring being sired by a male who is not the female's social partner, which 95% of these extra-pair matings being with a male from another territory (Hajduk et al. 2018, 2020). Females control this extra-group mating, undertaking pre-dawn forays to neighbouring territories to choose an extra-group mate (Double and Cockburn 2000). Males that complete their moult to blue breeding plumage earlier in the year gain more reproductive success, indicating that females may be making their choice of extra-pair mate well before the start of the breeding season, that they may be evaluating the endurance of male display over the year as males start displaying as soon as they turn blue, and/or that moult date may be strongly correlated with other unmeasured aspects of male phenotype on which a female is choosing at the time that she mates (Double and Cockburn 2000).

Total sample size comprised of 3254 moult records from 1110 males over 27 years (1992-2019). We measured moult date as the number of days before December 31st that a male completed his moult into blue breeding plumage. A higher value therefore indicates an earlier moult completion date and greater expression of the SST. A small number of males never completed moult into blue plumage in a given

year (156 observations, 5% of the sample). For these records, moult date was censored to one day later than the latest a male ever completed full moult in any breeding season (30 days before Dec. 31). In a different subset of 2% of cases (51 observations), males did not moult back into brown plumage following the end of one breeding season (typically in February), and instead remained in blue plumage throughout the whole year. For these males in these years, moult dates were censored to one day earlier than the earliest a male has ever moulted into blue plumage (309 days before December 31st).

Adult male fairy-wrens have an average lifespan of 3.4 years (2.2 SD) (excluding individuals that died before age 1 when they reach reproductive maturity). Maximum recorded lifespan is 12, but mortality risk increases continuously with age from age 1 onwards, and as a result less than half of adult males survive to the age of three, and only 14% of adult males survive to the age of six (Cooper et al. 2020). Due to the declining sample sizes with increasing age, we pooled males of ages 3-5 years into one class, and males aged 6+ years into another, for the purposes of our analysis. This resulted in four age classes: age 1 (1029 moult records and males), age 2 (727 moult records and males), age 3-5 (1113 moult records from 520 males), and age 6+ (385 moult records from 165 males).

Quantitative genetic analysis

We fitted a quantitative genetic 'animal model' in order to investigate how the genetic and environmental effects of moult date (co)varied amongst the age classes (Kruuk 2004). All ages were included within a single model. Prior to analysis, moult date was z-transformed (mean centered and divided by the standard deviation across all age classes) in order to facilitate model convergence. The model included age (in years) as a second order polynomial fixed effect. Random effects decomposed the total phenotypic variance for each age category into four components: additive genetic variance, "individual environment" variance, variance associated with the year of trait expression, and residual variance. "Individual environment" variance (sometimes known as "permanent environment" variance) is typically estimated from repeated measures of the same individuals across years (Kruuk 2004; Wilson et al. 2010), and in such models the residual variance term represents within-individual variance due to, for example, within-individual plasticity, stochasticity or measurement error. In our model this was possible only for the age classes which spanned more than one year (age 3-5 and age 6+). For the age classes that contained only one year (age 1 and age 2), the individual environment variance is indistinguishable from residual variance. To avoid this source of variance being arbitrarily split across the two terms, we fixed the residual variance components to effectively zero (1×10^{-5}), so that all variance

attributable to individual environment was assigned to the individual environment term in the model, and could therefore covary with the individual environment terms for other age classes.

Thus, the model of moult date (*z*) of male *i* in year *j* can be written:

$$z_{ij} = \mu + \beta_1 . age_{ij} + \beta_2 . age_{ij}^2 + \left(age_{class_{ij}} \cdot a_i\right) + \left(age_{class_{ij}} \cdot ID_i\right) + \left(age_{class_{ij}} \cdot y_j\right) + \left(age_{class} \cdot r_{ij}\right)$$

Where μ is an intercept, β_1 and β_2 are the coefficients for the fixed effects of age (for a second-order polynomial), age_{class} is a categorical variable which interacts with each random effect to give separate variance estimates for each age class (1, 2, 3-5, and 6+) for each of the random effects (a_i: additive genetic , ID_i: individual environment, y_j: year, r_{ij}: residual). Additive genetic values (*a*) are normally distributed as $(a_1, \ldots, a_n)^T \sim N(0, \sigma_A^2(z)\boldsymbol{A})$ where $\sigma_A^2(z)$ is the additive genetic variance for moult date, n is the number of males, and $\boldsymbol{A}$ is the relatedness matrix between individuals. We estimated the covariance between age class-specific variance estimates for each random effect and from this calculated the correlation between ages for each random effect (Wilson et al. 2010). The model was run in a Bayesian framework using the R package MCMCglmm (Hadfield 2010), with Gaussian errors. We report point estimates as posterior modes and uncertainty as the 95% highest posterior density credible intervals. For all random effects, we used parameter expanded priors belief parameters of 0.002, variances of 1, prior means of 0 and prior covariances of 1000. We ran the model for 9 million iterations, with a burn-in of 20,000 and a thinning interval of 1500. We checked mixing and convergence by visual inspection of trace plots for all parameters. Effective sample sizes were < 300 for some estimates, as fixing the residuals for age classes 1 and 2 resulted in difficulty in mixing. To insure the model was reasonable, we reran the model twice more for a shorter number of iterations (3 million) and found qualitatively the same results each time.

Selection models

We estimated selection on age-specific moult date from the covariances between fitness and moult date using four separate bivariate generalized linear mixed models, one for each age class, again fitted using MCMCglmm (Hadfield 2010). Lifetime reproductive success (LRS) was used as a proxy for fitness. We

measured LRS of males as the total number of chicks whose paternity was assigned to a given male from SNP genotyping of all nestlings within the study site, using a blood sample taken shortly after hatching (Cockburn et al. 2020). After exclusion of males for which LRS could not be confidently estimated (due to their living close to the study area border, poor quality blood sampling, or having only been tracked for part of their entire lifespan), our resulting sample size was 2795 moult records from 946 males. LRS was modelled as an over-dispersed Poisson trait (with log-link function) and moult date was modelled as a Gaussian trait. The models for the two age classes incorporating multiple ages (age 3-5 and age 6+) contain a fixed effect of age (years) as a categorical factor (3 and 6 levels, respectively). Random effects of year of trait expression ('year') for moult date and of an individual's birth ('cohort') for LRS were included in the models of moult date for each age class (Bennington and Thayne 1994).

From these models, we estimated four different metrics of selection (Lande and Arnold 2014; Arnold et al. 2019) on moult date in each age class:

1. *Selection differentials* (the change in mean phenotype due to selection, within-generation and within-age class) were estimated directly from each model. Usefully, because of the log-link function used in the GLMM, the estimate of the latent-scale covariance between the trait and *absolute* fitness is equal to the data-scale covariance between the trait and *relative* fitness (Bonnet et al. 2019; Morrissey and Bonnet 2019), and could therefore be taken as a measure of the selection differential (Lande and Arnold 2014).

2. We also estimated a '*force of selection*' for each age class as the selection differential weighted by probability of survival to the given age class (the population-level change in phenotype due to selection in a given age class). For the four age classes, these probabilities were 1.00, 0.70, 0.48, and 0.15 respectively.

3. *Univariate selection gradients* β_{UV} (the rate of increase in fitness with each unit increase in trait value; Phillips and Arnold 1989) were estimated by dividing the covariance by the total phenotypic variance for each age class (estimated from the quantitative genetic animal model, see above).

4. *Multivariate selection gradients* were calculated in an analogous way to the univariate selection gradients except using partial selection differentials for each age. We combined the four covariances from the four bivariate models of each age class with LRS to obtain a vector of selection differentials $\boldsymbol{S}$. We used the results from the animal model above to estimate phenotypic variances and covariances for the four age classes, generating a 4x4 phenotypic variance-covariance matrix $\boldsymbol{P}$ for moult date in the

four age classes. We then applied the formula $\boldsymbol{P}^{-1}\boldsymbol{S}$ to obtain the direct selection gradient β_{MV} for moult date in that age class, corrected for the indirect selection on moult date in each of the other age classes. Each age-specific β_{MV} thus gives an estimate of the 'direct' selection on age-specific trait values, independent of selection acting via correlated values at other ages (Phillips and Arnold 1989).

All models were run in a Bayesian framework using the R package MCMCglmm (Hadfield 2010), with Gaussian errors. We report point estimates as posterior modes and uncertainty as the 95% highest posterior density credible intervals. For random effect estimates we used parameter expanded priors belief parameters of 0.002, variances of 1, prior means of 0 and prior covariances of 1000. We ran each model for 240,000 iterations, with a burn-in of 7,000 and a thinning interval of 120. We checked mixing and convergence by visual inspection of trace plots for all parameters.

Results

Average male moult date became earlier with increasing age in fairy-wrens (Figure 1). Along with this phenotypic shift, there were also changes in both the genetic architecture, and the pattern of selection on the trait between the age classes.

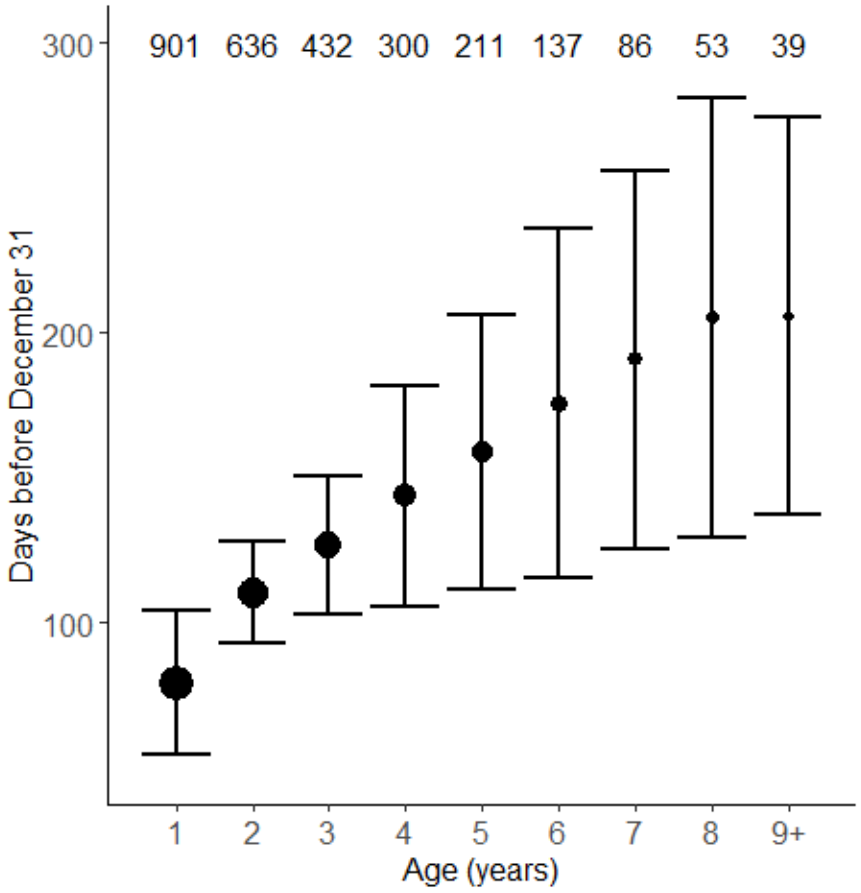


Figure 1. Raw data means and standard deviations for moult date at each age. Point size is log-proportional to the number of individuals in the sample, and the number of individuals is also denoted across the top of the graph. Moult records from individuals aged 9 – 12 are grouped together for illustrative purposes.

Animal Model: Components of variance and covariance in age-specific moult date

Using the animal model, we were able to quantify the (additive) genetic variance and environmental (individual environment, year, and residual) variances for moult date in each age class (figure 2, table 1,

2 & 3). Total phenotypic variance varied between the age classes ($V_{P\ age1}$ = 0.27, $V_{P\ age2}$ = 0.13, $V_{P\ age3\text{-}5}$ = 0.57, $V_{P\ age6+}$ = 1.76). Age class 6+ had the greatest total phenotypic variance in moult date and this was reflected in larger variance estimates for all random effects in this age class, but especially in the residual variance (figure 2). Age 2 had the least phenotypic variance in moult date, with especially small estimates of additive genetic and individual environment (residual) variances (figure 2). Note that residual variance for ages 1 and 2 was fixed to effectively zero in the model, in order to facilitate (co)variance estimates for individual environment (see Methods). The percentage of age-specific phenotypic variance attributable to additive genetic variance (i.e. the narrow sense heritability estimates) declined with increasing age, from 43% (CI: 31 – 57%) at age 1, to 18% (CI: 4 – 37%) at age 6+ (table 1). Conversely, the percentage of phenotypic variance attributable to 'individual environment' (i.e. the individual ID term and any residual variance), was smaller at age 1 (42%; CI: 32 – 57%) and age 2 (37%; CI: 24 – 54%), compared to the older ages 3-5 (61%; CI: 50 - 75%) and 6+ (60%; CI: 46 – 81%; table 2). The percentage of phenotypic variance attributable to year did vary across the age classes, but not in a way that suggested the variation was linearly related to age (table 3).

Using the animal model we quantified the covariances and correlations between age classes for each random effect. Both additive genetic and year effects were strongly positively correlated amongst early age classes (age 1, 2, and 3-5), while these effects in age 6+ were only weakly positively correlated with age 3-5, and there was no significant correlation with ages 1 or 2 (table 1, 3; though all estimates of additive genetic covariances were positive). Correlations between ages in individual environment effects were only significant between ages 1 and 2, and between ages 2 and 3-5, and these positive correlations were all weaker than those found for additive genetic variance components, or year variance components (table 2).

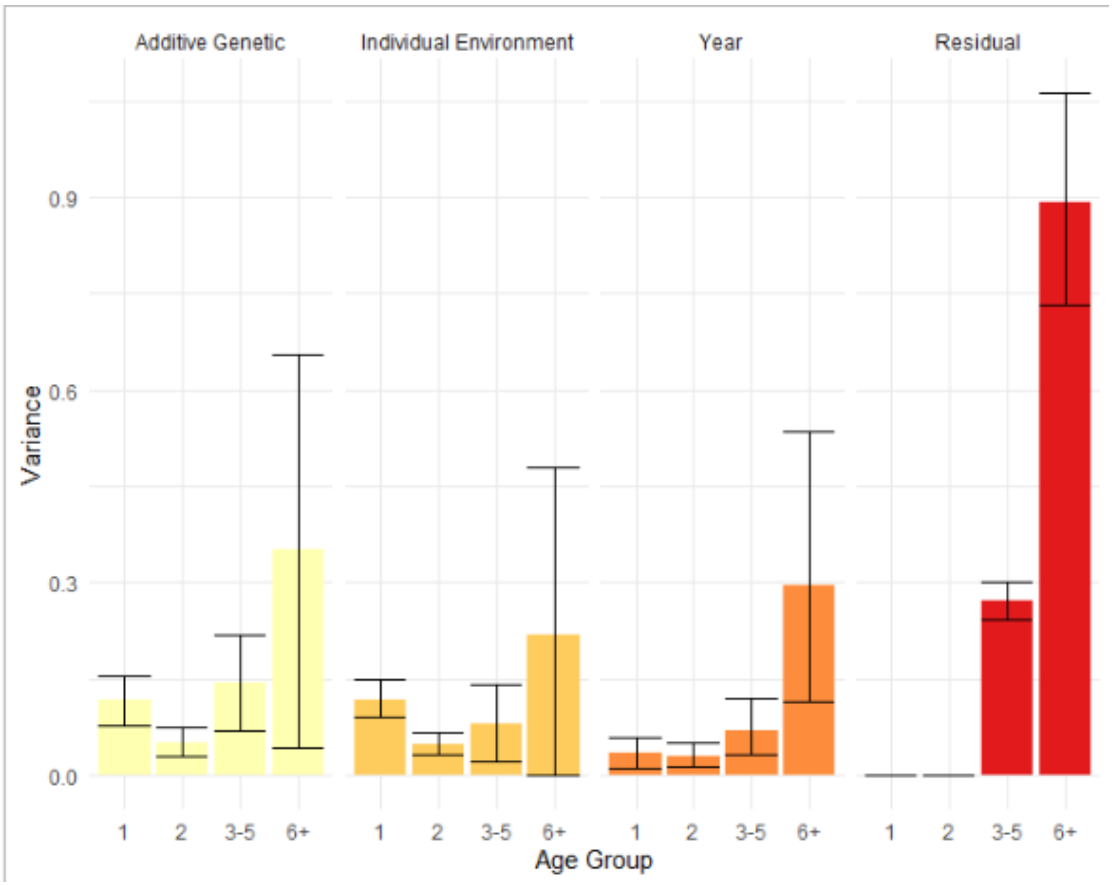


Figure 2. Variance estimates for year, additive genetic, and individual environment effects, as well as residual (within-individual environment) variance for each age class from the animal model of male moult date. Values shown are posterior means and 95% credible interval from MCMCglmm models. (Note that because age classes 1 and 2 only had one measure, the individual environment variance is equivalent to the residual variance.)

		Covariances			
	$\left(\frac{V_A}{V_P}\right)$	**Age 1**	**Age 2**	**Age 3-5**	**Age 6+**
Correlations	**Age 1**	0.12 [0.08; 0.15] *43% [31 - 57%]*	0.06 [0.04; 0.09]	0.1 [0.06; 0.14]	0.05 [-0.03; 0.14]
	Age 2	0.82 [0.64; 0.94]	0.05 [0.03; 0.07] *39% [24 - 56%]*	0.07 [0.04; 0.1]	0.05 [-0.01; 0.12]
	Age 3-5	0.75 [0.53; 0.96]	0.8 [0.64; 0.96]	0.14 [0.07; 0.22] *25% [14 - 38%]*	0.13 [0.01; 0.26]
	Age 6+	0.28 [-0.2; 0.77]	0.41 [-0.07; 0.85]	0.59 [0.2; 0.93]	0.35 [0.04; 0.66] *18% [4 - 37%]*

Table 1. Additive genetic variances and percentage of phenotypic variance associated with individual environment for each age class (diagonal), as well as covariances (above the diagonal) and correlations (below the diagonal) between age classes. Estimates are from the animal model. Square brackets contain the 95% credible intervals.

		Covariances			
	$\left(\frac{V_{ID}}{V_P}\right)$	**Age 1**	**Age 2**	**Age 3-5**	**Age 6+**
Correlations	**Age 1**	0.12 [0.09; 0.15] *42% [32 - 57%]*	0.02 [0; 0.04]	-0.01 [-0.04; 0.02]	-0.05 [-0.12; 0.02]
	Age 2	0.22 [0.01; 0.41]	0.05 [0.03; 0.07] *37% [24 - 54%]*	0.03 [0; 0.06]	0.02 [-0.03; 0.07]
	Age 3-5	-0.12 [-0.53; 0.26]	0.49 [0.17; 0.76]	0.08 [0.02; 0.14] *61% [50 - 75%]*	0.07 [-0.01; 0.16]
	Age 6+	-0.33 [-0.8; 0.16]	0.16 [-0.39; 0.68]	0.5 [-0.02; 0.96]	0.22 [0; 0.48] *60% [46 - 81%]*

Table 2. Individual environment variances and percentage of phenotypic variance associated with individual environment for each age class (diagonal), as well as covariances (top) and correlations (bottom) between age classes. Estimates are from the animal model. Square brackets contain the 95% credible intervals. Note these (co)variances and correlations do not include residual variance, which was estimated as a non-zero value only for ages 3-5 and 6+ (see methods).

		Covariances			
	$\left(\frac{V_Y}{V_P}\right)$	**Age 1**	**Age 2**	**Age 3-5**	**Age 6+**
Correlations	**Age 1**	0.03 [0.01; 0.06] *10% [5 - 21%]*	0.02 [0.01; 0.04]	0.03 [0.01; 0.06]	0.02 [-0.03; 0.07]
	Age 2	0.8 [0.56; 0.98]	0.03 [0.01; 0.05] *21% [11 - 33%]*	0.03 [0.01; 0.06]	0.02 [-0.02; 0.07]
	Age 3-5	0.68 [0.39; 0.93]	0.74 [0.48; 0.94]	0.07 [0.03; 0.12] *10% [6 - 20%]*	0.07 [0; 0.15]
	Age 6+	0.2 [-0.25; 0.62]	0.24 [-0.21; 0.63]	0.46 [0.09; 0.81]	0.3 [0.11; 0.53] *15% [7 - 27%]*

Table 3. Year variances and percentage of phenotypic variance associated with year for each age class (diagonal), as well as covariances (top) and correlations (bottom) between age classes. Estimates are from the animal model. Square brackets contain the 95% credible intervals.

Selection Analysis

We estimated four different metrics of selection for each of the age classes in order to ascertain a comprehensive description of age-specific selection on moult date. Selection differentials increased with increasing age, with a notably large increase in selection in the age 6+ group, although the credible intervals are large (figure 3A). The survival-weighted selection differentials (figure 3B) illustrate that once survival probability to each age class is accounted for, there is little apparent difference between

ages in how much the trait is displaced by age-specific selection at the level of the population. The selection gradient measures (figure 3C) indicate that the slope of selection is roughly equal for ages 1, 3-5, and 6+, and the steepest selection gradient occurs at age 2. Lastly, estimating 'direct' selection from multivariate selection gradients (i.e. controlling for the effects of phenotypic covariance between age classes), there was no evidence that selection on moult date was significantly different from zero in any age class, although it approaches significance for ages 6+ (figure 3D; est. 16.84; 95% CI: -0.67, 32.93), and the smallest estimate was for age one (est. 3.91; 95% CI: -18.51, 27.50).

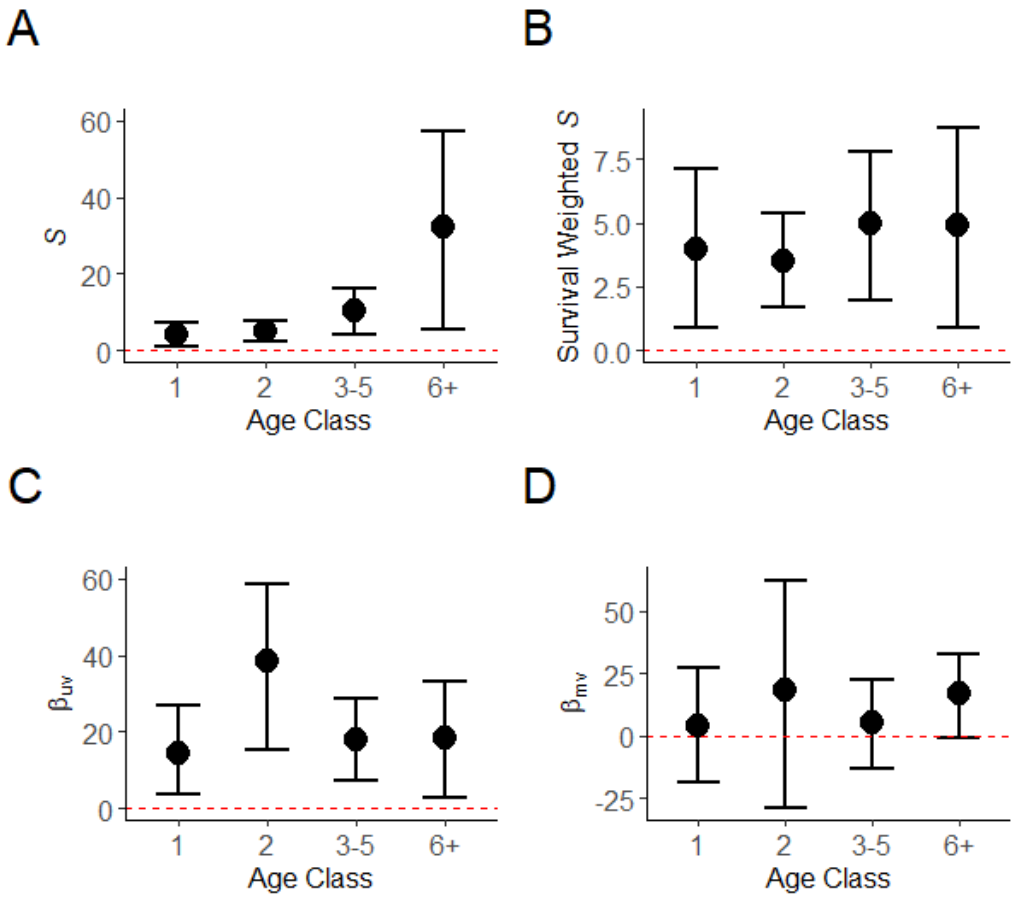


Figure 3. Estimates of selection on moult date within each age class with 95% credible intervals. The four metrics of selection shown for each age class are: (A) selection differentials *S*, (B) the force of selection (selection differential (*S*) multiplied by the probability of survival to that age class), (C) univariate selection gradients β_{UV}, and (D) multivariate selection gradients (controlling for phenotypic covariance between moult dates between age classes), β_{MV}. Estimates that span the red dotted line are not significantly different from 0.

Discussion

Our analyses of age-specific variance components and selection pressures for the male secondary sexual trait of timing of moult into breeding plumage showed changing patterns across the lifespan. There was

evidence of additive genetic variance in each age class. This rules out the possibility that the age-related improvement in moult date is driven by an absolute genetic constraint preventing younger individuals from moulting earlier. Additionally, the evidence of additive genetic variance in each age class suggests that the trait has not already reached an evolutionary equilibrium at an optimum fitness peak within each age class. Thus, age-specific estimates of selection may help us better understand the age-dependent pattern of trait expression.

Our estimates of selection considered the covariance between annual moult date (within an age class), and a male's lifetime reproductive success, thus not only considering how moult date is associated with current reproductive success, but also future and past reproductive success and survival. Selection differentials increased with increasing age class, which indicates a larger effect of the selection on moult date at later ages, relative to earlier ages (Figure 3A). That is to say, a male at age one that completes his moult relatively earlier than the average one-year-old will have a higher lifetime reproductive success than the population average, but, if that same male instead moulted equally relatively early at age 6+ compared to the average 6+ male, the fitness benefit for that male would be greater. This is likely to be driven by the much greater variance in the trait in the oldest age classes (age 6+, Figure 2): a 'relatively early' male will be much further from the population mean in the older ages, than amongst the young ones. The increase in selection differentials in the older age classes could also be a consequence of changes in reproductive strategy, and consequently the importance of sexual advertisement, that are correlated with age in male fairy-wrens. Fairy-wrens are cooperative breeders and males queue for the dominant position on a territory based on age, with the eldest male on a territory assuming the dominant position (Cockburn et al. 2008b). The younger subordinate male fairy-wrens primarily achieve extra-pair success parasitically, by occupying hidden leks near the dominant male during the dawn chorus (Double and Cockburn 2003). Thus, the benefits of signaling quality through an early moult date may be more prominently realized by the (older) dominant males that gain extra-pair paternity through advertisement of their quality to females, rather than through parasitizing another male.

However, when considering selection on age-specific trait expression, it is important to also consider an individual's declining probability of surviving to each subsequent age class due either to senescence or simply the constant risk of extrinsic causes of mortality. Our metric of the strength of selection on moult date at each age does consider this by weighting the age-specific selection differentials by population-level survival probability (Figure 3B). As a consequence of this declining survival probability with increasing age, the effective 'force' of selection does not change with age. Further, when considering

selection gradients – the rate of change of LRS with increasing trait values, or effectively the slope of the regression of LRS on the trait – there was no evidence of changes with age (Figure 3C). This indicates that for a shift of moult date by a single day, the increase in LRS was the same, and illustrates the subtle difference in information conveyed by the selection differentials vs. gradients. When we extended these analyses to estimate multivariate selection gradients, accounting for the correlations across ages, all estimates remained positive, but with much larger uncertainty such that all credible intervals overlapped zero (with age 6+, very marginally). In summary, all our approaches to estimating selection indicate consistently positive directional selection at all ages, but that, aside from the simplest estimates of the selection differentials, little evidence that selection increased in magnitude with age: earlier moult would be favorable for both young and old males. It will also be valuable to compare the estimates here with selection coefficients calculated considering only reproductive success at that age, rather than considering lifetime reproductive success.

Although it is not possible to make inferences about the past evolution of a trait given contemporary selection (Wilson et al. 2010), these results do still beg the question: how did the observed age-related improvement in moult date evolve? It is possible that moult date is expressed at low values (i.e. late moult date) at younger ages because the apparent selection for early dates does not have any evolutionary effect. In particular, it could be that the observed phenotypic covariance between fitness and moult date (i.e. selection) at early ages is not due to any causal effects of the trait on fitness, but instead is the result of both higher fitness and earlier moult date being correlated with improved environmental conditions at young ages (Kruuk et al. 2002). This is plausible in the general sense, as SSTs are expected to be plastic, and to change in response to environmental conditions (Badyaev and Duckworth 2003; Badyaev and Young 2004; Hooper et al. 2018). There is also empirical evidence of this occurring. By estimating environmental and genetic selection gradients separately, apparent selection on a SST in red deer was shown to be driven by environmental covariance, possibly explaining the evolutionary stasis of the trait (Kruuk et al. 2014). In fairy-wrens moult date does vary with environmental conditions, with males moulting earlier when conditions are good (Cockburn et al. 2008a). However, in order for the age-specific covariance between moult date and environmental conditions to adequately explain the *age-related improvement* in moult date, there should be a decline in this environmental source of variance with increasing age, and/or an increase in the genetic sources of variance with increasing age. However, our results here actually suggest the opposite is true. Overall phenotypic variance in moult date is greatest for the oldest age class, followed by the second oldest age class. Additionally, the proportion of overall variance attributable to additive genetic variance (i.e.

heritability) declines with increasing age across the classes. Comprehensive test of this issue would require estimation of the respective genetic and environmental selection gradients (in addition to the phenotypic selection gradients presented here), which would be the subject of future analyses. However the analyses here indicate that an age-related change in the extent to which the association between moult date and LRS is driven by confounding environmental variation is not likely to adequately explain the age-related improvement in moult date.

Another possibility is that moult date co-varies with some other unmeasured trait(s), and the selection on these trait(s) is what drives moult date phenotype. Either the correlation between moult date and the unmeasured trait(s) must change with age, or, selection on the unmeasured trait(s) must change with age if it is to adequately explain the age-related improvement in moult date. For example, it could be that moult date at young ages is positively correlated with a trait that is under negative selection (or that moult date is negatively correlated with a positively selected trait, generating a trade-off at early ages), and uncorrelated with this same trait at older ages. Or, there could be a scenario whereby the correlation between moult date and the unmeasured trait(s) does not change with age, but selection on the unmeasured trait(s) is greater at older ages. Further investigation would be required to explore these possibilities further, but one obvious candidate additional trait could be survival, which shows an exactly opposite pattern of age-related change to moult date. By the above scenario, a negative genetic correlation between moult date and survival in early ages could possibly generate the observed pattern, and could be tested for with age-dependent estimates of genetic correlations.

It is interesting to note that moult dates are generally highly correlated across the earlier age classes, but correlations of the younger age classes with the age 6+ class are of a much lesser magnitude. This indicates that the environmental and genetic processes determining moult date differ in these older ages from those at younger ages. Thus, the difference in trait expression between the age classes could be a consequence of the different past evolutionary development of moult date in different age classes, for example, if the genes determining moult date at younger ages are under greater evolutionary constraint than the genes that determine moult date at older ages (Teplitsky et al. 2014).

FInally, it is worth noting that increasing additive genetic variance with increasing age is hypothesized to provide evidence for evolutionary models of senescence (Charlesworth 1990; Moorad and Promislow 2009), as shown for example in two ungulate populations (Wilson et al. 2007). Moult date is a trait that shows continual improvement with age and no senescence, and yet we observe the largest amount of additive genetic variance in the oldest age class, followed by the second oldest age. This finding

indicates that increasing additive genetic variance with age does not necessarily indicate trait senescence. Overall, our analyses here do not provide a definitive explanation for the improvement in a sexually-selected trait. However, combined with the results of Chapter 2 (Cooper et al. 2020), we can probably discount three possible explanations: selective disappearance, early-life constraints, and age-specific selection. We therefore suggest that life-history trade-offs between different fitness-related traits are likely to play an important role in determining the age-related expression of this trait.

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Chapter 4

Do the ages of parents or helpers affect offspring fitness in a cooperatively breeding bird?

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Abstract

Age-related changes in parental phenotypes or genotypes can impact offspring fitness, but separating germline from non-germline transgenerational effects of ageing is difficult for wild populations. Further, in cooperatively-breeding species, in addition to parental ages, the age of 'helpers' attending offspring may also affect juvenile performance. Using a 30-year study of a cooperative breeder with very high rates of extra-pair paternity, the superb fairy-wren (*Malurus cyaneus*), we investigated the effects of maternal, paternal, and helper ages on three measures of offspring performance: nestling weight, juvenile survival to independence, and recruitment to the breeding population. Mothers with a longer lifespan had offspring with higher juvenile survival, indicating selective disappearance, but the effect of maternal age on juvenile survival was of similar magnitude but negative. For extra-pair offspring, there was no evidence of any effect of the ages of either the genetic sire or the cuckolded 'social' father. However, for within-pair offspring, there was a positive effect of paternal age on juvenile survival, which we suggest may be driven by sexual selection. There were positive associations between the average age of helpers attending a nest and two of the three aspects of offspring performance; these effects were stronger than any of the effects of parental age. In general, the multiple associations between offspring fitness and the ages of adults around them appeared to be driven more by age-related changes in environmental effects than by age-related changes in the germline.

Introduction

Identifying how parents influence the fitness of their offspring is central to understanding evolution by natural selection. In iteroparous animals, the age of parents can affect their offspring either because of changes with age in the parental germline, or because of changes in the environment that parents provide (Priest *et al.*, 2002; Schroeder *et al.*, 2015; Lemaître & Gaillard, 2017). Negative effects of ageing in human parents on offspring fitness have been recognized for over 100 years (Bell, 1918), and similar deleterious effects are increasingly being observed across the animal kingdom (Priest *et al.*, 2002; Fox *et al.*, 2003; Bouwhuis *et al.*, 2010; Carnes *et al.*, 2012; Api *et al.*, 2018). These negative transgenerational effects of ageing parents are typically attributed to age-related germline changes, such as *de novo* mutations and epigenetic changes that occur over time (Soubry *et al.*, 2014; Sharma *et al.*, 2015; Markunas *et al.*, 2016). However, the environment that offspring experience can also vary with the age of their parents, with changes in parental care or in the quality of the external environment shared by parents and offspring potentially having substantial effects on offspring performance. For example, physiological senescence of parents could result in poorer provisioning with increasing age, both pre-natal and post-natal (Moorad & Nussey, 2016; Lemaître & Gaillard, 2017). Alternatively, improvement of parental caring ability through experience, or accumulation of resources, could result in increases in the provision of care as parents age (Daunt *et al.*, 2007; Froy *et al.*, 2017). Pair duration may also co-vary with age and impact offspring fitness (Spoon *et al.*, 2006; Nisbet & Dann, 2009). The different germline and environmental components of parental age effects may also not be mutually exclusive: for example there could be germline-level deterioration with age co-occurring with age-related changes in the effectiveness of parental care (Monaghan *et al.*, 2020). As a result of these multiple potential effects, inferring the direction of causation of changes in offspring performance with parental age is notoriously difficult, as for example when favorable environmental conditions result in both longer-lived parents and higher offspring performance without there being any underlying causal association between parental age and offspring performance.

In wild populations, the relative importance of these multiple different components of parental age effects are especially poorly understood. Most previous research has either isolated germline effects, or quantified combined germline and environmental effects (Lemaître & Gaillard, 2017; Monaghan *et al.*, 2020). To our knowledge, only one study to date has differentiated between germline and environmental parental age effects within the same population. Using cross-fostering experiments in a wild population of house sparrows (*Passer domesticus*), a study by Schroeder et al. (2015) found

negative effects of the age of the genetic parents on chick fitness, but no effects of the age of the rearing parents. However, a cross-fostering manipulation necessarily removes potentially interesting aspects of natural variation in mating success and rearing ability. In particular, it removes any potential role of female choice, sexual selection, and any differential allocation in the natural breeding dynamics, as females are not raising extra-pair chicks from sires they themselves chose (Burley, 1988; Sheldon, 2000). Germline changes may result in some males producing offspring of lower quality as they age. However, such senescence is likely to vary between males (Charlesworth, 1990; Moorad & Promislow, 2009). If female choice discriminates against senescent males so only older males that do not exhibit senescence are able to mate, negative effects of male germline senescence may not be apparent in natural conditions (Bowers *et al.*, 2015). This may explain the paradox that females often demonstrate a preference for older sires, despite the evidence for negative effects of paternal germline (Johnson *et al.*, 2015; Gaillard & Lemaître, 2017). It is therefore also useful to investigate germline and environmental paternal age effects observationally in a natural environment, without impeding any potential role of sexual selection. This can be done by using observational data on a population with biparental care of offspring, but where females are often unfaithful to their social mate – such that some offspring will be cared for by an unrelated male. In such systems, extra-pair mating allows the germline and environmental effects of paternal ages to be separated.

In cooperative breeders, it is not only the ages of the parents that may influence offspring fitness. Ages of the group members that act as helpers in the rearing of offspring may also potentially be of importance. The fitness of the young may be affected by the presence (Covas *et al.*, 2011; Hammers *et al.*, 2019b), number (Sparkman *et al.*, 2011; Brouwer *et al.*, 2012), sex (Stacey & Koenig, 1984; Hailman *et al.*, 1994), behavior (Russell *et al.*, 2007; Hammers *et al.*, 2019a), or relatedness (Green *et al.*, 2016) of helpers . There is also evidence from several species that helpers become more effective in provisioning young with increased experience. For example, in purple gallinules (*Porphyrula martinica*) and El Oro parakeets (*Pyrrhura orcesi*), older or more experienced helpers feed chicks more frequently (Hunter, 1987; Klauke *et al.*, 2014), and in white-winged choughs (*Corcorax melanorhamphos*) and apostlebirds (*Struthidea cinerea*), older helpers spend more time incubating chicks (Heinsohn & Cockburn, 1994; Woxvold *et al.*, 2006). However, despite the evidence that the presence of helpers can affect offspring fitness, and the above evidence that helper behavior towards juveniles may change with their age, we are not aware of any study to date that has explicitly tested the impact of the age of helpers on fitness-related traits of offspring.

The gaps in our understanding of both parental and helper age effects in wild populations are likely a consequence of the difficulties associated with investigating the effects of the age of care-givers on fitness. Longitudinal tracking of individuals is typically required so that both parents and helpers can be accurately aged. Additionally, models of age-related effects are at risk of being biased by 'selective disappearance' if the lifespan of individuals is correlated with other aspects of individual quality (van Noordwijk & De Jong, 1986; van de Pol & Verhulst, 2006; Hayward *et al.*, 2013). This selective disappearance can be modelled by including parental lifespan as a covariate in models of offspring performance (van de Pol & Verhulst, 2006). In addition, including both parental age (at the time of breeding) and parental total lifespan as covariates allows for *within-individual* effects of ageing *per se* to be disentangled from *between-individual* effects whereby longer-lived individuals have different parental effects than shorter-lived individuals (i.e. an effect of lifespan). *Between-individual* effects can be confused for *within-individual* effects of age in cross-sectional studies where it is not possible to control for parental lifespans in analyses (van de Pol & Verhulst, 2006). As an additional logistical challenge of estimating transgenerational effects of age in the wild, offspring must also be tracked so that metrics of their fitness can be estimated, and genetic testing of both offspring and adult males in the population is necessary to confirm parentage.

The superb fairy-wren (*Malurus cyaneus*; hereafter 'fairy-wren') offers an excellent system with which to investigate both germline and environmental effects of parental age, as well as effects of helper age. The species is a cooperatively breeding passerine endemic to south-eastern Australia. Fairy-wrens occupy year-round territories, living in groups composed of a breeding female, a dominant male, and between zero and five sexually-mature male helpers (Cockburn *et al.*, 2016). The breeding female and the dominant male are aided in provisioning young by the helpers residing on their territory. Despite the socially-monogamous relationship between the dominant female and dominant male on a territory, fairy-wrens have high rates of infidelity: 61% of chicks are sired by an extra-pair male that almost always (95%) resides on a different territory (Hajduk *et al.*, 2018; 2020a).

In this study, we aimed to quantify the effects of maternal, paternal and helper ages on three components of chick fitness in a wild population of fairy-wrens: (*i*) weight as a nestling (known to be under positive selection) (Hajduk *et al.*, 2020b), (*ii*) survival to foraging independence, and (*iii*) recruitment of male offspring into the breeding population in the year after hatching. We included the lifespan of each parent in our models as a test for selective disappearance of parents of differing performance, as well as to distinguish within-individual effects of parental ageing from between-

individual effects of differences between parents. Using the naturally-occurring instances of extra-group matings, we were able to separate and quantify age-related effects of both paternal germline and paternal environment without impeding any influence that sexual selection (i.e. female choice) may have had on these paternal age effects.

Methods

Study Population

Our study population of superb fairy-wrens is located in and around the Australian National Botanic Gardens, Canberra, Australia (35°16 S, 149°06 E) and has been intensively monitored since 1988 (Cockburn *et al.*, 2003, 2016). The study site, approximately 60 hectares in area, contains 40-90 territories encompassing between 120-230 year-round resident adults. Fairy-wrens are multi-brooded, with females having an average of 1-2 fledged nests per year within each breeding season, which runs from September through February (Cockburn *et al.*, 2016). Broods contain 2-5 chicks, with the large majority having 3 chicks. Brood size (number of chicks) typically equals clutch size (number of eggs) as only 5% of eggs are infertile and partial predation of a nest is rare (Cockburn *et al.*, 2016). Shortly after hatching, all chicks within the study area are colour-banded, and a blood sample taken to assign parentage using SNP genotyping (van de Pol *et al.*, 2020).

The life history of both male and female adult fairy-wrens facilitates easy tracking of their age and eventual lifespan (Cockburn *et al.*, 2008b, 2016). Males are philopatric, with 72% of males recruited as adults on their natal territory, where they usually remain for their entire life (Cockburn *et al.*, 2008b). Males that do disperse move to an immediately neighbouring territory 95% of the time (Cockburn *et al.*, 2008b). Females disperse from their natal territory and must establish themselves on a new territory as the dominant female for their first breeding season at the age of one. Thus, between reaching independence from their parents at six weeks old, and before the age of one year, female disappearance from the study area cannot be distinguished from dispersal. However, all surviving females occupy their own breeding territory by the age of one year, and remain on their first breeding territory for their entire lives 80% of the time (in the rare cases that they do move subsequently, it is most common for them to move directly to an adjacent territory (Cockburn *et al.*, 2016)). Juvenile females that immigrate into the study area are known to be juveniles, as they only immigrate during a narrow time period of the calendar year (see Cockburn *et al.*, 2003 for a more detailed description of

female immigration). Thus, female age and lifespan is reliably tracked for females aged one year and above.

Males are fertile and can sire offspring from age one. Dominant males may gain reproductive success as either the dominant male on the territory ('within-pair sire'), or as an 'extra-pair sire' for females on other territories (figure 1). Helper males may gain reproductive success as extra-pair sires both with the breeding female on their own territory (though this is rare, see below) and with females on other territories. Helpers queue for the dominant male breeding position based on age: when the dominant male dies, the eldest of any helpers on the territory will assume the dominant position (Cockburn *et al.*, 2008b). Helpers can either be the sons of the dominant female on the territory, or be unrelated to the dominant female if their mother has died or dispersed and been replaced by another female (Cockburn *et al.*, 2008b, 2016). Due to age-related queueing for dominance, the dominant female is occasionally socially paired with her son as the dominant male on a territory. In these situations inbreeding is avoided, and all offspring in the clutch are extra-pair (Hajduk *et al.*, 2018).

Only 45% of territories contain any helpers, and most territories with help only have a single helper. Territories with help are associated with higher productivity (Hajduk *et al.,* 2020b). Helpers are equally likely to be the sons of the dominant female, or be unrelated. The number of son helpers and the number of unrelated helpers on a territory indicates slightly different information about that territory. The presence of unrelated helpers is indicative of a high quality territory, since it indicates that the territory is capable of supporting more than two adults (the dominant male and female). The presence of son helpers would similarly indicate high chick and adult survival, but it could also be indicative of a high quality mother who is capable of rearing offspring that survive beyond maturity. Recent evidence also suggests that female extra-pair mate choice is affected in different ways by the presence of son vs unrelated helpers (Hajduk *et al.*, 2020a). Because of the slightly different information conveyed by the numbers of son and unrelated helpers, we fitted each as its own variable (rather than the more usual approach of considering the total number of helpers of any type, e.g. (Hajduk *et al.*, 2018). Since having more than two son or unrelated helpers on a territory is rare (in this dataset only 2% and 1% of chicks had more than two son and unrelated helpers, respectively), we included the number of helpers as a three-level factor of 'none', 'one' or 'two or more' for unrelated and son helpers separately.

Dataset

We used data from breeding events spanning the 1988 – 2018 breeding seasons, during which a total of 8210 chicks were hatched within the study area. Our data set included only those chicks with complete records of the following information: hatch date; the identities, ages and lifespans of the mother, the social father, and, if different, the genetic father, and the presence and ages of any helpers in the group. This reduced the sample down to 4912 chicks (60% of the initial sample). All hatch dates of offspring are accurate to ± one day. All the lifespans of parents are accurate to within the year, thus we used year as the level of precision for parental ages. All chicks have a 'genetic' father (the male that sires the chick), and a 'social' father (the dominant male on the natal territory, who provides parental care). In the case of chicks sired extra-pair, the genetic father (hereafter the 'extra-pair genetic father') and the social father (hereafter the 'extra-pair (cuckolded) social father') are different individuals. For chicks sired within-pair, the genetic father and the social father are one individual (hereafter simply the 'within-pair father'). In the cases where the dominant male on a territory was the son of the dominant female (see *Study Population* above), this resulted in a social father who was not the genetic father of the offspring but was still genetically related to them (most likely as half siblings). As a consequence, separating genetic from environmental effects was more difficult in these cases and so we excluded any chicks in such clutches (141 chicks). We also excluded chicks whose genetic father was a helper on their natal territory since again these individuals share both genes and environment with the chicks, even though the chicks are extra-pair (165 chicks). The final sample therefore comprised 4538 chicks from 1691 clutches over 30 cohorts, with 537 mothers, 562 genetic fathers (within-pair and extra-pair), and 482 cuckolded social fathers. The identities of the social father and the genetic father were the same for chicks sired within-pair (45% of the sample). There were approximately equal numbers of male (2369) and female chicks (2153), and 25 chicks were of unknown sex, which were all included in analysis.

Statistical Analysis

We measured effects of adult ages on offspring performance using three mixed effects models which tested the effects of *maternal age*, *within-pair father age* (for within-pair chicks), *cuckolded social father age* (for extra-pair chicks), *extra-pair genetic father age* (for extra-pair chicks), and *mean helper age* (for chicks with helpers) on each fitness-related trait in the chicks (nestling weight, survival to independence, and recruitment). Recruitment (i.e. survival to adult breeding age, at one) could only be accurately assessed for male offspring due to the juvenile dispersal of females (see *Study Population* above).

The three fitness-related traits in offspring analyzed were *nestling weight*, *juvenile survival to independence*, and *male survival to recruitment*, defined as follows:

- *Nestling weight:* Nestling weight was measured in grams when nestlings were briefly removed from their nest to be banded and bled for SNP genotyping. The majority of weights were measured seven days after hatching, but sometimes one or two day(s) earlier or later. To control for this, the age of the chick (in days) at weighing was included in this model as a covariate. We also fitted a two-level factor '*pre-1992*', indicating whether the cohort was before 1992 or not. This term controlled for a change in protocol in the time of day at which chicks were weighed from this year forward (Kruuk *et al.*, 2015). We included *clutch size* as a covariate to control for any potential reduction in chick weight resultant from a larger number of chicks being present in the nest. We excluded 226 chicks from this analysis for which weight was not measured during the nestling phase or measurements were deemed unreliable. This resulted in a sample size of 4310 chicks from 1688 nests. Weight had an approximately Normal distribution and so a linear model with Gaussian error structure was used.

- *Juvenile Survival to Independence:* Early-life survival was measured from the late nestling stage (approximately seven days old, when chicks are banded and bled to assign parentage), until four weeks after fledging (which occurs at 12 days, so in total, when chicks reached an age of 40 days from hatching). This is the earliest age at which chicks reach foraging independence from their parents, as indicated by rare dispersal events observed at this age. The total sample size was 4538 chicks from 1771 nests. Individual survival probability was modeled using a Bernoulli distribution (fitted with a logit-link function).

- *Male Survival to Recruitment:* Survival from the late nestling stage to recruitment (measured as being alive at the start of the next year's breeding season) was only estimated in males, as recruitment cannot be confidently tracked in juvenile females (see *Study Population* above). After excluding 96 males for which emigration or death was uncertain due to them living close to the study area border, 2252 males from 1394 nests were used in this analysis. Recruitment probability was again modeled using a Bernoulli distribution (fitted with a logit-link function). For this model, social father was not included as a random effect as doing so led to non-convergence of the random effect estimates given the relatively smaller sample size.

To compare the paternal germline and environmental age effects separately (using the genetic father and the social father of extra-pair chicks) as well as the combined age effects of paternal germline and environment (fathers of within-pair chicks), we included all three 'types' of father ages in each model. To do this, we created a dummy variable (0 = within-pair chick, 1 = extra-pair chick) and fitted an interaction between this dummy variable and *cuckolded social father age* and *extra-pair genetic father*

age, so only extra-pair chicks contributed to the estimates of these terms. Similarly, we fitted the term *within-pair father age* in an interaction with the reverse dummy variable (0 = extra-pair chick, 1 = within-pair chick), so that only within-pair chicks contributed to the estimate of this term. The model structure that results from this dummy variable method is described in detail in the supplementary material (S1).

Non-linear parental age effects are possible, and have been observed in other study systems (Beamonte-Barrientos *et al.*, 2010; Torres *et al.*, 2011; Hammers *et al.*, 2012). However, fairy-wrens live considerably shorter lives than these species in which quadratic effects have been identified. The majority of females and males that survive to adulthood subsequently die before their third and fourth birthday, respectively (Cooper et al., 2020). Additionally, in both sexes, survival senescence begins at age 1, just as they reach sexual maturity (Cooper et al., 2020). As a result, we do not expect to observe substantial differences in early life *vs* later life age-related changes in the fairy-wrens, and given their shorter lifespans, modelling within-individual quadratic effects of ageing is challenging. Thus, in this study, we only investigate the linear effects of parental ages.

For 40% of chicks (45% of territories), the dominant breeding pair was assisted by at least one helper, while the remaining 60% had no helpers. To include both these groups of chicks within each model, we used an analogous method to that used for the paternal age terms, fitting an interaction between the term *mean helper age* and a dummy variable (0 = no helpers, 1 = helper(s) present; supplementary S1). *Mean helper age* was calculated as the mean age of all the helpers residing on a chick's natal territory at the time of their hatching. To separate any effects simply due to the presence of helpers and not their age, we also controlled for the number of *unrelated helper(s)* (indicative of a higher quality territory) and the number of son *helper(s)* (indicative of a higher quality territory and/or a higher quality mother – see above under *Study Population*) (Cockburn *et al.*, 2008c), each as a three level categorical effect (0, 1, 2+).

We included the *lifespans* of the mother and each type of father to control for and quantify potential 'selective disappearance', as well as to distinguish *within-individual* (age) from *between-individual* (lifespan) parental effects (van de Pol & Verhulst, 2006). Lifespans of each father type were fitted using the same dummy variables as father age effects (see above, and see supplementary material S1). Julian *incubation date* (the number of days counted from 1 January of the calendar year of the cohort on which incubation began) was included to control for any potential changes in chick performance across the breeding season (Kruuk *et al.*, 2015; Lv *et al.*, 2019). Julian incubation date was z-transformed (to zero mean and unit standard deviation) in all models, so that values were on a similar scale to values of

the other fixed effect variables, to help convergence. Random effects of each adult ID (mother, social father, and genetic father) were included to control for the non-independence of repeated measures from the same adults across chicks. Cohort was also included as a 30-level random effect to control for any potential heterogeneity between years.

Parental age effects sometimes vary with offspring sex (Priest *et al.*, 2002; Fox *et al.*, 2003; Carnes *et al.*, 2012; Bouwhuis *et al.*, 2015; Schroeder *et al.*, 2015), with parents potentially influencing the fitness of one sex more than that of the other. To test for this, we reran the *weight* and *juvenile survival to independence* models including an additional interaction between each parental age term and chick sex (excluding the 25 chicks of unknown sex). The differences between the sexes were minimal and did not change interpretation of any results (supplementary material S2), and so from herein results refer to the base models without fitting offspring sex or its interaction with parental ages.

We assessed the degree of age-assortative mating in the population using the (pseudo) R-squared values obtained from GLMMs testing the association between mother age and both within-pair and extra-pair father ages, separately (Nagelkerke, 1991). Variance inflation factors calculated from R-squared values quantify the increase in standard error due to correlation between predictors (Marquardt, 1970). Variance inflation factors were low (1.07 for within-pair and 1.01 for extra-pair mates), indicating that there was enough variation in mating pairings that the partial effects of each parental age can be assessed with adequate precision. This is expected as new breeding females are recruited at the age of one year, regardless of the age of the male.

All statistical analyses were fitted in R version 3.5.0 (R Core Team, 2018) using the lme4 package for mixed models (Bates *et al.*, 2015).

Results

We estimated effects of parental and average helper age on the three different metrics of early-life performance (nestling weight, survival to independence, and male recruitment probability) using a total of 4538 individual chicks from 1771 nests across 30 cohorts. There were, on average, 8.5 repeated measures of at least one metric of chick performance for each mother, 4.8 for each within-pair father, 6.0 for each cuckolded social father, and 6.1 for each extra-pair genetic father.

For mothers of the chicks, the mean maternal age at chick hatching was 2.6 years (1.6 SD) and the mean lifespan was 4.2 years (2.2 SD). For fathers, within-pair fathers had the youngest mean age (3.5 years, 1.9 SD) and the shortest mean lifespan (5.3 years, 2.4 SD), followed by extra-pair cuckolded social

fathers (age: 3.8 years, 2.1 SD, lifespan: 5.6 years, 2.6 SD), with extra-pair genetic fathers having both the oldest mean age at chick hatching (4.1 years, 2.1 SD), and longest mean lifespan (5.8 years, 2.3 SD). The mean of the 'average helper age' variable was 1.7 years (1.0 SD). The distribution of the frequency of parental and helper ages is illustrated in figure 1.

In all three models of chick performance, there were strong effects of variables that were not directly related to the ages of parents or helpers. *Incubation date* was positively associated with all three metrics of chick performance, likely owing to improved environmental conditions through the first half of the breeding season (table 1). The chick's age at weighing and being pre-1992 (see *Methods*) both had strong positive effects on chick weight, but there was no evidence of clutch size being associated with chick weight (table 1). The 'extra-pair dummy variable' was significantly positive in the model for chick weight, however this does not indicate that extra-pair chicks necessarily weigh more than within-pair chicks, since the dummy variable is included in a higher-level interaction (see details of model construction in S1). Below we describe the rest of the results, as they apply to maternal, paternal, and helper effects on each of the three metrics of chick performance.

Nestling Weight

There was no evidence of any effects of parental ages (representing *within-individual* effects), or parental lifespans (representing *between-individual* effects, table 1) on nestling weight. Although there was no effect of mean helper age, there was some evidence of an effect of helper presence. In comparison to having no son helpers, there was a marginally positive effect of having two or more son helpers (table 1). However, there was no support for any other effects of helper presence on nestling weight.

Juvenile Survival to Independence

There was a positive association between maternal lifespan and chick survival to independence (table 1; figure 2; log-odds 0.086, $p = 0.03$). The (non-significant) association between chick juvenile survival to independence and maternal age was of a similar magnitude, but in the opposite direction (table 1; log-odds -0.077, $p = 0.06$). This indicates that chicks from mothers with longer lifespans had a higher probability of surviving to independence, but that there was also an indication that chicks hatched in their mother's late life had lower survival than those hatched by the same mother at an earlier stage of her life. For example, for a one-year-old mother, the model predicts only a 37% chance of chick survival if the mother's total lifespan was one year, but a 54% chance of survival if the mother's total lifespan

was nine years. However, once the mother with a lifespan of nine years reaches the age of nine, her predicted probability of chick survival has declined to 38%.

The *between-individual* maternal lifespan effect indicates that there was selective disappearance of low quality mothers in older age groups. Since only mothers with longer lifespans are alive to produce chicks at later ages, the counteracting *between-individual* effect of maternal age and *within-individual* effect of maternal lifespan results in little apparent change in chick survival with increasing maternal lifespans in the raw data. For this reason, to illustrate the effect of maternal lifespan graphically (figure 2), we separated the raw data into two maternal age categories: mothers of one to three years old (with three years being the average lifespan of adult female fairy-wrens), and mothers of four years and older (up to the maximum recorded lifespan of ten years, with lifespans of nine and ten combined in figure 2). When plotted separately for each of the two maternal age groups, the mean chick survival probability for each maternal lifespan illustrates that the positive effect of maternal lifespan is primarily driven by differences between the mothers occurring at relatively young ages (age 1-3), and that, for long-lived mothers, chick survival is lower in late life (ages four and above; figure 2).

For chicks sired within-pair, paternal age was weakly positively associated with juvenile survival probability (table 1; figure 3A; log-odds 0.095, $p = 0.04$). The paternal age effects on these within-pair chicks represents the combined age-related effects of paternal germline and paternal environment. Surprisingly, despite this positive association between survival and age of the father in the within-pair chicks, for extra-pair chicks there was no evidence of any effect of either their genetic father's age (representing just the paternal germline; table 1; figure 3B) or their social father's age (representing paternal environment; table 1; figure 3C). Additionally, there was no evidence of any paternal lifespan effects, indicating that there were no *between-individual* differences in fathers' quality associated with their lifespans, and that the within-pair paternal age effect represents a *within-individual* change associated with ageing in these fathers.

There were no significant effects of the presence of either son helpers or unrelated helpers. However, amongst chicks who had helpers at the nest, chicks with older helpers were on average more likely to survive to independence (table 1; figure 4; log-odds 0.214, $p < 0.01$).

Male Survival to Recruitment

For male recruitment, the negative effect of maternal age and the positive effect of maternal lifespan were of comparable magnitude to the effects on juvenile survival to independence, but were both non-

significant (table 1; maternal age log-odds -0.067, p = 0.31; maternal lifespan log-odds 0.103, p = 0.07). This is likely owing to the smaller sample size and thus increased uncertainty in the effect estimates for male recruitment, in comparison to those for juvenile survival probability (for both sexes). The direction of the effects indicates that, similarly to juvenile survival probability, there may be counteracting positive *between-individual* effects of maternal lifespan (i.e. selective disappearance of lower quality mothers in older age groups) and negative *within-individual* effects of maternal age (i.e. declining quality of mothers as they age).

There were no significant within-pair father, cuckolded social father, or extra-pair genetic father age effects on male survival to recruitment, indicating that there was no evidence of either germline or environmental *within-individual* changes of fathers, associated with ageing. There were also no effects of any of the fathers' lifespans, indicating no *between-individual* differences (i.e. selective disappearance) amongst fathers of any group.

Similar to chick survival probability, there were no effects of helper presence on male recruitment probability. However, amongst males who did have helpers, mean helper age was positively associated with recruitment probability (table 1; figure 4; log-odds 0.443, $p < 0.01$). It is worth noting that the raw-data mean values for the associations between mean helper age and chick performance (filled circles, figure 4) suggest non-linear relationships with male recruitment into the breeding population, as well as juvenile survival probability. These measures of juvenile performance are relatively high when mean helper age is one, followed by a drop to low but increasing values beyond the mean age of one (figure 4). There is a bias towards younger helpers being primarily the sons of the female on the territory (the mother), rather than unrelated to the chicks, since younger helpers are more likely to have their mother still alive on their territory. We explored the raw data to see if this bias towards a higher proportion of son helpers at younger mean helper ages could be contributing to the surprisingly high average effect of one-year-old helpers. However, the effects of average helper age were similar for both son and unrelated helpers, indicating that this was not the case (supplementary material S3).

Table 1. Effects on *(i)* nestling weight, (*ii*) chick survival to independence (four weeks post-fledging), and (*iii*) male recruitment probability (survival to the breeding season after their hatching). Chicks sired both extra-pair and within-pair are included in each model. Interactions with dummy variables (0 or 1) are employed so that only extra-pair chicks contribute to estimates related to the extra-pair genetic fathers and cuckolded social fathers, while only within-pair chicks contribute to estimates related to within-pair fathers. This dummy variable method is also employed so that only chicks with helpers on the territory contribute to the estimate of mean helper age. (Please note that the dummy variable parameters are not relevant in themselves. See Methods for model details, and suppl. material for further description of dummy variables.)

	(i) Nesting Weight		*(ii)* Survival to Independence		*(iii)* Male Recruitment	
Fixed Effects	Log-Odds (95% CI)	p-value	Log-Odds (95% CI)	p-value	Log-Odds (95% CI)	p-value
Intercept	**0.771 (0.504 – 1.037)**	**<0.001**	**-1.948 (-2.442, -1.454)**	**<0.001**	**-5.462 (-6.399, -4.525)**	**<0.001**
Age (in Days) at Weighing	**0.852 (0.823 – 0.881)**	**<0.001**	-	-	-	-
Pre-1992	**0.411 (0.079 – 0.744)**	**0.015**	-	-	-	-
Clutch Size	-0.017 (-0.050 – 0.015)	0.293	-	-	-	-
Incubation Date (Days Past Jan. 1)	**0.316 (0.181 – 0.450)**	**<0.001**	**3.292 (2.819, 3.764)**	**<0.001**	**14.534 (12.891, 16.177)**	**<0.001**
Extra-Pair Dummy [yes]	**0.198 (0.045 – 0.350)**	**0.011**	0.335 (-0.165, 0.835)	0.189	0.295 (-0.643, 1.233)	0.537
(A) Maternal effects						
Mother Age	-0.006 (-0.030 – 0.018)	0.616	-0.077 (-0.157, 0.003)	0.059	-0.067 (-0.196, 0.063)	0.314
Mother Lifespan	0.001 (-0.022 – 0.024)	0.930	**0.086 (0.010, 0.163)**	**0.028**	0.103 (-0.009, 0.215)	0.071
(B) Paternal effects						
Within-pair Father Age	0.023 (-0.005 – 0.050)	0.108	**0.095 (0.002, 0.188)**	**0.044**	0.121 (-0.039, 0.281)	0.138
Within-pair Father Lifespan	-0.001 (-0.026 – 0.024)	0.910	-0.039 (-0.123, 0.045)	0.361	-0.085 (-0.224, 0.053)	0.227
Cuckolded Social Father Age	0.001 (-0.024 – 0.025)	0.948	-0.022 (-0.103, 0.059)	0.597	-0.086 (-0.229, 0.057)	0.239
Cuckolded Social Father Lifespan	-0.004 (-0.026 – 0.018)	0.716	-0.035 (-0.111, 0.040)	0.361	-0.029 (-0.142, 0.084)	0.619

Extra-pair Genetic Father Age	-0.009 (-0.032 – 0.014)	0.454	-0.053 (-0.132, 0.026)	0.185	-0.119 (-0.264, 0.026)	0.108
Extra-pair Genetic Father Lifespan	-0.001 (-0.026 – 0.024)	0.910	0.026 (-0.044, 0.097)	0.466	0.081 (-0.053, 0.215)	0.238
(C) Helper effects						
Son Helper Presence [1]	0.008 (-0.081 – 0.097)	0.853	-0.123 (-0.421, 0.175)	0.418	-0.145 (-0.680, 0.389)	0.594
Son Helper Presence [2+]	**0.113 (0.001 – 0.225)**	**0.049**	-0.208 (-0.581, 0.165)	0.275	0.017 (-0.648, 0.683)	0.959
Unrelated Helper Presence [1]	0.003 (-0.108 – 0.114)	0.954	-0.371 (-0.745, 0.003)	0.052	-0.436 (-1.069, 0.196)	0.177
Unrelated Helper Presence [2+]	-0.032 (-0.180 – 0.115)	0.666	-0.362 (-0.866, 0.142)	0.159	-0.430 (-1.266, 0.405)	0.313
Mean Helper Age	0.038 (-0.006 – 0.081)	0.089	**0.214 (0.065, 0.363)**	**0.005**	**0.443 (0.179, 0.706)**	**0.001**
Random Effects	Number of levels	Variance	Number of levels	**Variance**	Number of levels	**Variance**
Mother ID	531	0.077	537	**0.855**	492	**1.298**
Genetic Father ID	562	0.035	570	**0.145**	497	**1.158**
Social (Cuckolded) Father ID	481	0.050	490	**0.772**	-	**-**
Cohort	30	0.018	30	**0.095**	30	**0.092**
Residual	-	0.450	-	**-**	-	**-**

Note: Cuckolded social father ID was not included as a random effect for male recruitment as there was inadequate statistical power to estimate this term.

(ii) and (iii) are binomial GLMMs for which residual variance term was not estimated.

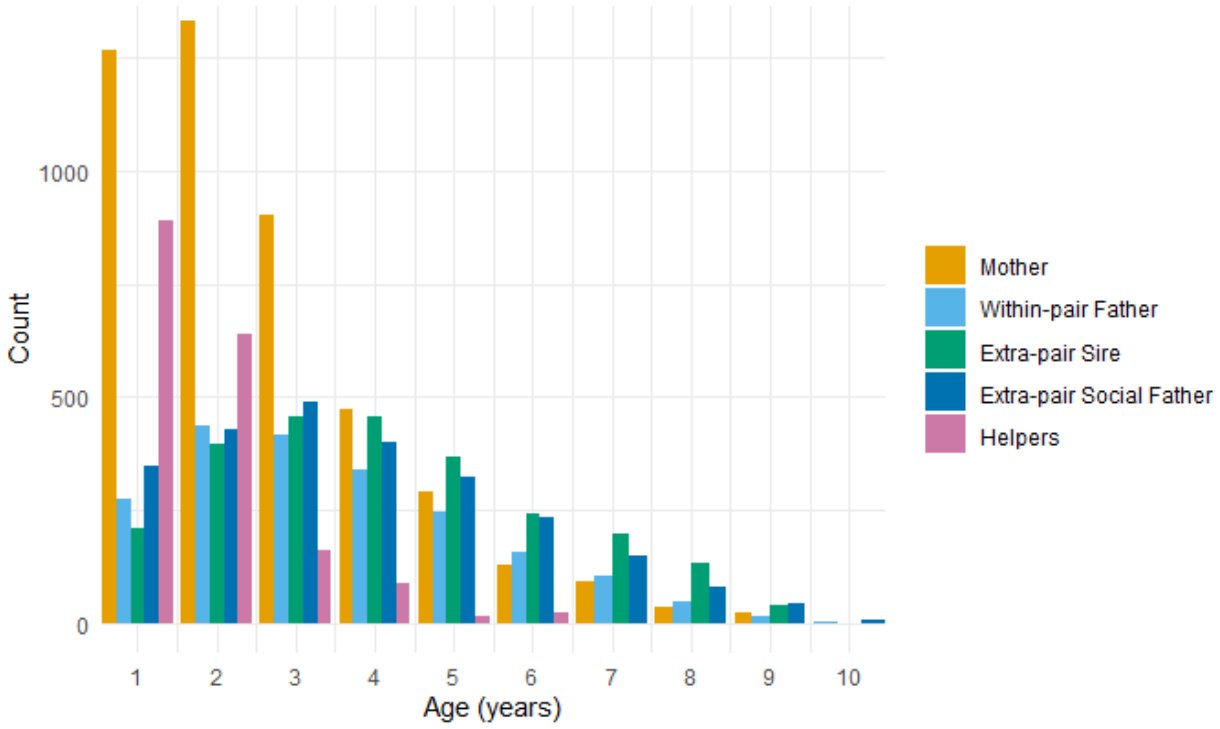

Figure 1 The age distribution of maternal, paternal and mean helper ages for all 4538 chicks used in analyses. There are a larger number of maternal ages overall than there are for within-pair fathers, extra-pair sires, extra-pair (cuckolded) social fathers or helpers. This is because there is a maternal age associated with each chick (each data point) but there are only within-pair paternal ages associated with chicks sired within-pair (45% of sample), only extra-pair sire and social father ages associated with chicks sired extra-pair (55% of sample), and only mean helper ages associated with chicks with at least one helper on their territory (40% of sample). Mean helper ages are rounded to the nearest integer for illustrative purposes.

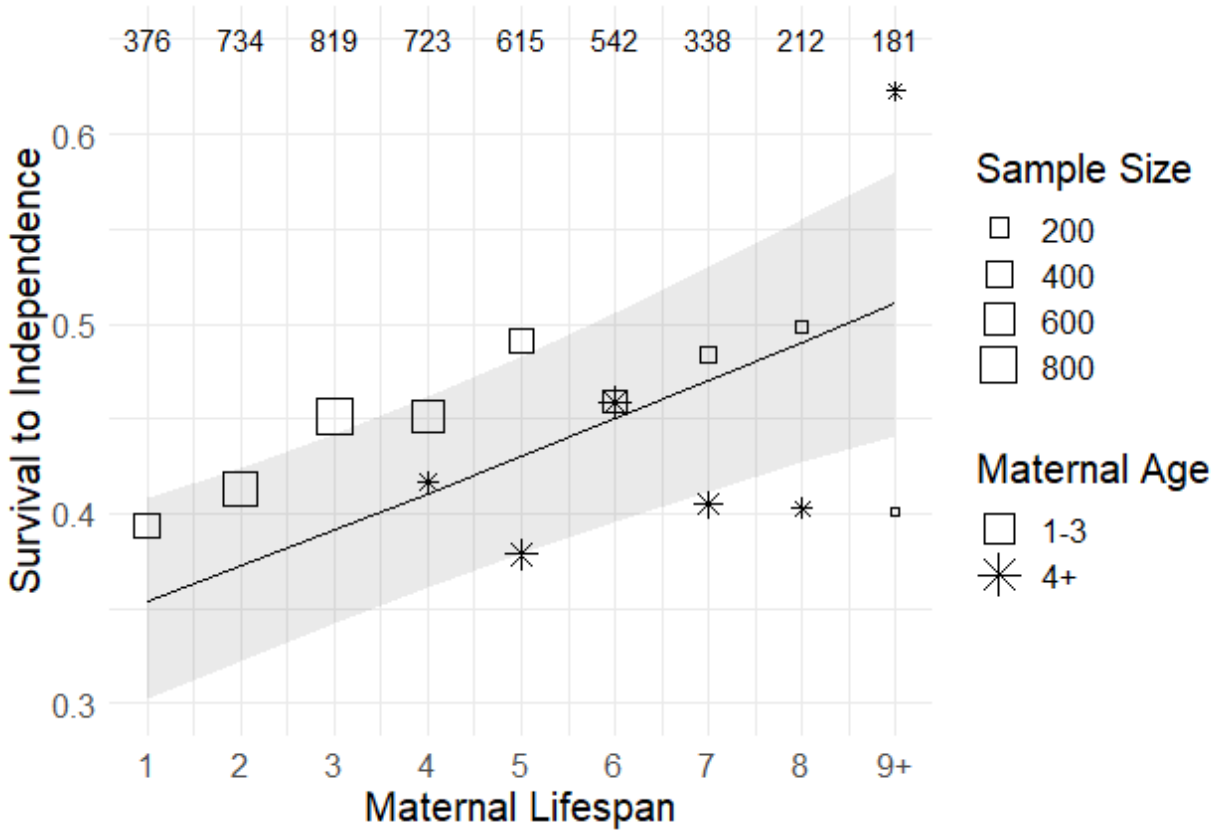

Figure 2 The effect of maternal lifespan (in years) on the probability of chick juvenile survival to independence, with the solid line representing the predicted model fit (Table 1), the shaded area representing the 95% confidence interval and the symbols showing raw-data mean values. The raw-data means are separated into two categories by maternal age at the time of chick hatching (squares, maternal age 1-3 years; asterisks, maternal age 4+ years), to illustrate that the positive effect of maternal lifespan is primarily driven by chicks produced by young mothers (increasing values of squares). The size of the squares and asterisks is (log)-proportional to the number of data points for that maternal lifespan within that age group, and the total sample sizes (for both age groups combined) for each maternal lifespan (number of chicks) are included across the top of the graph. Maternal lifespans of nine and ten have been combined for illustrative purposes.

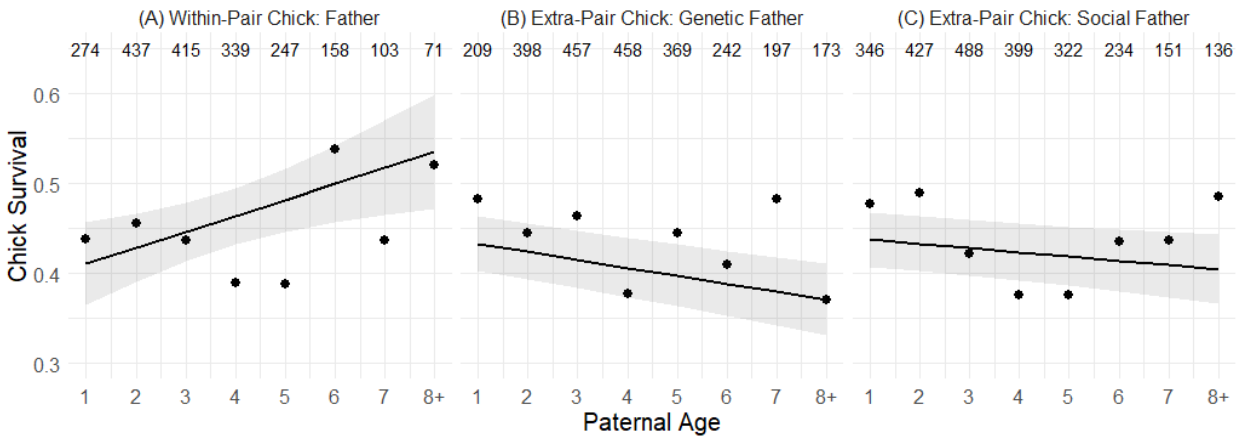

Figure 3 The effects of (A) within-pair father's age, (B) extra-pair genetic father's age, and (C) extra-pair chick's social father's age on the probability of chick survival to independence. Lines represent model predictions and the shaded areas are the 95% confidence intervals. Points represent raw mean values, uncorrected for other variables in the models. The sample sizes (number of chicks) for all three father types together are included across the top of each graph. Paternal ages eight to ten are combined for illustrative purposes.

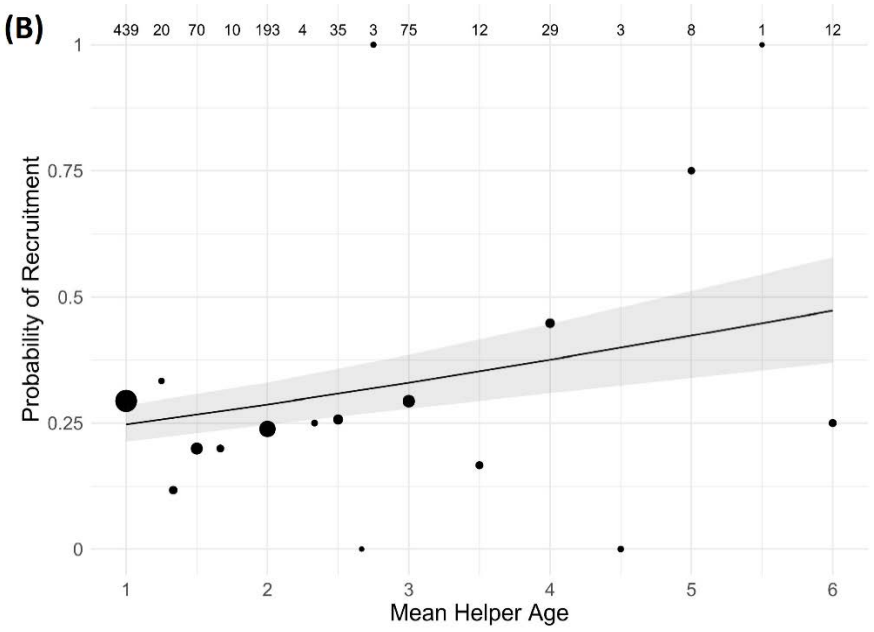

Figure 4 The effect of the mean age of helper(s) in a group on the probability of (a) chick survival to independence and (b) male recruitment. Lines represent model predictions and shaded areas are the 95% confidence intervals. Circles represent the raw-data mean values, uncorrected for other variables in the models. The size of each circle is log proportional to the number of data points for that mean helper age. Sample sizes (number of chicks) are included across the top of the graph. (Note that mean helper age was not necessarily an integer value because groups could contain 1-5 helpers.)

Discussion

In this study, we investigated the transgenerational effects of parental and helper ages in the cooperatively breeding superb fairy-wren by testing how maternal, paternal, and helper ages influenced three different components of chick performance. Chicks of mothers that lived longer had increased survival to independence, but there was evidence that maternal ageing was concurrently associated with reduced chick survival to independence. There was evidence of improvement in juvenile survival to independence with father age, but surprisingly only for within-pair fathers and not for extra-pair genetic fathers or extra-pair social fathers. Survival to independence and male recruitment probability improved with the mean age of helpers on the natal territory; to our knowledge, this study is the first to demonstrate that the ages of cooperatively breeding helpers are associated with components of offspring fitness. We discuss each of these results and their potential evolutionary and ecological implications below.

1. Maternal Age Effects

There was evidence of maternal age effects on chick survival to independence, but not on nestling weight or male survival to recruitment. Mothers with longer lifespans had chicks with higher survival, irrespective of the age of the mother at the time the chicks were hatched. Conversely, there was a negative association between the maternal age at the time of hatching and chick survival. It is worth noting that the counteracting directionality of these two effects would obscure the association between maternal lifespan and chick survival if data were cross-sectional rather than longitudinal. It is only when maternal age is controlled for that we are able to see that, at early ages, mothers who will live longer produce chicks with higher survival compared to those with shorter lifespans (figure 2). The associations between lifespan and chick performance constitute *between-individual* differences in mothers, which would be recognized as 'maternal effects' in a variance-partitioning analysis (Räsänen & Kruuk, 2007). Conversely, the effects of maternal age constitute *within-individual* change in the effect of a mother on her offspring, in a manner that would not be picked up in an analysis testing simply for differences between mothers. The results thus illustrate both the importance of maternal effects on offspring, but also that they may not be consistent over an individual mother's lifetime – and hence why it is

important to be able to control for both within- and between-individual effects when investigating questions related to ageing.

It is likely that the positive effect of maternal lifespan is due to an association between either individual quality and lifespan, or between territory quality and lifespan. However, if it were an association between territory quality and lifespan, we might expect that extra-pair social father and within-pair father lifespans would also be positively associated with chick survival, which they were not. Thus, it is most likely that mothers that live longer are inherently better 'quality' than those living shorter lives, and this allows them to produce chicks with higher survival irrespective of their current age (van Noordwijk & De Jong, 1986; de Jong, 1993; Wilson & Nussey, 2010). The lack of an effect of maternal lifespan on nestling weight suggests longer lived mothers are not better at early life provisioning of their offspring, but may instead be better at protecting their young fledglings from predation, which is the primary cause of juvenile death in fairy-wrens (Cockburn *et al.*, 2016).

It is difficult to ascertain the proximate causes of a maternal age effect on offspring fitness. Any decline in chick survival with increasing maternal age could be a consequence of deterioration of the maternal germline (Wong *et al.*, 2016), or non-germline-related aspects of senescence such as deterioration in maternal care (Lemaître & Gaillard, 2017). Here, we found a negative effect of having an older mother on the *per-chick* survival rate. In a previous study of this population, which considered effects of a female's age on her own performance, there was no evidence of decline in the *per-female* production of offspring at later ages (Cooper et al., 2020). This apparent paradox between the *per-chick* vs. the *per-female* effects of maternal age can be explained by females producing a larger number of chicks as they age, to compensate for the chicks' reduced survival. Female fairy-wrens start breeding earlier and increase their average clutch size as they age (Cooper *et al.*, 2020), so this is likely what drives this increase in the absolute number of independent chicks produced.

2. <u>Paternal Age Effects</u>

Increasing age of the father was associated with higher probability of juvenile survival for chicks sired within-pair, although the effect appeared to be largely driven by higher survival of chicks sired by males aged 6+ (fig 3; 16% of sample), for whom the sample sizes are relatively small. We found no equivalent effect for nestling weight or probability of recruitment. There were no effects of the ages of the genetic or the social father on the performance of chicks sired extra-pair (table 1). Thus, if there is a positive

effect of father age on within-pair chick survival, the mechanism driving it is not entirely clear. It is unlikely to be a consequence of germline-level changes with age, for two reasons. First, we saw no effect of the genetic father's age on extra-pair offspring performance here (figure 3B). Second, it has now been shown in at least some other species that sperm DNA damage increases with paternal age (Velando *et al.*, 2011; Johnson *et al.*, 2015), and that, if there are any effects of sperm age on offspring fitness, these are typically negative (Johnson & Gemmell, 2012; Lemaître & Gaillard, 2017) (see below). We believe it is therefore more likely that any effect of paternal age for within-pair chicks is related to non-germline changes that in some way differ from the effects of social father age for extra-pair chicks.

It is possible that differences between dominant males associated with the extent to which they are cuckolded generate this difference between within-pair and extra-pair sired chicks in paternal age effects. In particular, it is plausible that the degree of cuckoldry a male experiences is negatively correlated with some aspect of his overall 'quality', and also with the quality of his offspring. During their fertile period, female fairy-wrens copulate with their social partner soon after they have mated with their preferred extra-group male (Cockburn *et al.*, 2016). The outcome of the resultant sperm competition must influence within-pair siring success (Calhim *et al.*, 2011). If variation in male quality increases in older age groups, as is predicted by evolutionary theories of senescence (Charlesworth, 1990; Moorad & Promislow, 2009), sperm competition may play a greater role in determining siring success for these older males. Thus, rather than any effect of ageing *per* se, the apparent improvement in chick performance with within-pair sire age could simply be a consequence of sperm competition biasing the sample of successful older dominant males. In other words, within-pair success at old age would be indicative of a high quality dominant male, who might then produce higher quality offspring. Note that the 'inheritance' of quality need not be genetic, but could also reflect correlations driven by shared environments. The raw-data means indicate that the positive effect of within-pair paternal age is driven by males above age five (figure 3A), which few males survive to (Cooper *et al.*, 2020), and so the sample size is relatively low in comparison to data on younger fathers (figure 1). Thus, more work on this system will be required to investigate this paternal age effect further. Since our study is (to our knowledge) the first to attempt to disentangle age effects of both genetic and naturally-occurring 'foster' fathers on offspring performance, additional work on other species will also be valuable for assessing the robustness of this result across other systems.

It is also interesting to note that, while controlling for lifespan does allow correlation to be distinguished from causation in the specific case of selective disappearance, in the case where age-related biases in

reproductive success are a consequence of any process other than mortality (as described above), a correlation between parental age and offspring performance is not indicative of causation. Thus, even using longitudinal studies where selective disappearance caused by mortality can be controlled for (i.e. accounting for some between-individual differences), parental age effects may still reflect correlations rather than causative *within-individual* changes in the parents as they age, as they are often interpreted.

As there was no support for effects of the age of the genetic father of extra-pair chicks in our analyses, there was no evidence of germline deterioration with age in this population. Although sperm has been shown to deteriorate in quality with male age in other systems (Johnson *et al.*, 2015; Lemaître & Gaillard, 2017), the effects of senescent sperm carrying over to influence offspring fitness are contentious. 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; Nybo Andersen & Urhoj, 2017), but many others have not found any such associations (Fox *et al.*, 2003; Fricke & 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 in competition with less senescent males (Vuarin *et al.*, 2019). Similar to a potential contribution to the positive effect of within-pair sire age as discussed above, this could also result in the sample of older males that are successful extra-pair sires being biased towards only high quality males (Pizzari *et al.*, 2008; Fitzpatrick & Lüpold, 2014), which may result in an overall null effect of extra-pair genetic father age. It is interesting to note that, to our knowledge, the studies to date which have found negative effects of paternal age on offspring fitness have all been in situations where both female choice and sperm competition are likely to be limited: either in controlled laboratory experiments or in a cross-fostering experiment where female choice and sperm competition are constrained (Priest *et al.*, 2002; Ducatez *et al.*, 2012; Schroeder *et al.*, 2015), in species with high genetic monogamy where female choice and sperm competition play little to no role (Bouwhuis *et al.*, 2015), and in modern-day humans (Nybo Andersen & Urhoj, 2017) where adaptive female choice and sperm competition are likely to be rendered irrelevant by societal and cultural factors. Female superb fairy-wrens are highly promiscuous (Cockburn *et al.*, 2008a; Hajduk *et al.*, 2018), and female choice and sperm competition may result in a reduction in senescent males being successful sires. Regardless of the mechanism underlying the results presented here, the lack of any negative effects of father age suggests that any female preference for older males is neither adaptive nor maladaptive in the context of offspring early-life fitness.

3. Effects of Helpers' Age(s)

We found evidence for positive associations between the mean age of helpers on a territory and both chick survival to independence and male recruitment. There are two non-mutually exclusive mechanisms that could be driving these results. First, it is possible that the effect is driven by helper age *per se*, whereby helpers become better at providing care to chicks as they gain experience with age. This is plausible as it has been shown in several cooperatively-breeding bird species that the age of helpers is associated with their level of contribution towards chick provisioning and predator defense (Lawton & Guindon, 1981; Hunter, 1987; Heinsohn & Cockburn, 1994; Woxvold *et al.*, 2006; Klauke *et al.*, 2014). It has even been argued that learning the skills necessary for effective parental care is a selective force favouring helping behaviour (Dixon, 1966; Komdeur, 1996) and there is evidence in some species that birds with helping experience are superior parents when they gain a breeding position (Komdeur, 1996).

A non-mutually exclusive, and arguably more plausible, cause of the effect of helper age is that there is a correlation between helper survival and territory quality, which drives a correlation with offspring performance. Helpers may enjoy increased survival until later ages as a consequence of their natal territory having lower predation risk or greater food availability, which may be associated with the fitness of chicks hatched on this same territory. Since we found no evidence that helper age affects nestling weight, a trait which might be expected to respond strongly to helper provisioning, this suggests that predator avoidance is the more likely source of the older helper advantage. Distinguishing cause and effect in associations between helper number and survival in systems like this has proved notoriously difficult (Cockburn *et al.*, 2008c; Brouwer *et al.*, 2020), and the hitherto uninvestigated association between helper age and offspring survival adds further complexity to that puzzle. However, the weight of evidence in this case suggests that benefits to chicks associated with older helpers attending the nest may be a consequence of conditions favoring the survival of both chicks and helpers, rather than the case of the helpers themselves increasing productivity with age.

In contrast to helper age, there were not strong or consistent effects of helper presence on chick performance. When compared with the absence of helpers, there was a marginally significant positive effect of the presence of two or more son helpers on chick weight, but no apparent effects of the presence of only one son helper, or any unrelated helpers (table 1). Previous work on the effects of helper presence has found consistently positive effects of helpers on chick weight (Kruuk *et al.*, 2015; Hajduk *et al.*, 2018). However, these studies did not separate unrelated and son helpers, and did not control for helper age effects, which may explain the difference in results. We found no associations

between helper presence and chick survival to independence or male recruitment. Our results suggest that any benefits of the presence of helpers are not passed on to the chicks themselves, despite the fact that helper presence is associated with higher territory productivity (Cockburn *et al.*, 2008c; Brouwer *et al.*, 2020).

Conclusions

Our study found evidence that the age of the different adults in an offspring's early life can influence its fitness-related traits. There were counteracting within-individual (ageing) and between-individual (lifespan) effects associated with mothers on chick survival to independence, which illustrate the importance of longitudinal measurements in investigating questions related to ageing. The ages of fathers had a positive effect on chick survival to independence, but only for chicks sired within-pair. The lack of effect of social father and genetic father ages for extra-pair sired chicks suggests that the dynamics of sexual selection, and especially female choice, may play an important role in the evolutionary ecology of transgenerational age effects. Our study is also the first, to our knowledge, to demonstrate that the average age of helpers in cooperatively breeding groups is associated with increased chick performance, with increasing helper age improving chick survival to independence and recruitment probabilities. These results suggest the effects of parent and helper ages on the early-life fitness of the next generation appear to be primarily related to environmental changes in superb fairy-wrens. They thus indicate that negative germline effects of parental age may not be ubiquitous.

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Chapter 4 Supplementary Material

Contents

S1. Use of dummy variables for father-type and helper-presence

Each chick performance trait was modelled using a mixed effects regression model, described below:

$$f_i = \beta_0 + EP + EP.(\beta_{EPgen}.age_{Fgen} + \beta_{EPsoc}.age_{Fsoc}) + WP.\beta_{WP}.age_{Fgen} + H + H.\beta_{help}.age_{help} + EP.(\beta_{EPgen}.lifespan_{Fgen} + \beta_{EPsoc}.lifespan_{Fsoc}) + WP.\beta_{WP}.lifespan_{Fgen} + (other\ effects)$$

where f is the fitness metric of an individual chick (*i*). EP is a dummy term of 1 for a chick sired extra-pair and 0 for a chick sired within-pair. The age of the genetic father is given by age_{Fgen} (for both within- and extra-pair sired chicks) and the age of the cuckolded social father by age_{Fsoc} (for extra-pair sired chicks only).The dummy term *WP* is the inverse of *EP*, where a value of 1 denotes a chick sired within-pair, and a value of 0 denotes a chick sired extra-pair. As a result, the coefficients for extra-pair genetic father age β_{EPgen} and cuckolded social father age β_{EPsoc} are only estimated for extra-pair chicks, and the coefficient for within-pair father age β_{WP} is only estimated for within-pair chicks. The same dummy variable process is used to estimate lifespan effects of the three father types.

An analogous dummy variable (H) was used to estimate the mean helper age coefficient (β_{help}) only using chicks for which helper(s) were present at the nest. The estimate produced for the lower-level (non-interacting) dummy variable EP was not interpretable given the higher order interactions containing this term. The dummy variable WP was not included as a lower-level additive term as it correlates perfectly with EP. WP was also excluded as a lower-level term since it correlates with two other variables in the model, son helper presence and unrelated helper presence. All additional fixed effects and random effects included in each model are described under *Statistical Analysis* of the main text.

S2. Testing for sex-specific effects of parent and helper age on chick fitness

In order to investigate if the effects of parental or helper age differed between chicks of each sex, we ran the same models presented in the main text for nestling weight and juvenile survival to independence but also including interaction terms of *chick sex* with each parental age, parental lifespan, and helper age effect (a total of ten interaction terms in each model). Since the third measure of chick fitness, survival to recruitment, was only measured in males (main text, *Methods*), we did not test for sex-specific effects in this trait. 25 chicks of unknown sex were excluded from the analysis. This resulted in a sample of 2260 males and 2036 females for nestling weight, and a sample of 2369 males and 2153 females for juvenile survival to independence.

We found a single statistically significant interaction, where the effect of maternal lifespan was more positive on male chick weight than female chick weight ($\beta_{\text{mat. lifespan} \cdot \text{sex}} = 0.033$, $p = 0.02$). However, there was no significant effect of maternal lifespan on the combined male and female sample of nestling weights (main text table 1). We modeled male and female nestling weight separately and found a non-significant negative effect of maternal lifespan on female nestling weight ($\beta_{\text{mat. lifespan}} = -0.013$, $p = 0.30$) and a non-significant positive effect of maternal lifespan on male nestling weight ($\beta_{\text{mat. lifespan}} = 0.020$, $p = 0.13$). Since both effects were non-significant, the interpretation of our main results remain unchanged. There were no other statistically

significant interactions between parental lifespan, parental age, or helper age terms and sex for either nestling weight or chick survival to independence.

S3. Helper mean age effects

The raw age-specific means in figure 4 of the main text suggests that, when not controlling for other factors in the model, chicks with helper(s) of a mean age of one year displayed the highest juvenile survival and recruitment probability. This was unexpected given that the linear models predicted a positive effect of mean helper age on chick fitness. Since our *a priori* hypothesis on the effect of helper age was that it would be improve chick fitness linearly, we did not test for a quadratic effect in our models. However, the raw means appear to display a quadratic effect of mean helper age, with the youngest and the oldest helpers offering the greatest effect on chick fitness.

One potential cause of this unexpected pattern in the data is a difference between related and unrelated helpers. Most one-year-old helpers are related (the sons of the current dominant female), while older helpers are more likely to be unrelated (as more time has passed for their mother to die and a new dominant female to assume the territory). However, when the raw mean data was separated into chicks that had at least one related helper (triangles, figure S2.1), and chicks for which all the helper(s) were unrelated (circles, figure S2.1), both groups demonstrated high levels of chick survival to independence and recruitment when helper mean age was one year.

We can hypothesize about other possible reasons why territories with one-year-old helper(s) have higher rates of chick survival and recruitment. It is possible that one-year-old helpers are a better indicator of a high quality territory than helpers of later ages if territory quality changes over time. Alternatively, it is possible one-year-old helpers put more effort into provisioning and protection of chicks than older helpers, possibly because first year helpers need to demonstrate their value to the dominant breeding pair. However without directly testing either of these hypotheses we can only speculate on their validity. Further work, beyond the scope of this paper, is needed to further explore this pattern.

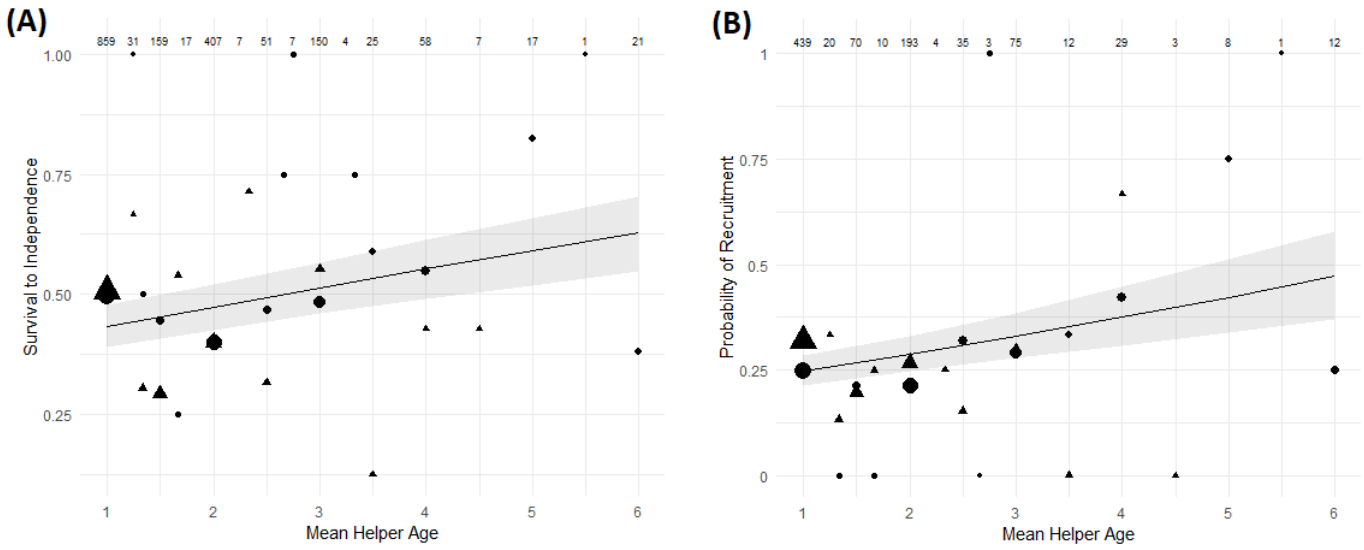

Figure S3.1 The effect of the mean age of helper(s) on the probability of (a) chick survival to independence and (b) male recruitment. Triangles represent the raw mean values for chicks that had at least one related helper, and circles represent the raw mean values for chicks where all the helper(s) were unrelated. The size of the points is log proportional to the number of data points for that mean helper age and sample sizes (total number of chicks) are included across the top of the graph. Lines represent model predictions and the shaded areas are the 95% confidence intervals.

Conclusion

Unlike genotype, the phenotype of any multicellular organism can vary dramatically over its adult lifespan. These age-related phenotypic changes occur in response to a myriad of internal physiological processes and external environmental variables. Broadly defined as 'ageing', understanding the evolutionary and ecological drivers that determine these age-related changes is central to our understanding of life-history theory. However, given the complexity of processes and factors that shape ageing, it is unsurprising that it is likely one of the most poorly understood processes occurring in the natural world (Bronikowski and Promislow 2005; Monaghan et al. 2008; Gaillard and Lemaître 2020). This thesis uses empirical data from wild animal populations to investigate the eco-evolutionary dynamics of ageing from various angles, with the aim to further our understanding of the ecological causes and the evolutionary consequences of ageing.

Senescence, which is the period of late-life ageing characterized by a generalized deterioration in physiological functioning, coupled with declining fertility and increasing mortality risk, is perhaps the most mysterious aspect of the ageing process. Why is natural selection apparently ineffective at extending reproductive physiology and somatic maintenance beyond certain age limits? Additionally, why is there often considerable variation in the rates of senescent deterioration between individuals within a population (Nussey et al. 2013)? In part, the answer lies in the fact that senescence is highly context-dependent. There is good evidence that environmental conditions play a large role in the rates of organismal senescence (Austad 1993; Kawasaki et al. 2008; Lemaitre et al. 2013; Holand et al. 2016). However, how past environmental conditions, and in particular environmental conditions experienced during early-life development, impact late-life ageing has been less well studied, particularly in the wild. Chapter 1 of my thesis offers the first cross-species synthesis of early-environmental effects of ageing on wild animals. We found that there is indeed an observable impact of early-life environmental quality on the rate of senescent decline at the end of life. Interestingly, this influence of early-life environment only carried over to impact the senescence rate in one aspect of phenotypic fitness, reproduction, but did not impact survival probability senescence.

This finding of differences between senescence rates of different fitness components illustrates an important point in understanding the complexity of organismal senescence, which is that different traits can express different ageing trajectories, even within the same individual. Understanding how different fitness-related traits change with age can help us better understand how adaptive trade-offs (Lemaître

et al. 2015), constraints (Cohen et al. 2020), and the relative roles of sexual and natural selection (Bonduriansky et al. 2008) all shape life-history. This was the focus of my second chapter, where I investigated how a suite of different fitness-related traits change with age using longitudinal data collected on a wild population of superb fairy-wrens (*Malurus cyaneus*). Here, a difficulty I encountered early on was deciding how to quantify ageing. Unfortunately, at the present there is no standardized method for quantifying ageing that is universally followed. As a consequence, despite the growing number of studies of ageing across different fitness-related traits in wild populations, this research is difficult to synthesize, and it's difficult to draw comparisons and make larger inferences about the eco-evolutionary dynamics of trait-specific ageing (Monaghan et al. 2008; Nussey et al. 2008; Lemaître and Gaillard 2017). For this reason, I sought to use a methodology that was widely applicable and allowed for direct comparison of ageing across traits, populations, and species. As an important part of this chapter, I've created a detailed tutorial guide on the methods I've used, in hopes to better unify future ageing research, and allow for greater synthesis and comparisons between species.

Chapter 2 identified that fairy-wrens show asynchronous ageing across traits, and these differences are most dramatic when comparing traits driven by natural selective pressures versus those driven by sexual selection pressures. Particularly notable, the date males complete their annual moult into breeding plumage was the only trait that showed not only a lack of senescence, but actually continuous improvement with age, up until maximum ages. Age-related improvements in sexually selected traits such as moult date, even despite senescence in other fitness-related traits, appear to be common in wild animal populations (Galvan and Møller 2009; Nussey et al. 2009; Potti et al. 2013). The modelling of moult date in Chapter 2 indicated that the age-related improvement seen at the population level was not a consequence of selective disappearance, but rather within-individual changes in trait expression that occur as males age. Why young males would not display the trait at high levels, and similarly, how as the males get older they are capable of displaying the trait more strongly, are questions that are not well understood.

In Chapter 3 I attempted to determine the underlying evolutionary forces shaping the curious age-related improvement observed for fairy-wren male moult date using a quantitative genetic 'animal model' (Kruuk 2004) as well as selection models (Phillips and Arnold 1989) which quantified sources of genetic and non-genetic variance, and selection within age groups. With these models I was able to test two competing hypotheses which may explain the age-related improvement in moult date: (1) a genetic constraint, or, (2) adaptive plasticity in trait expression. Ultimately, the results do not provide support

for either hypothesis. It is most likely that the age-related improvement in moult date is a consequence instead of more complex trade-offs between fitness components occurring with age, however future work is needed to confirm this theory.

One important finding from my third chapter, which was perhaps unexpected, was that additive genetic variance was greatest in the oldest age class. This finding could be considered surprising given that increasing additive genetic variance at old ages is hypothesized to be a consequence of phenotypic senescence having a genetic basis (i.e. as a consequence of declining strength of selection with increasing age; Charlesworth 1990; Moorad and Promislow 2009). Previous quantitative genetic studies of age-specific genetic variance have focused on traits that demonstrate senescence, and here increased additive genetic variance in the oldest age classes has been lauded as evidence of a genetic basis of the phenotypic senescent declines (Charmantier et al. 2006; Wilson et al. 2007; Chantepie et al. 2015). However, as Chapter 3 demonstrates that there is increased genetic variance at old ages in a trait that actually shows greater improvement of trait expression into late life, rather than senescence, indicates that increasing additive genetic variance with age is not sufficient as evidence that senescence is a genetically driven process. Additional analyses of the age-related changes in additive genetic variance and selection on other phenotypic traits in the fairy-wren population that did show senescence (e.g. survival) would be interesting as a comparison to the patterns found for moult date.

It is possible that ageing does not only have consequences for the phenotype of the individual, but ageing could have broader ecological consequences if age impacts offspring fitness , as this could create knock-on effects impacting demography, and even the evolution of life-history (Monaghan et al. 2020). Chapter 4 investigated the prevalence of intergenerational effects of ageing on the early-life performance of fairy-wren chicks. Some features specific to the fairy-wren system offered novel avenues for investigation of the intergenerational effects of age. Firstly, because fairy-wrens are socially monogamous, but with high rates of extra-pair paternity, I was able to separately quantify paternal ageing effects associated with the germline (i.e. from extra-pair fathers that provide only their sperm) and paternal ageing effects associated with the environment (i.e. from cuckolded fathers that provide only parental care). Secondly, because fairy-wrens are cooperative breeders, I was able to also investigate if there was any influence of the ages of adult male subordinate ‘helpers’ that aide in provisioning and protection of chicks. Surprisingly, the mean ages of any helpers attending the nest had a stronger impact on chick fitness than either the ages of mothers or any type of father did. This study is the first to demonstrate that helper age may impact chick fitness in a cooperative breeding species.

Likely, it is that high-quality territory conditions are associated with older helpers residing on the territory, rather than a within-individual influence of helpers improving their care of chicks as they get older. This finding highlights the complexity of mechanisms that can drive age-related effects.

Overall, the findings in this thesis illustrate the importance of incorporating life-history theory and eco-evolutionary feedbacks into our understanding of phenotypic ageing. Chapter 1 demonstrates the plasticity of individual senescence rates, in that not only current environment, but even environmental conditions from previous life stages can influence the trajectory of an individual's ageing rate. Chapter 2 provides an demonstration of how differently different aspects of phenotype change with age within a population. The asynchronous patterns of fairy-wren ageing across traits suggest that adaptive trade-offs, life-history, and sexual selection all play an important role in determining for which traits there is senescent deterioration, and for which traits there is not. Chapter 3 indicates that age-related shifts in trait expression may not be adequately explained by either an age-related genetic constraint or adaptive plasticity in trait expression, but rather it is perhaps a more complex relationship involving shifting trade-offs between reproduction and somatic maintenance that contribute to the observed pattern. Chapter 4 illustrates that caregiver age can impact the fitness of the next generation, however these influences are varied between types of caregivers, and environmental variables that may co-vary with age of mothers, fathers, and helpers need to be considered carefully.

Seminal theorists on the evolution of ageing predicted senescence would not be observable in the wild (Medawar 1952; Williams 1957; Kirkwood and Austad 2000). Decades of longitudinal individual-based research on wild animals has proven this to be incorrect (Nussey et al. 2013). As a consequence, the evolutionary ecology of ageing has since flourished as a field of study, providing novel insight into the complexity of processes that modulate ageing. More than anything, this body of research has highlighted the importance of life-history theory as the essential framework on which to hypothesize and test empirically how and why such a complexity and diversity of ageing patterns between populations, between individuals, and between different fitness components in seen in the natural world. The findings in this thesis are also quite illustrative of this complexity, highlighting the range of diverse variables that need to be considered to understand the realized patterns of phenotypic change that occur with age. It is an incredibly exciting time to be engaging in this field of research, with many new insights, and equally as many new questions, being raised in the study of the evolutionary ecology of ageing in the present age.

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