Maternal and paternal age effects on male antler flies: a field experiment

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Key Words: Lansing effect, Mark-recapture, Maternal effect, Paternal effect, Senescence, Survival

Type of Article: Note

Word Count: 3,352

Online material includes: Supplementary methods, Tables A1-A8, Fig. A1

Abstract

In many species, parental age at reproduction can influence offspring performance and lifespan, but the direction of these effects and the traits affected vary among studies. Data on parental age effects are still scarce in non-captive populations, especially insects, despite species such as fruit flies being models in laboratory-based aging research. We performed a biologically relevant experimental manipulation of maternal and paternal age at reproduction of antler flies (*Protopiophila litigata*) in the laboratory and tracked the adult lifespan and reproductive success of their male offspring released in the wild. Increased paternal, but not maternal, age somewhat increased sons' adult lifespan, while parental ages did not influence sons' mating rate or reproductive senescence. Our results indicate that while parental age effects do exist in an insect in the field, they may be beneficial in such a short-lived animal, in contrast to results from most wild vertebrates and laboratory invertebrates.

Introduction

Offspring produced by old parents often suffer survival or performance costs due to genetic deterioration in the parental germline (Monaghan and Metcalfe 2019), senescence in maternal and paternal effects (i.e., age-related declines in offspring provisioning, care, or epigenetic factors; Moorad and Nussey 2016), and/or genetic or phenotypic differences caused by selective disappearance (Monaghan et al. 2020). Parental age effects are reported from laboratory studies (e.g., Lansing 1947; Priest et al. 2002; Fox et al. 2003, but see Ivimey-Cook and Moorad 2018) and natural populations (e.g., Bouwhuis et al. 2010; Schroeder et al. 2015; Fay et al. 2016), including humans (Gillespie et al. 2013; Arslan et al. 2017).

Parental age effects vary in the traits affected and the nature of the effect (Fay et al. 2016). Increasing parental age often decreases juvenile survival and performance (Fay et al. 2016), and effects can carry over into adulthood, reducing offspring lifespan (Lansing 1947; Priest et al. 2002; Wylde et al. 2019) and reproductive success (Bouwhuis et al. 2010; Schroeder et al. 2015; Arslan et al. 2017).

Deleterious parental age effects are common, but in some studies offspring quality peaked at intermediate parental age before declining (Wang and vom Saal 2000; Rödel et al. 2009; Reichert et al. 2019) while, in others, offspring quality increased with parental age (Fox et al. 2003; Kroeger et al. 2020). Parental age effects can also depend on parental sex (Fay et al. 2016; Wylde et al. 2019) and can interact with offspring sex (Fox et al. 2003; Schroeder et al. 2015). Maternal and paternal age may even interact, if, for example, paternal germline mutation load makes offspring more sensitive to poor egg provisioning by older mothers. However, we know of no study that tested such an interaction.

Although Lansing (1947) conceptualized a "transmissible... factor in aging", subsequent studies of parental effects focused on offspring longevity (Monaghan et al. 2020, but see Wylde et al. 2019). However, longevity depends not only aging rate, but also baseline mortality and the timing of the onset of senescence (Péron et al. 2019). Extrapolating effects on aging from longevity, or *vice versa,* can be misleading if these other parameters also vary. Statistical methods that estimate both aging rate and baseline mortality are therefore more informative than analyses of lifespan alone.

Most laboratory studies of parental age effects have used short-lived model organisms, especially invertebrates, while studies of natural populations have largely, if not entirely, involved vertebrates. However, senescence differs strikingly across taxa (Jones et al. 2014) and between laboratory and natural environments (Kawasaki et al. 2008; Hämäläinen et al. 2014; Mautz et al. 2019). Maternal age effects appear to differ between wild and semi-captive contexts, but so far, laboratory and field studies remain highly confounded with phylogeny (Ivimey-Cook and Moorad 2020). Furthermore, manipulations of parental age in laboratory studies often vastly exceed the typical lifespan of the organism in the wild. Insect studies have often used parents ≥ 30 days old (Priest et al. 2002; Bloch Qazi et al. 2017; Wylde et al. 2019), while estimates of average adult lifespan in wild insects range from 2.1–28.9 d (Fincke 1982; Bonduriansky and Brassil 2002; Kawasaki et al. 2008; Zajitschek et al. 2009).

We test whether parental breeding age influences male offspring performance in an insect, the antler fly, *Protopiophila litigata* (Diptera: Piophilidae), that lacks parental care. Male (but not female) antler flies have high site fidelity on the shed cervid antlers that host their mating aggregations, facilitating collection of longitudinal data under natural conditions (Bonduriansky and Brassil 2002). Taking advantage of this, we mated young (1–3 d) and old (10–13 d) parents of both sexes from a laboratory stock (derived from a wild population 11 generations previously) in a two-way factorial design and recorded survival and mating success of male offspring released into the wild. This age manipulation is ecologically relevant; senescence in wild antler flies begins soon after eclosion, and our "old" age lies beyond median adult lifespanin nature (6–8 d; Mautz et al. 2019), but is well below the maximum recorded wild lifespan for this species (32 d; Bonduriansky and Brassil 2002). Only ~20% of wild antler flies survive beyond 13 d (Bonduriansky and Brassil 2005), at which point mortality has increased 9% and mating rate has decreased 13% (Bonduriansky and Brassil 2002). If laboratory studies reflect ecological reality, we expected decreased longevity and mating success, and/or faster senescence, in sons of old parents. However, due to the diversity of reported parental age effects and our less extreme age manipulation, other relationships are also plausible.

Methods

Experimental procedure

Our laboratory stock was derived from >200 antler flies collected at the Algonquin Wildlife Research Station (AWRS), Algonquin Provincial Park, Ontario, Canada in 2017. They were maintained as a large, outbred, mixed-age population for 11 generations prior to the experiment following Oudin et al. (2015). Beginning 1 May 2018, we created offspring from all four factorial combinations of young (1-3 d) and old (10-13 d) parents in three genetically distinct temporal blocks (see Appendix A; Table A1). Offspring were relocated to the AWRS on 22 May 2018, where they were housed in portable Reptibator incubators (ZooMed Laboratories, Inc., USA) set to 23 °C, with ambient humidity and light.

Upon eclosion, offspring were immobilized without anaesthesia (Bonduriansky and Brooks 1997) and photographed under a microscope. Flies that eclosed in the evening were held overnight in separate vials with moistened coco peat and *ad libitum* sugar prior to processing. Sugar supplementation has negligible effects on adult survival and mating success (Mautz et al. 2019). We measured wing length, a proxy for body size (Angell et al. 2020), using ImageJ v1.51 (Schneider et al. 2012). Males—which, unlike females, have high site fidelity and can be tracked in the wild (Bonduriansky and Brooks 2002)—were marked with individual codes on their thorax using enamel paint (The Testor Corporation, USA) following Bonduriansky and Brooks (1997).

Marked males were released onto one of four shed moose (*Alces alces*) antlers (Table A2), collected within the park and placed on 0.8 m-tall wooden stands, 13–42 m apart, in a natural forest environment at the AWRS. Antlers were not enclosed, so focal males were unrestricted in their movement and activities, and they were exposed to natural weather, predation, and variation in sex ratio and population density. We observed antlers concurrently every 2 h from 9:00–19:00 between 10 June and 5 July 2018, recording the presence and mating status (single vs. copulating or mate guarding) of marked males, and the number of antler flies present, including unmarked wild females and males. Copulation and mate guarding last 2.3 h on average (Bonduriansky and Brooks 1998), so we observed nearly all matings. Males that did not survive at least 24 h after release were excluded from analyses to minimize handling effects on mortality. Our analyses include 147 males (young mother × young father: 41; young mother × old father: 43; old mother × young father: 32; old mother × old father: 31; Table A3).

Statistical analyses

Statistical analyses were performed in R v3.6.3 (R Core Team 2020). All continuous independent variables were standardized to mean of zero and standard deviation of one (Schielzeth 2010).

Early Life Variables

First, we investigated whether parental age treatment (young vs. old) affected offspring cohort size (number of emerging adults per jar), development time, and wing length. Early life environmental quality, development time, and body size can influence adult performance and senescence in antler flies (Bonduriansky and Brassil 2005; Angell et al. 2020). Thus, if parental ages influenced these variables, they could mediate effects on offspring performance. We analyzed cohort size (n = 24) with a linear model including maternal age, paternal age, and block, with significance determined via permutation, given observed heteroscedasticity, using *lmPerm* (Wheeler and Torchiano 2016). Development time (n = 448) and wing length (n = 433) were each analyzed with a linear model that included fixed effects of parental ages, offspring sex, cohort size, and block. We performed type-III F-tests on each parameter using *car* (Fox and Weisberg 2011).

Survival and Actuarial Senescence

We analyzed offspring survival and actuarial senescence with an interval-censored parametric survival regression using the "survreg" function in the *survival* package (Therneau 2015). This function can use one of six survival distributions: the (non-senescent) single-parameter exponential distribution, which has only a single parameter representing mortality rate, and five two-parameter distributions that model senescence (Weibull, Gaussian, log-normal, logistic, and log-logistic). The latter have "location" and "scale" parameters, representing average mortality and senescence rate, respectively. Any number of variables can be accommodated on the location parameter, but only a single categorical factor can be fit on the scale parameter via the "strata" function.

Individuals were considered to have died in the inclusive interval between the day of their last sighting and the following day. Four males alive at the end of the experiment were right-censored. We performed the survival analysis in three steps: 1) distribution selection via AICc (Hurvitch and Tsai 1996); 2) selection of a scale parameter factor based on likelihood ratio tests (LRT); 3) testing the significance of all variables in the resulting model using LRT.

In steps 1 and 2 (Appendix A), we determined that a log-normal distribution fitting a single global scale value (i.e., containing no scale factor) was the best fit to our data (see Results). The location parameter of the log-normal distribution (μ_{log}) represents mean lifespan on a log scale, and the scale parameter (σ_{log}) represents the standard deviation of lifespan on a log scale. In step 3, we tested the significance of all variables using type-II LRT implemented in *car*, as the design was not balanced and contrasts cannot be set properly for type-III tests in *survival*. We included the following variables on the location parameter: maternal and paternal age, their interaction, development time, wing length, offspring cohort size, lifetime average adult density (flies/cm^2 on the antler), and block. Development time, body size (e.g., wing length), adult density, and larval environmental quality (e.g., cohort size) have been previously shown to affect longevity, mating success, and/or aging in this species (Bonduriansky and Brassil 2005; Angell et al. 2020). Block was treated as a fixed effect because there were only three levels, which is not sufficient to accurately estimate a random effect variance (Harrison et al. 2018).

Mating Success and Reproductive Senescence

We analyzed parental age effects on mating rate (the likelihood of mating at a given observation) and reproductive senescence using a binomial generalized linear mixed model (GLMM) implemented in *lme4* (Bates et al. 2015), in which each male was either not mating (0) or mating (1) at each observation period. When a male was mating in consecutive observations (n = 9 observations), the second record (and, in one case, third) was disregarded to avoid multiple counting. We included fixed effects of maternal and paternal age, their interaction, linear and quadratic effects of offspring age (to quantify reproductive senescence) and their two-way interactions with parental ages (to quantify treatment effects on reproductive senescence). Offspring adult density (flies/cm^2 on the antler at time of observation), cohort size, development time, wing length, antler, and block were also included as fixed effect covariates. To limit model complexity, we chose not to fit higher-order interactions. We included

random effects of male identity and observation nested within day. We tested the significance of our parameters using type-III Wald χ^2 tests in *car*.

Lifetime mating success (LMS; total number of matings observed) was analyzed using a negative-binomial generalized linear model (GLM) implemented in *MASS* (Venables and Ripley 2002), including the same variables as those on the location parameter of the survival regression as fixed effects. We tested the significance of our parameters using type-III LRTs in *car*.

Results

Old mothers had smaller offspring cohort sizes (number of emerging adults per jar; permutation test: $p = 0.026$), indicating lower fecundity and/or reduced juvenile viability of their lab-reared offspring (mean number of offspring ± SD: old, 58.5 ± 58.4;young, 134.5 ± 104.5; Fig. A1A). There was no effect of paternal age on cohort size ($p = 0.514$), nor did paternal age interact with maternal age ($p = 0.883$). Parental ages did not affect offspring development time or wing length (Table A4; Fig. A1B,C), but larger cohorts were associated with slower development ($F_{1,440} = 8.51$, $p = 0.004$; Fig. A1D).

Survival of sons in the wild was best described by a log-normal distribution (ΔAICc = 0.1–1.8 for the next best supported distribution, Weibull; Table A5). Unlike the Weibull distribution, in which mortality rate increases continuously with age, the log-normal distribution describes an initial increase in mortality rate, followed by a decrease, which was small in this case (Fig. 1A). However, a non-senescent exponential model was a poor fit (ΔAICc = 115.4), providing evidence of actuarial senescence.

Sons of old fathers lived slightly longer on average (location parameter, μ_{log}) than sons of young fathers (LRT: $\chi^2_1 = 4.39$, $p = 0.036$; Fig. 1C; Table A6), but there was no significant effect of maternal age ($\chi^2_1 = 1.51$, $p = 0.220$). The effect of paternal age was more prominent among sons of young mothers (Fig. 1B,C), although the interaction was not significant ($\chi^2_1 = 2.07$, $p = 0.151$). Actuarial senescence rate (scale parameter, σ_{log}) did not differ significantly among treatments (maternal age: $\chi^2_1 =$

192 0.0005, $p = 0.982$; paternal age: $\chi^2_1 = 0.963$, $p = 0.326$; maternal × paternal age: $\chi^2_3 = 0.966$, $p = 0.809$)
193 or other groups (binned development time: $\chi^2_1 = 0.010$, $p = 0.922$; binned wing length: $\chi^2_1 = 0.150$, $p =$
194 0.699).

195 There were significant linear (Wald $\chi^2_1 = 4.15$, $p = 0.042$) and quadratic effects of age ($\chi^2_1 =$
196 11.93, $p < 0.001$) on male mating rate (Table A7). Mating rate increased from release until age 9 d and
197 subsequently declined (Fig. 1D). When excluding observations prior to the apparent peak at 9 d, the
198 linear effect of age (i.e. actuarial senescence) was not significant ($\chi^2_1 = 1.55$, $p = 0.213$).

199 Average mating rate did not change significantly with maternal (Wald $\chi^2_1 = 0.308$, $p = 0.579$) or
200 paternal age ($\chi^2_1 = 0.047$, $p = 0.828$), nor their interaction ($\chi^2_1 = 0.017$, $p = 0.897$; Fig. 1F). The change
201 in mating rate with age also did not differ among parental age treatments (maternal age × offspring age:
202 $\chi^2_1 = 0.105$, $p = 0.747$; maternal age × offspring age^2: $\chi^2_1 = 0.060$, $p = 0.807$; paternal age × offspring
203 age: $\chi^2_1 = 0.014$, $p = 0.970$; paternal age × offspring age^2: $\chi^2_1 = 0.681$, $p = 0.409$).

204 Finally, male LMS did not change significantly with maternal age (LRT: $\chi^2_1 = 0.030$, $p = 0.863$),
205 paternal age ($\chi^2_1 = 2.21$, $p = 0.138$), nor their interaction ($\chi^2_1 = 0.515$, $p = 0.473$; Fig 1E; Table A8).
206

207 **Discussion**

208 Parental age effects on offspring longevity and performance are well known in humans and laboratory
209 populations of other taxa (Bell 1918; Lansing 1947; Wang and vom Saal 2000), but investigation in
210 wild animals has started only recently (reviewed in Fay et al. 2016). We used a manipulative field
211 experiment to measure parental age effects on survival and reproduction of male antler flies under
212 natural conditions. Overall, we report improved longevity in sons of old fathers, and no other
213 significant effects—including, notably, no apparent costs—of parental age at reproduction in antler
214 flies.

215 When adult male offspring were released in the field, increased paternal age improved longevity
216 (location parameter, μ_{log}), although this effect was primarily driven by differences in offspring of young

mothers. At 5 d, when the difference was greatest, we estimate 40% higher mortality in sons of young compared to old fathers (Fig. 1A), and we find an 18% difference in mean lifespan overall (Fig. 1C). Differences in offspring survival were some of the earliest parental age effects reported (Bell 1918; Lansing 1947), suggesting these may be the strongest and easiest to detect. Frequently, offspring of old parents are short-lived (Bell 1918; Lansing 1947; Reichert et al. 2019), but some studies have found no effect (Schroeder et al. 2015; Arslan et al. 2017; Ivimey-Cook and Moorad 2018) or a positive effect of parental age on offspring longevity (Priest et al. 2002; Fox et al. 2003), consistent with our findings in fathers. Such positive effects of paternal age should favor female preferences for old mates (Kokko and Lindström 1996).

Consistent with previous studies (Bonduriansky and Brassil 2002), we detected actuarial senescence in wild male antler flies (Fig. 1A). Mortality increased rapidly then plateaued, which could reflect selective disappearance of frail males before reaching old age. However, there was no effect of parental age on senescence rate (scale parameter, $\sigma_{\log}$). Parental age effects on senescence rate *per se* have rarely been quantified. Wylde et al. (2019) found modest and inconsistent differences between (grand)offspring of old and young captive neriid flies. Thus, parental age may primarily affect offspring survival through differences in overall frailty rather than senescence.

There was no evidence for parental age effects on offspring mating rate, reproductive senescence, or LMS (Fig. 1D-F). Previous studies have reported parental age effects on reproduction in the wild, but these typically measure offspring production (e.g., brood size or recruitment) rather than mating success (Bouwhuis et al. 2010; Schroeder et al. 2015; Kroeger et al. 2020).

Parental age effects can differ by parental sex (Fay et al. 2016) and can also interact with offspring sex (Fox et al. 2003; Schroeder et al. 2015). In wild house sparrows (*Passer domesticus*), increased parental age decreased lifetime reproduction of only same-sex offspring (Schroeder et al. 2015). Similarly, our results suggest stronger effects of paternal than maternal age on sons' survival and

LMS, although we cannot say whether maternal age has a parallel effect on daughters. Female antler flies lack males' territorial behavior and can therefore only be studied in the laboratory.

Overall, these data demonstrate that large, costly parental age effects do not occur in wild male antler flies, at least over the ages used in our experimental manipulation. The only significant effect was a benefit of decreased mortality in sons of old fathers (Fig. 1A,C). This could represent a fundamentally positive relationship (Kroeger et al. 2020) or the beginning of an ultimately convex surface (Rödel et al. 2009; Ivimey-Cook and Moorad 2018). Older parental ages would be required to verify that, but considering the natural history of antler flies—wild flies' median lifespan is 6–8 d (Mautz et al. 2019) and only ~20% survive beyond 13 d (Bonduriansky and Brassil 2005)—the biological importance of effects at such ages is likely small. Parental age manipulations of months performed in laboratory studies of short-lived species (Priest et al. 2002; Wylde et al. 2019) are hard to extrapolate to late-life reproduction in wild insects.

Under laboratory conditions, older females produced fewer adult offspring, indicating reproductive senescence of female parents (i.e. a decline in fecundity with age; Moore and Moore 2001) and/or maternal effects on egg to adult viability (Bloch Qazi et al. 2017), but we cannot separate these effects. There were no parental age effects on offspring size or development time.

Parental age effects can be caused by age-related changes in the parents (Moorad and Nussey 2016) or by selective disappearance of individuals differing in genetic makeup or reproductive strategy (Ivimey-Cook and Moorad 2018; Monaghan et al. 2020). For example, alleles conferring longevity may have been overrepresented in those fathers that survived to old age, and thus in their offspring. Because our experiment was cross-sectional (i.e., using separate old and young cohorts) rather than longitudinal (i.e., tracking age-related changes within individuals), we cannot distinguish between these.

In conclusion, our study begins to bridge the gap between laboratory-based understanding of parental age effects in short-lived invertebrates and vertebrate-based understanding of the consequences

of parental age in the wild. Our results suggest that apparent costs of increasing parental age that are often detected in the lab may be less prevalent in the wild. Studies of senescence in wild insects remain rare (Zajitschek et al. 2020), and further work will hopefully reveal whether parental age effects in insects are as common under natural conditions as they appear to be in the laboratory.

Acknowledgments

We are grateful to the antler flies and to the moose whose shed antlers made our work possible. This research was supported by a grant from the Natural Sciences and Engineering Research Committee of Canada to HDR.

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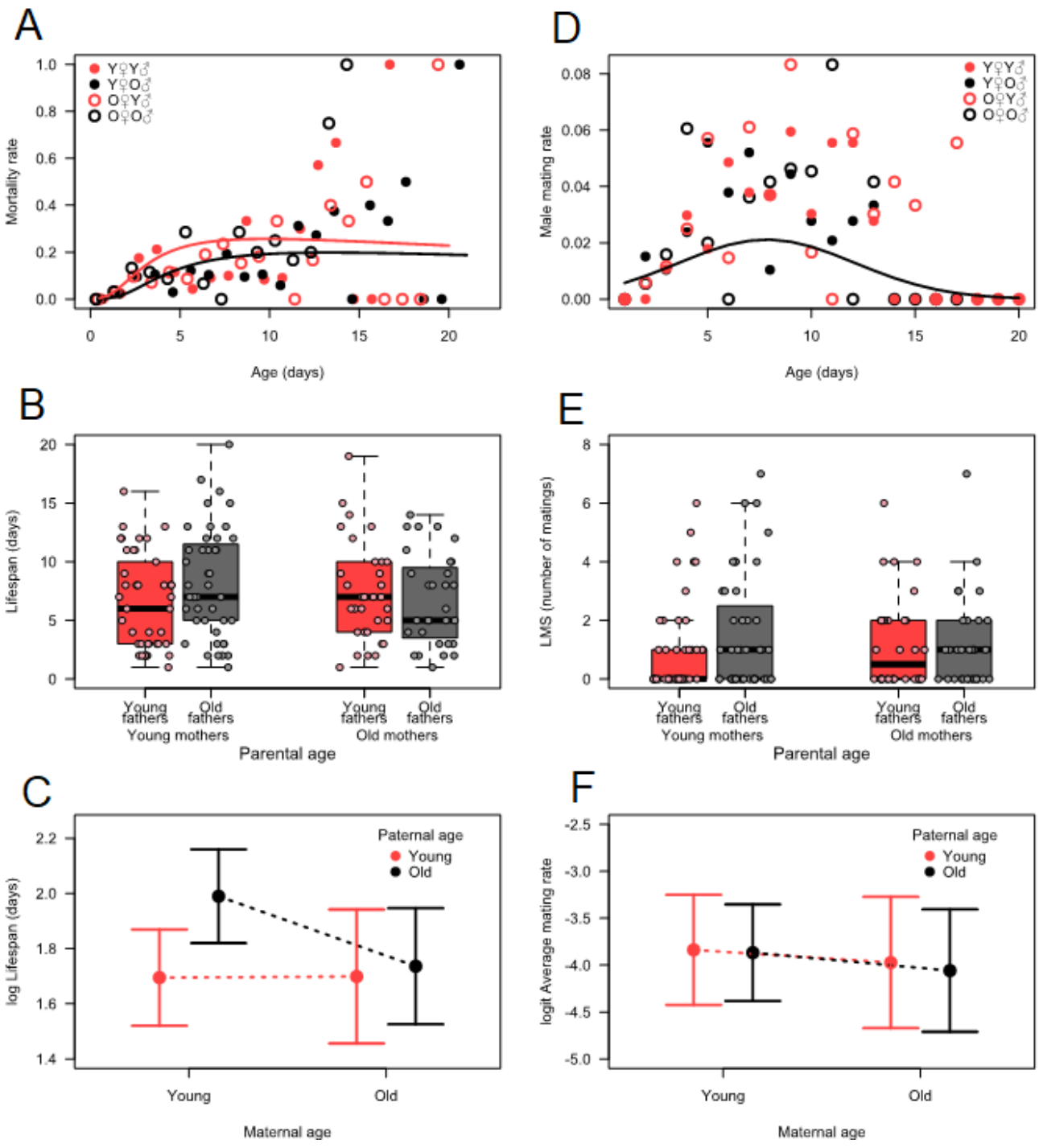
A
Y♀Y♂
Y♀O♂
O♀Y♂
O♀O♂
Mortality rate
Age (days)
D
Male mating rate
Age (days)
B
Lifespan (days)
Young fathers
Old fathers
Young mothers
Old mothers
Parental age
E
LMS (number of matings)
C
Paternal age
Young
Old
log Lifespan (days)
Maternal age
F
logit Average mating rate

Figure 1. Effects of parental age on survival, mating success, and senescence of male antler flies in the wild. *A*, age-specific mortality rate (actuarial senescence). Points represent observed daily mortality rates and curves are predicted values based on the log-normal survival model (red: sons of young fathers; black: sons of old fathers). *B*, observed lifespan. Thick lines represent the median and boxes demark the first and third quartiles. *C*, estimated marginal means (95% CI) for maternal and paternal age (i.e., accounting for the effects of other variables) on the location parameter ($\mu_{\log}$, representing mean lifespan on a log scale) from the log-normal model, shown on a log scale. *D*, *c*hange in mating rate with age (reproductive senescence). Points represent observed mating rates (per observation period) in each treatment and the curve is predicted values from the binomial GLMM (weighted mean across treatments, antlers, and blocks), back-transformed from the logit scale. *E,* lifetime mating success. Thick horizontal lines are the median and the boxes demark the first and third quartiles. *F,* estimated marginal means for maternal and paternal age on average mating rate, shown on a logit scale. Estimated marginal means were calculated with the R package *emmeans* (Lenth 2020).

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Appendix A

Supplementary Methods

Parental age manipulation

Beginning 1 May 2018, we collected newly eclosed males and females, to serve as parents, from the stock over a period of 3–4 d. Adults were held in mixed-sex cages (35M:35F, 1.9–2.7 L) that contained sugar, water, and oviposition dishes consisting of ground beef and a sponge soaked with "beef solution" (Oudin et al. 2015). Young parents were collected 9 d after old parents, so they were 1–3 d old when the old parents were 10–13 d old. Parents of each sex and age were mated to each other for 48 h in a 2×2 factorial design in 100M:100F cages (2.7 L, one per treatment) containing two oviposition dishes. Dishes were collected and replaced after 24 h, yielding two sets of two dishes per cage. Oviposition dishes were placed as pairs in glass jars (n = 24 jars: 12 parental cages × 2 days of collection each) with a mesh cap and 3–5 cm of coco peat for pupation. Dishes were supplemented with up to 1.5 ml beef solution three times/week during larval development. We repeated the procedure twice more with different parents, 3–4 d apart, creating three staggered, genetically distinct blocks of offspring.

Selection of Survival Distribution and Scale Factor

First, we used AICc model selection (Hurvitch and Tsai 1996), implemented in the R package *MuMIn* (Bartoń 2016), to choose which of the six survival distributions supported by *survival* provided the best fit to our data. We fit a series of survival regressions using each distribution, each containing the following variables on the location parameter: maternal and paternal age, their interaction, development time, wing length, offspring cohort size, lifetime average adult density (flies/cm^2 on the antler), and block. Block was treated as a fixed effect because there were only three levels, which is not sufficient to accurately estimate a random effect variance (Harrison et al. 2018).

We tested the fit of the two-parameter distributions (i.e. all except the single-parameter exponential) using each of the following scale-parameter factors separately: maternal age, paternal age,

maternal × paternal age, binned development time, and binned wing length. Development time and wing length, which have previously been shown to affect survival and senescence in this species (Bonduriansky and Brassil 2005; Angell et al. 2020), were converted to categorical variables for use on the scale parameter by binning them above and below the median value. Regardless of the scale factor, log-normal was always the top ranked survival distribution (Table A3), so subsequent survival analyses used a log-normal distribution.

Second, we selected among the above scale parameter factors using LRT. For each factor, we compared a model where the scale parameter value was able to vary by the level of the factor to a model fitting a single global scale value.

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346 Table A1. Cohort sizes (number of emerging adults) for each larval jar.

		Maternal age			
		Young mothers		Old mothers	
		Paternal age			
Block	Laying Date	Young fathers	Old fathers	Young fathers	Old fathers
1	15 May	223	269	120	92
1	16 May	261	295	34	4
2	19 May	24	47	0	3
2	20 May	118	105	66	86
3	21 May	97	149	29	198
3	22 May	1	47	25	23

347

Table A2. Number of males in each treatment and block marked and released on each antler.

Overall	Maternal age				
	Young mothers		Old mothers		
	Paternal age				
Antler	Young fathers	Old fathers	Young fathers	Old fathers	*Total*
A	16	19	13	15	*63*
B	16	23	12	15	*66*
C	18	18	14	12	*62*
D	16	18	12	12	*58*
Total	*66*	*78*	*51*	*54*	***249***
Block 1	Maternal age				
	Young mothers		Old mothers		
	Paternal age				
Antler	Young fathers	Old fathers	Young fathers	Old fathers	*Total*
A	11	12	7	5	*17*
B	10	14	7	3	*34*
C	14	12	7	5	*38*
D	12	6	6	4	*28*
Total	*47*	*44*	*27*	*17*	***117***
Block 2	Maternal age				
	Young mothers		Old mothers		
	Paternal age				
Antler	Young fathers	Old fathers	Young fathers	Old fathers	*Total*
A	3	4	2	2	*11*
B	5	6	4	3	*18*
C	1	1	2	1	*5*
D	3	3	3	3	*12*
Total	*12*	*14*	*11*	*9*	***46***
Block 3	Maternal age				
	Young mothers		Old mothers		
	Paternal age				
Antler	Young fathers	Old fathers	Young fathers	Old fathers	*Total*
A	2	3	4	8	*17*
B	1	3	1	9	*14*

C	3	5	5	6	*19*
D	1	3	3	5	*12*
Total	*7*	*14*	*13*	*28*	***62***

Table A3. Number of males released in each treatment, block, and antler that were resighted at least once. There was no difference among treatment groups in the proportion of males resighted at least once ($\chi^2 = 1.10$, d.f. = 3, $p = 0.288$).

Overall	Maternal age				
	Young mothers		Old mothers		
	Paternal age				
Antler	Young fathers	Old fathers	Young fathers	Old fathers	*Total*
A	9	11	8	9	*37*
B	11	10	6	8	*35*
C	10	10	9	8	*37*
D	11	12	9	6	*38*
Total	*41*	*43*	*32*	*31*	***147***
Block 1	Maternal age				
	Young mothers		Old mothers		
	Paternal age				
Antler	Young fathers	Old fathers	Young fathers	Old fathers	*Total*
A	6	8	4	3	*21*
B	6	7	3	3	*19*
C	7	5	6	4	*22*
D	9	8	5	3	*25*
Total	*28*	*28*	*18*	*13*	***87***
Block 2	Maternal age				
	Young mothers		Old mothers		
	Paternal age				
Antler	Young fathers	Old fathers	Young fathers	Old fathers	*Total*
A	1	2	1	1	*5*
B	5	1	3	0	*9*
C	1	1	2	0	*4*
D	2	2	2	1	*7*
Total	*9*	*6*	*8*	*2*	***25***
Block 3	Maternal age				
	Young mothers		Old mothers		

	Paternal age				
Antler	Young fathers	Old fathers	Young fathers	Old fathers	*Total*
A	2	1	3	5	*11*
B	0	2	0	5	*7*
C	2	4	1	4	*11*
D	0	2	2	2	*6*
Total	*4*	*9*	*6*	*16*	***35***

Table A4. Linear models testing for effects of parental age on development time and wing length of offspring.

	Development time (days)			Wing length (mm)		
Factor	Estimate ± SE	F †	P	Estimate ± SE	F ‡	P
(Intercept)	30.0 ± 0.302	2110.1	< 0.001	2.27 ± 0.014	309.2	<0.001
Cohort size (adults emerging)	1.05 ± 0.360	8.51	0.004	-0.009 ± 0.008	1.49	0.223
Sex (Female)	-0.151 ± 0.244	0.382	0.537	0.049 ± 0.005	80.3	<0.001
Maternal age (Old)	0.184 ± 0.300	0.376	0.540	0.004 ± 0.007	0.342	0.559
Paternal age (Old)	0.085 ± 0..254	0.111	0.739	0.007 ± 0.006	1.57	0.211
Maternal × paternal age (Old-Old)	-0.457 ± 0.243	3.55	0.060	-0.003 ± 0.005	0.238	0.626
Block (2)	1.29 ± 0.400	7.39	< 0.001	-0.002 ± 0.009	4.32	0.014
Block (3)	-0.111 ± 0.467			-0.023 ± 0.010		

† D.f. are 1, 440 for all variables except block, which has d.f. 2, 440.

‡ D.f. are 1, 425 for all variables except block, which has d.f. 2, 425.

Table A5. Model selection by AICc to choose a parametric survival distribution. ΔAICc values are calculated relative to the best supported distribution (log-normal in each case) using the same scale factor.

	Scale parameter factor											
	Intercept (single level)		Development time (two levels)		Wing length (two levels)		Maternal age (two levels)		Paternal age (two levels)		Maternal × paternal age (four levels)	
	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc	AICc	ΔAICc
Log-normal	779.9	0.0	782.2	0.0	782.2	0.0	782.3	0.0	781.7	0.0	786.5	0.0
Weibull	781.7	1.8	784.0	1.8	782.3	0.1	783.9	1.6	782.5	0.8	787.2	0.7
Log-logistic	783.6	3.7	786.0	3.8	785.6	3.4	786.0	3.7	785.0	3.3	789.9	3.4
Gaussian	802.7	22.8	803.9	21.7	805.0	22.8	805.1	22.8	805.0	23.3	807.3	20.8
Logistic	804.0	24.1	805.2	23.0	806.3	24.1	806.3	24.0	806.3	24.6	809.0	22.5
Exponential	885.5	105.6	-	-	-	-	-	-	-	-	-	-

Table A6. Summary of the log-normal parametric survival model. All continuous independent variables were standardized to a mean of zero and a standard deviation of one prior to analysis. Location parameter estimates are on a log scale.

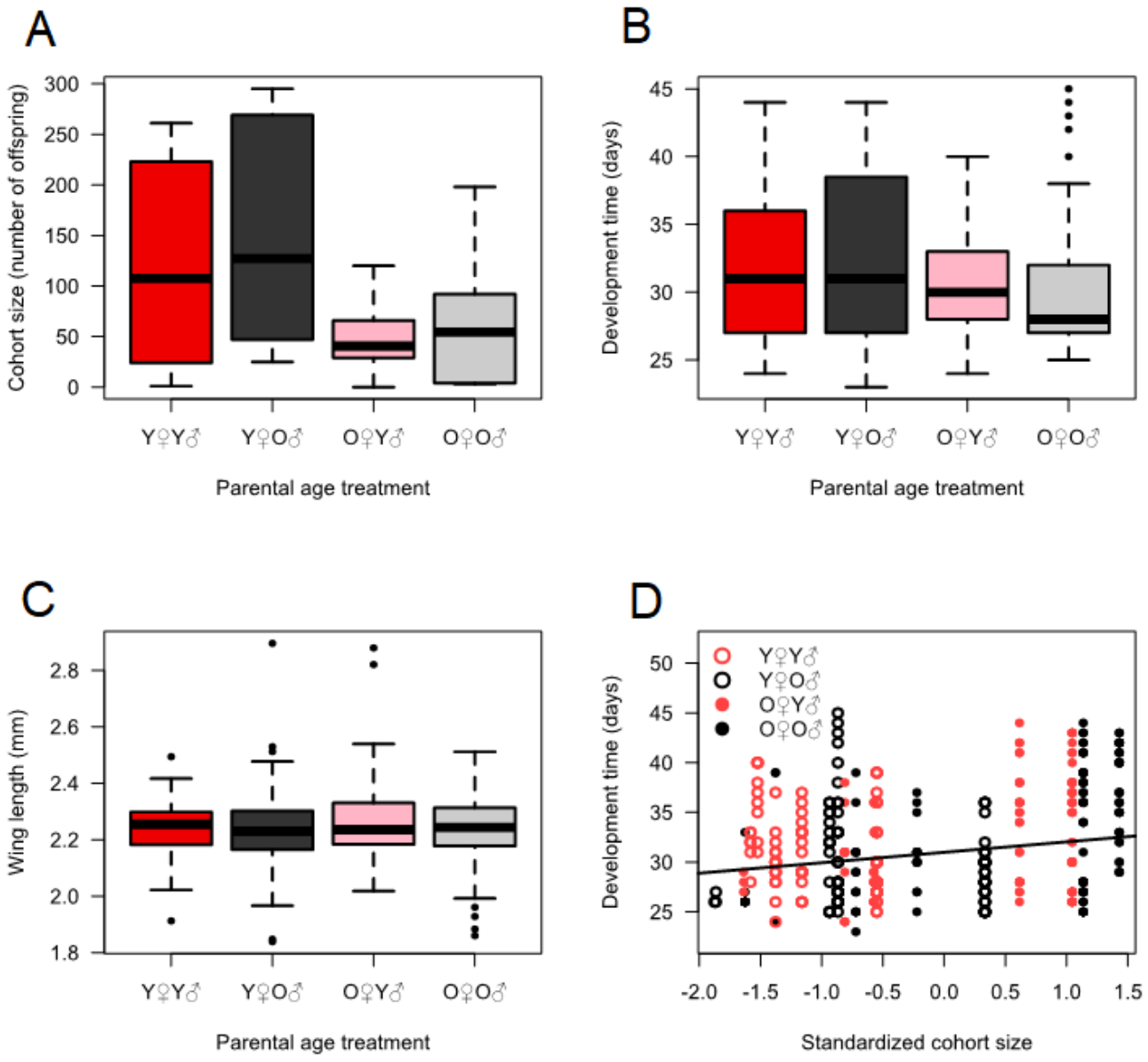
	Estimate	SE	*z*	*p*
Location effect (μ_{log})				
Intercept	1.943	0.100	19.4	< 0.001
Maternal age (Old)	0.004	0.149	0.03	0.979
Paternal age (Old)	0.295	0.115	2.56	0.010
Maternal × paternal age (Old-Old)	-0.258	0.179	-1.44	0.150
Offspring cohort size	-0.148	0.070	-2.12	0.034
Development time	-0.166	0.051	-3.25	0.001
Wing length	0.062	0.044	1.40	0.161
Mean adult density	0.354	0.058	7.35	< 0.001
Block (2)	-0.341	0.149	-2.29	0.022
Block (3)	-0.403	0.125	-3.23	0.001
Scale effect ($ln(\sigma_{log})$)				
Intercept	-0.666	0.061		

Table A7. Summary of the binomial GLMM for mating rate. All continuous independent variables were standardized to a mean of zero and a standard deviation of one prior to analysis. Estimates are on a logit scale.

Fixed effect	Estimate	SE	*z*	*p*
Intercept	-3.93	0.219	-17.9	< 0.001
Age	0.273	0.134	2.04	0.042
Age^2	-0.343	0.099	-3.45	< 0.001
Maternal age (Old)	0.081	0.147	0.555	0.579
Paternal age (Old)	0.029	0.133	0.217	0.828
Maternal × paternal age (Old-Old)	-0.014	0.109	-0.129	0.897
Age × maternal age (Old)	-0.038	0.118	-0.323	0.747
Age^2 × maternal age (Old)	0.025	0.102	0.245	0.807
Age × paternal age (Old)	0.004	0.115	0.038	0.970
Age^2 × paternal age (Old)	-0.086	0.104	-0.825	0.409
Offspring cohort size	-0.128	0.166	-0.771	0.441
Development time	-0.008	0.138	-0.060	0.952
Wing length	0.053	0.110	0.481	0.630
Adult density	0.316	0.147	2.14	0.032
Antler (B)	0.218	0.201	1.09	0.277
Antler (C)	0.143	0.246	0.581	0.561
Antler (D)	-0.651	0.344	-1.89	0.058
Block (2)	-0.044	0.201	-0.220	0.826
Block (3)	-0.212	0.229	-0.925	0.355
Random effect	Variance	SD		
Male identity	0.550	0.664		
Observation (nested within day)	0.450	0.670		

371 Table A8. Summary of the negative binomial GLM for LMS (dispersion parameter $\theta = 1.32$). All
372 continuous independent variables were standardized to a mean of zero and a standard deviation of one
373 prior to analysis. Estimates are on a log scale.
374

Fixed effect	Estimate	SE	*z*	*p*
Intercept	0.053	0.258	0.167	0.868
Maternal age (Old)	-0.065	0.372	-0.173	0.862
Paternal age (Old)	0.431	0.290	1.49	0.137
Maternal × paternal age (Old-Old)	-0.319	0.445	-0.714	0.475
Offspring cohort size	-0.282	0.167	-1.68	0.092
Development time	-0.178	0.136	-1.31	0.190
Wing length	0.085	0.112	0.845	0.398
Mean adult density	0.512	0.119	4.314	< 0.001
Block (2)	-0.437	0.381	-1.15	0.252
Block (3)	-0.126	0.302	-0.417	0.676



375 Figure A1. Offspring cohort size, development time, and body size (wing length) across parental age
376 treatments. *A.* cohort size (adult emergence per larval jar) in each treatment. *B*, egg-to-adult
377 development time in each treatment. *C*, wing length in each treatment. Heavy horizontal lines represent
378 the median, and the boxes demark the first and third quartiles. *D,* relationship between number of
379 offspring per jar and development time across treatments. Regression line based on the partial effect of
380 cohort size on development time in Table A4.