Coevolution of longevity and female care of male gametes

Julian Baur[1], Mareike Koppik[1,2], Uroš Savković [3], Mirko Đorđević [3], Biljana Stojković [4] & David Berger[1],†

† Corresponding author: david.berger@ebc.uu.se.

1: Department of Ecology and Genetics, Uppsala University, Sweden.

2: Animal Ecology, Department of Zoology, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany; mareike.koppik@zoologie.uni-halle.de

3: Department of Evolutionary Biology, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade, Serbia

Uroš Savković – savkovic.uros@ibiss.bg.ac.rs +381112078376

ORCID: https://orcid.org/0000-0001-9430-4619

Mirko Đorđević – mirko.djordjevic@ibiss.bg.ac.rs +381112078376

ORCID: https://orcid.org/0000-0002-5768-0081

4: Institute of Zoology, Chair of Genetics and Evolution, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia

Biljana Stojković – bilja@bio.bg.ac.rs +381112635627

ORCID: https://orcid.org/0000-0001-9978-2249

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Abstract

An often-overlooked aspect of life-history optimization is the allocation of resources to protect the germline and secure safe transmission of genetic information. While failure to do so renders significant fitness consequences in future generations, germline maintenance comes with substantial costs. Thus, germline allocation should trade-off with other life history decisions and be optimized in accord with an organism's reproductive schedule. Here we tested this hypothesis by studying germline maintenance in lines of seed beetle, selected for early (E) or late (L) reproduction for 350 and 240 generations, respectively. Female animals provide maintenance of male gametes in their reproductive tract and oocytes. Here, we revealed the ability of young and old E and L-females to provide this form of germline maintenance by mating them to males with ejaculates with artificially elevated levels of protein and DNA damage. We find pronounced evolved differences in this form of germline maintenance between E and L females that accord with the age of reproduction in respective regime. These findings identify the central role of allocation to secure germline integrity in life history evolution and highlight how females can play a crucial role in mitigating effects of male germline decisions on mutation rate and offspring quality.

Introduction

The interplay between growth, reproduction, and survival is a central focus of life history theory (Roff, 1993; Stearns, 1992). Trade-offs among these three fundamental aspects of an organism's life play key roles in dictating the evolution of reproductive schedules, with cascading effects on organismal aging and demography (Flatt & Heyland, 2011; Monaghan et al., 2008; Promislow & Tatar, 1998). Indeed, some species invest heavily in early reproduction at the expense of longevity, while others prioritize growth and survival before reproducing, and these alternative strategies are expected outcomes of differences in mortality risk and age-related changes in the strength of natural selection (Roff, 1993; Stearns, 1992).

Another, often less explicitly defined, dimension of life history optimization involves the allocation of resources to protect the germline (Maklakov & Immler, 2016; Monaghan & Metcalfe, 2019). The integrity of the germline is essential for the transmission of genetic information to subsequent generations, and hence, the evolutionary consequences of germline maintenance strategies can be considerable both at the individual and population level (Agrawal & Whitlock, 2012; Lynch, 2010; Muller, 1950). The costs of germline maintenance may be substantial (Maklakov & Immler, 2016), in part due to the expensive repair machinery required to keep the rate of deleterious germline mutations at levels several magnitudes lower than the somatic mutation rate (Lynch, 2008). This sets the stage for trade-offs between the germline and other life history traits, where costly maintenance of the germline may secure the genetic quality of future generations at the cost of reduced reproduction and/or survival of the parent (Maklakov & Immler, 2016; Monaghan & Metcalfe, 2019).

Thus, theories of aging not only predict age-specific optimization of classic life history traits, but also concerted evolution of processes aimed at protecting the germline. For example, the mutation accumulation theory of aging (Medawar, 1952), from hereon: “MA”, predicts a decline in germline maintenance past an organism’s reproductive peak due to weakened selection and the accumulation of alleles with deleterious effects late in life. Indeed, ageing not only leads to somatic deterioration (Partridge & Barton, 1996), but also negatively

affects the maintenance of the germline (Monaghan & Metcalfe, 2019), shown, for example, by the fact that *de novo* germline mutation rates in primates seem to be foremost associated with advancing age (Kong et al., 2012; Rahbari et al., 2016a; Venn et al., 2014). Ageing-trajectories can also be driven by antagonistic pleiotropy (Williams, 1957), from hereon: "AP", whereby alleles with beneficial effects on germline maintenance at the peak of reproduction (e.g. via increased allocation of energy to germline repair) are favoured by selection despite potential deleterious side-effects on somatic maintenance (Chen et al., 2020), reproduction (Baur & Berger, 2020; Berger et al., 2017), and/or germline maintenance at other ages.

There is now growing evidence that germline decisions can be studied through a life-history lens. For instance, germline maintenance strategies can differ between environments that place different needs on maintenance versus reproduction (Berger et al., 2021; Chu et al., 2018; Crean & Immler, 2021; Drake, 2009; Kurhanewicz et al., 2020; Puurtinen et al., 2016; Svetec et al., 2016), or between individuals that differ in their overall energy budgets and allocation decisions (Agrawal & Wang, 2008; Baur & Berger, 2020; Berger et al., 2017; Koppik et al., 2023; Sharp & Agrawal, 2012). Interestingly, female animals possess the ability to provide maintenance not only of their own germline cells, but also of male ejaculates and haploid male DNA, by synthesizing anti-oxidants in the reproductive tract to neutralize mutagenic reactive oxygen species, and by including mRNA and proteins in the oocyte to detect and repair DNA damage in the early zygote (Horta et al., 2020; Jaroudi et al., 2009; Stringer et al., 2018). This sets the stage for an intricate evolutionary dynamic, where care over transferred ejaculates and newly formed zygotes is the ultimate decision of the female, but the need for it might be dictated by the germline decisions of the male partner, or, like in human populations, his age (Gao et al., 2019a; Rahbari et al., 2016b; Ségurel et al., 2014).

Even though there now is a good understanding of the mechanisms that cause age-dependent variation in DNA-repair and maintenance of the germline, little is known about the evolutionary potential of such mechanisms. In humans, for example, changes to environmental (Klass, 1977), pharmaceutical (Johnson et al., 2013) and genetic (Guarente & Kenyon, 2000) factors can increase longevity substantially within one or a few generations, and the peak of reproduction has in many populations changed drastically over the last

108 century (Khandwala et al., 2017a; Mills et al., 2011a). However, the long-term genetic
109 consequences of lifespan extension depend on whether selection on reproductive schedules
110 are coupled with concomitant changes in germline repair and maintenance, which remain
111 unexplored. Here we harnessed the power of long-term experimental evolution to
112 investigate how the evolution of lifespan affects age-dependent germline maintenance in
113 the seed beetle, *Acanthoscelides obtectus.* Using a set of lines that were established in 1989
114 and selected for early or late reproduction for more than 350 and 240 generations,
115 respectively, we examined germline maintenance in young and old females receiving male
116 ejaculates with artificially inflated levels of free radicals and DNA and protein damage (Fig.
117 1). This allowed us to explore i) whether female maintenance of male gametes evolves in
118 concerted fashion with reproductive schedules and other life history traits, and ii) whether
119 the AP (Williams, 1957) or the MA (Medawar, 1952) theory of ageing best explains the
120 evolutionary patterns observed.
121

122 **Methods**

123 *Study species*

124 The seed beetle, *Acanthoscelides obtectus* (Coleoptera; Chrysomelidae; Bruchidae) is a pest
125 of stored legumes, foremost, the common bean, *Phaseolus vulgaris* (Thakur, 2012). Females
126 lay eggs either directly under or in close proximity of host seeds and the hatched larvae
127 burrow into a seed where juvenile development takes place (Tucić et al., 1996). After
128 pupation, adults emerge from the seed and typically become reproductively active within 24
129 hours. Adult beetles are aphageous, meaning they don´t need nutrition nor water to be able
130 to reproduce and finish their lifecycle.
131

132 *Experimental populations*

133 The original base population, from which the experimental evolution lines were derived,
134 was created by mass-mating wild-caught beetles from three locations in the area of
135 Belgrade, Serbia, in 1983 (Tucić et al., 1996). Subsequently, the population was maintained
136 on *P. vulgaris* in the laboratory for 3 years (27 generations) at large population size (ca. 5000
137 individuals), until the experimental evolution lines were created in 1986. Experimental

evolution was performed in dark incubators at 30°C. No food or water was offered to experimental beetles.

During experimental evolution, one line was maintained under the same conditions as the base population (from here on referred to as the "base" line); after 40 days of development, 400-500 adult beetles were chosen at random and provided with fresh host seeds and allowed to reproduce for the next generation. Each of four replicate lines selected for early reproduction (E-lines) was created by taking 400 adults at random on the peak of adult emergence. To propagate the next generation, these adults were transferred to a new bottle containing host seeds *ad libitum* and were there allowed to reproduce and lay eggs for 48h, after which all adults were removed. Another four replicate lines were selected for late reproduction (L-lines) by taking 100 newly emerged (0-24h) adults every day for up to 10 days. Each of these subgroups was kept in a vial without seeds for 10 days. On the 10th day of each subgroup all the surviving beetles of a given replicate line were added to a single bottle containing fresh host seeds to propagate the next generation. For more information on the maintenance of the experimental evolution lines, see (Tucić et al., 1996, 1997). Development time of E-lines has evolved to be 29 days and the average virgin lifespan around 13 days in aphagous conditions, while L-lines emerge after 32 days and virgin beetles live on average 26 days for females and 22 days for males (Đorđević et al., 2015). The regimes have also diverged in other traits tied to rate-dependent life-history, such as the day of first oviposition (E: 1 day after eclosion, L: 4 days after eclosion) (Tucić et al., 2004), metabolic rate, and body size (Arnqvist et al., 2017).

Experimental design

The experiment was started in 2020, after 240 (L-lines) and 350 (E-lines) generations of experimental evolution. To estimate the evolution of age-dependent germline maintenance we compared females of all lines mated to males when aged 1-3 days (young age class) or 6-8 days (old age class). Note that 6-8 days is not truly an old age for L-lines and should be interpreted in relative terms. However, since we wanted to compare E- and L females, and E females age rapidly, 6-8 days were chosen to avoid any bias in form of selective disappearance (van de Pol & Verhulst, 2006) of low quality individuals in the E-lines (Fig. 1).

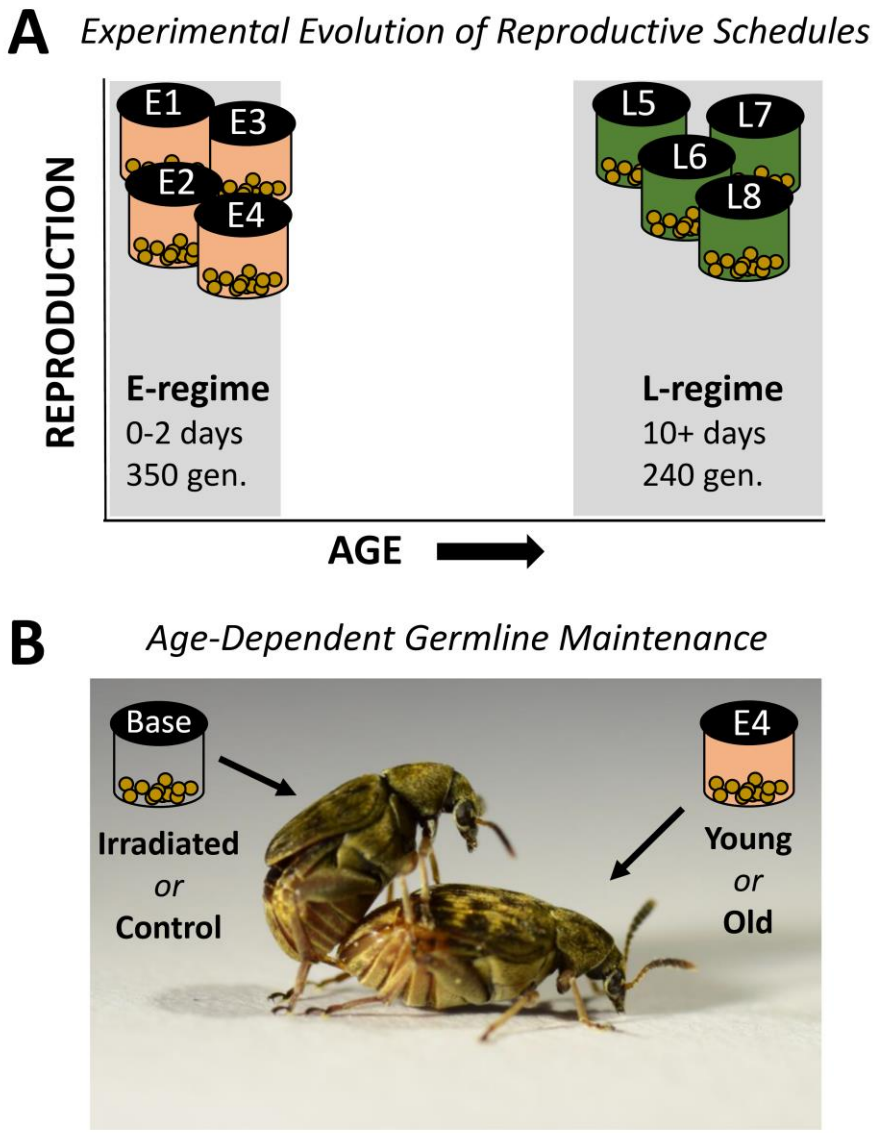

Figure 1: Experimental design.

A) The experiment included eight experimental evolution lines; four from the E- (early reproduction; salmon) and four from the L- (late reproduction; green) regime. The E-regime was propagated by harvesting eggs laid after only 2 days of adult reproduction, whereas the L-regime was propagated by providing individuals that had aged 10 days with host seeds and harvesting the eggs. The E and L regime had been kept for 350 and 240 generations, respectively, prior to the experiment. **B)** Females from all lines, that were either young (1-3 days) or old (6-8 days), were mated to standard males from the base population. These males were either serving as controls or they had been irradiated to generate a damaged ejaculate that would challenge maintenance in the female reproductive tract and oocytes. Offspring resulting from the matings to control males were counted to estimate age-dependent reproductive schedules of the F0 females in the experiment (Fig. 2). F1 offspring were then crossed within line and treatment group using a middle-class neighbourhood design to produce F2 offspring. From these F2 offspring counts, age-specific female care of male gametes could be deduced for each line by comparing counts from lineages deriving from young and old F0 females, mated with either irradiated or control males (Fig. 3). Photo credit: Mareike Koppik.

186 All lines were reared in staggered fashion by setting up several rearing containers of each
187 line in the two generations prior to the experiment, so that emergence of females would
188 happen continuously during the experiment and females assigned to the two age classes
189 could be assayed simultaneously and mated to standard males from the same cohort (see
190 below). We collected females on the day of their emergence, randomly assigned them to
191 the young or old age class, and then isolated them in perforated 0.5ml Eppendorf tubes and
192 left them to age. Females of both age classes were then mated to young (1-2 days) virgin
193 males from the base population in 60 mm petri-dishes placed on heating plates kept at
194 30°C. Couples were observed to avoid multiple mating. Males were removed after a mating
195 behaviour had been observed and females were allowed to lay eggs in a new 60 mm petri-
196 dish containing host seeds *ad libitum*.
197
198 The base males were either untreated (controls) or had been exposed to a 10 Gy dose of γ-
199 radiation, which causes substantial cellular damage to the male germline (Friedberg et al.,
200 2005) and impairs male fertility in seed beetles (Baur & Berger, 2020; Berger et al., 2017;
201 Koppik et al., 2023). Hence, the female repair and maintenance of damaged male gametes
202 in each line could be assessed by comparing the offspring production in lineages stemming
203 from females mated to control and irradiated males. γ-radiation produces free radicals with
204 mutagenic properties and causes direct damage to DNA within sperm cells (Friedberg et al.,
205 2005) and to proteins within the ejaculate (Kempner, 2001). Hence, our protocol captures a
206 multitude of effects that occur naturally in male ejaculates (Dowling & Simmons, 2009;
207 Maklakov & Immler, 2016), but that will have been greatly amplified by the irradiation
208 treatment.
209
210 As our interest lies in capturing effects of variation in female care that transcend
211 generations, we scored the fitness consequences of variation in female care by counting the
212 F2 offspring produced by all descending lineages. To achieve this, F1 males and females
213 stemming from the same treatment group and line were paired (while avoiding sib-mating)
214 and allowed to mate and produce F2 offspring in petri-dishes with ad libitum host seeds.
215 The emerging F2 offspring were counted to estimate each couple's fecundity, which was
216 used to estimate germline maintenance of the their F0 grandmothers (see below:
217 "Statistical analysis").

218 About half of the F0 couples produced no offspring. This was likely due to two reasons. First,
219 the mating latency of *A. obtectus* can be relatively long, and because the time in between
220 male irradiation and mating needed to be standardized, many couples did not mate in this
221 time. Secondly, even when a mating was thought to have been observed, several couples
222 did still not produce any offspring. Using a binomial model, we tested whether the incidence
223 of zero fertility was related to any of the factors of interest in our experiment (i.e.,
224 irradiation treatment, selection regime, and age). However, despite large sample size, no
225 factor had a significant effect on infertility. Indeed, all effects including male irradiation
226 treatment were very weak and non-significant (all $P > 0.3$), and the proportion of infertile
227 couples was relatively equal across treatments (E: young ctrl = 0.43; young irr = 0.48; old ctrl
228 = 0.59; old irr = 0.61. L: young ctrl = 0.50; young irr = 0.39; old ctrl = 0.48; old irr = 0.50).
229 Hence, we carried out our downstream analyses based on the remaining data from the 830
230 F0 couples that produced offspring. Each line and age-class was represented by 10-20
231 females mated to control males, and 20-30 females mated to irradiated males.
232
233 To reduce the effect of selection against epigenetic and DNA mutations induced by the
234 irradiation we applied a Middle-Class Neighbourhood (MCN) design (Shabalina et al., 1997).
235 The MCN design restricts each F0 couple to contribute the same number of F1 offspring for
236 future study, irrespective of their overall productivity. We note, however, that F0 couples
237 that produced less than four F1 offspring were not represented in the final data, and hence,
238 selection on very strongly deleterious effects of irradiation could not be excluded by the
239 MCN approach. For those F0 couples that did produce offspring, we aimed at propagating a
240 single male and female from each couple, so that each F0 couple would replace itself.
241 However, due to variability in the timing of eclosion of F1 individuals, additional F0 couples
242 fell out of the experiment as we did not want to age F1 beetles before setting them up in
243 assays. These strict criteria resulted in 490 F1 couples being analysed for offspring
244 production. Each line and age class was represented by ca 20 and 10 F1 couples that
245 originated from irradiated and control lineages, respectively. The experiment was
246 conducted in three temporal blocks, 3 days apart, corresponding to the three occasions on
247 which we irradiated F0 males from the base population. In the F1 generation, when
248 offspring productivity assays were set up, there was considerable overlap between blocks
249 due to variable timing of egg laying and juvenile development time.

Statistical analysis

We analyzed data using generalized linear mixed effect models implemented in the package MCMCglmm (Hadfield, 2010) for R (R Core Team, 2020). Offspring counts were analysed assuming Poisson distributed error. We used flat and uninformative priors for all models. We ran all models for 1.05 million iterations with an initial burn in of 50k iterations and a thinning factor of 500 to avoid autocorrelations, resulting in a posterior sample of 2000. P-values for all comparisons were calculated based on model posterior distributions, where significance of main effects was calculated by comparing posteriors of marginal means of two given groups (e.g. overall difference in offspring production in irradiated and control lineages). We used ggplot2 for graphical illustration (Wickham, 2016).

To provide an estimate of the extent of age-specific optimization of reproductive schedules at the current stage of experimental evolution, we first compared counts of offspring produced by young and old F0 females of all E- and L-lines, when mated to the standard base males belonging to the control (un-irradiated) treatment (N = 307). This model included female age (young vs. old), evolution regime (E vs. L), and their interaction, as fixed effects of interest. We also added the main effect of male age (1 or 2 days old) as a blocking factor. Replicate line, crossed with age, were added as random effects.

To analyse differences in female care of male gametes we compared the F2 offspring counts of control and irradiated lineages. This model included fully crossed fixed effects of evolution regime (E vs. L), F0 female age (young vs. old) and male radiation treatment (control vs. irradiated). Note that the three-way interaction between these effects tests our focal hypothesis that the E- and L-regime have diverged in age-specific female care of male gametes. Replicate line crossed with age and radiation treatment were included as random effects. To provide an intuitive measure of germline maintenance for reporting effect sizes, we also calculated the relative reduction in fertility due to unattended damage in the male ejaculate for each replicate line at young and old age, according to: $\Delta\zeta = 1 - \zeta_{IRR,\,i} / \zeta_{CTRL,\,i}$, where $\zeta_{IRR,\,i}$ and $\zeta_{CTRL,\,i}$ is the number of F2 offspring produced by lineages deriving from F0 females of age *i*, mated to irradiated and control males, respectively (see: Fig. 3B).

281 ## Results

282 *Evolution of age-specific reproductive schedules*

283 There was no difference in F1 offspring production between young E and L females mated to
284 control males in the F0 generation (p_{MCMC} = 0.28). However, there were clear differences in
285 aging trajectories. As expected, females originating from the E-regime showed a sharp decline
286 in offspring production when mated at old age (p_{MCMC} < 0.001), while no such effect was seen
287 in the old L-females. As follows, the interaction between regime and female age was highly
288 significant (p_{MCMC} < 0.001) and accorded with predictions (Fig. 2).

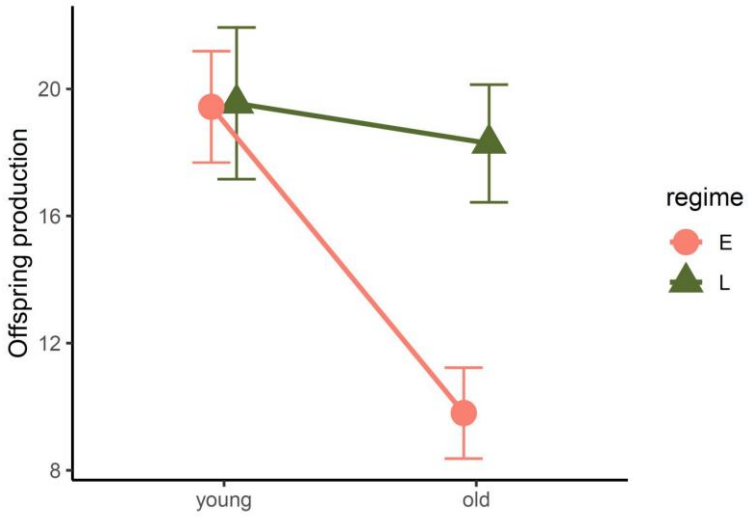

Figure 2: **Evolution of female reproductive schedules.**

The number of F1 offspring produced by young and old F0 females of the E (salmon) and L (green) regime, when mated to standard control males from the base line. Shown are means and 95% confidence limits.

289

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292 *Coevolution of reproductive schedules and female care of male gametes*

293 The irradiation treatment led to an overall decrease in F2 offspring numbers (effect of
294 irradiation in L-regime: p_{MCMC} < 0.001; effect of irradiation in E-regime: p_{MCMC} = 0.002). Taken
295 across both age-classes, there was no difference between evolution regimes in the effect of
296 irradiation on offspring production (regime*treatment interaction: p_{MCMC} = 0.74), providing
297 no evidence for strong divergence in female germline maintenance overall. However, in terms
298 of the age-dependency of female care, the two regimes seemed to behave very differently
299 (regime*age*treatment interaction; p_{MCMC} = 0.008, Fig. 3). This difference was due to that the
300 effect of irradiation was stronger in the young age-class compared to the old age-class for the
301 L-regime (p_{MCMC} = 0.004), while the aging trajectory showed the opposite pattern for E-lines
302 (although the effect of ageing was not significant, p_{MCMC} = 0.35). These results confirm that
303 female care of male gametes has evolved age-specific optimization.

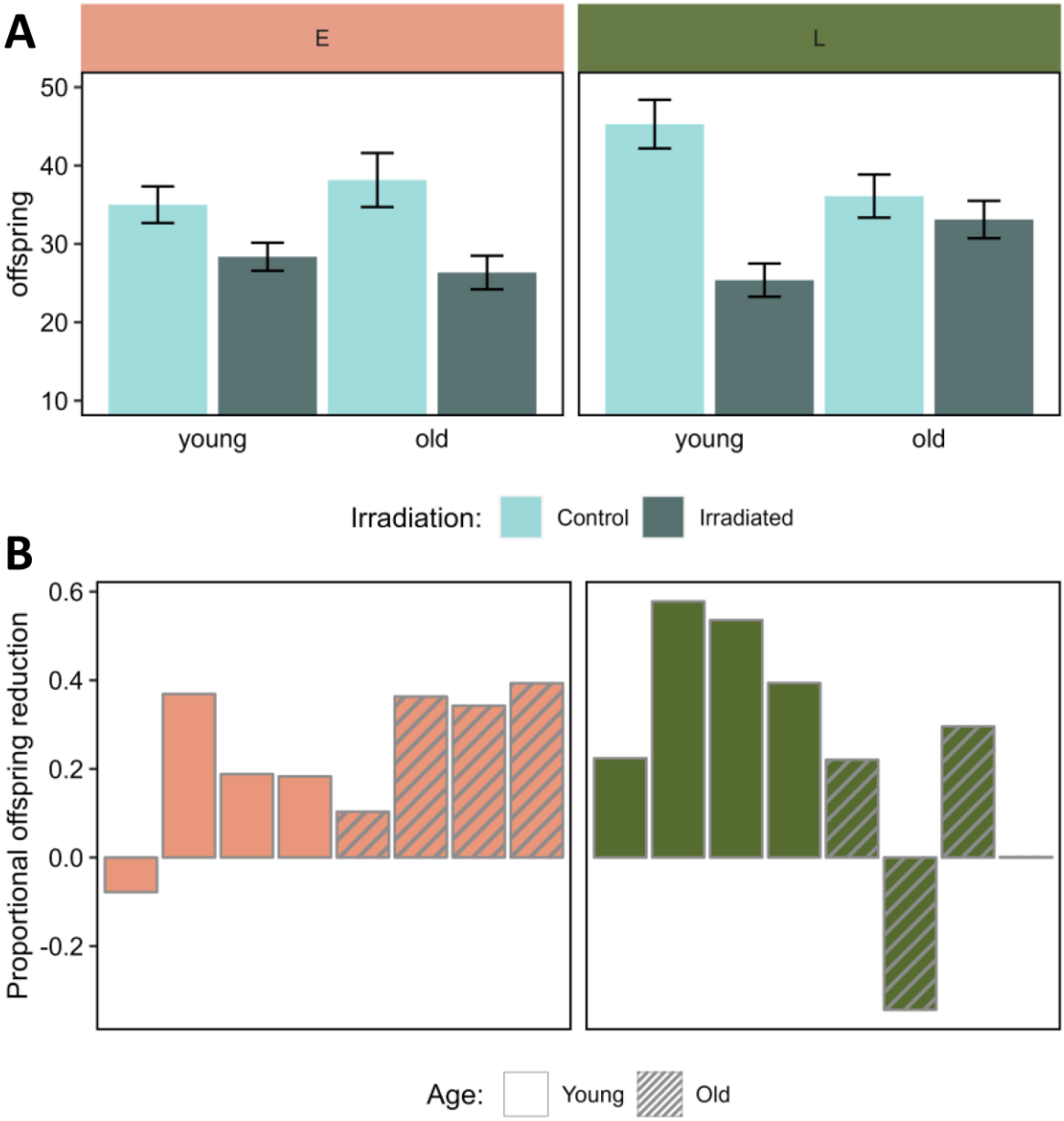

Figure 3: **Evolution of age-dependent female germline maintenance of male ejaculates.**

A) F2 offspring production in lineages stemming from F0 grandmothers mated to either a control (light bars) or irradiated (dark bars) male. Grandmothers were either young (1-3 days) or old (6-8 days) at mating, and belonged to either the E (salmon, left panel) or L (green, right panel) regime. Shown are means and standard errors. **B)** Line-specific estimates of the relative reduction in F2 offspring production induced by the irradiation treatment in F0 males, calculated as : $\Delta\zeta = 1 - \zeta_{IRR,\,i} / \zeta_{CTRL,\,i}$, where $\zeta_{IRR,\,i}$ and $\zeta_{CTRL,\,i}$ is the number of F2 offspring produced by lineages deriving from F0 females of age *i*, mated to irradiated and control males, respectively. This estimate is thus inversely related to the efficiency of germline DNA repair and maintenance in F0 mothers. Plain and striped bars represent lineages originating from young and old F0 grandmothers, respectively.

Discussion

Here we have shown that selection on reproductive schedules can lead to the evolution of age-specific maintenance of male gametes in the female reproductive tract. Such germline maintenance is a somewhat underestimated form of parental care with genetic consequences that can transcend generations. Our findings echo previous studies that have provided evidence that germline maintenance is costly and can trade off with life-history traits (Agrawal & Wang, 2008; Baur & Berger, 2020; Berger et al., 2017; Chen et al., 2020; Maklakov et al., 2012), emphasizing that germline maintenance should be considered a life-history trait in its own right (Bartosch -Harlid et al., 2003; Berger et al., 2017; Bromham, 2011; Ellegren, 2007; Maklakov & Immler, 2016).

The observed evolution of aging trajectories follows the general expectation that repair and maintenance in the oocyte evolves to optimize function at peak reproduction. The mutation accumulation (MA) theory of ageing (Medawar, 1952) predicts that E-lines should show efficient maintenance early in life, but a sharp decline in maintenance at old age, as old E-beetles have not been exposed to selection for >350 generations. In L-lines, a constant and efficient maintenance at both age-classes is expected, given that mature gametes have already formed at adult eclosion (Biemont, 1973) and should need continuous maintenance. Indeed, the observed reproductive schedules of young and old E- and L-females mated to control (unirradiated) males were qualitatively consistent with the MA hypothesis (Fig. 2). However, for female care of male gametes, E-females showed only a modest drop at old age, and instead it was young L-females that showed the lowest care (Fig. 3). L- females have not been provided with egg-laying substrate at young age for over 240 generations and egg-laying at this age thus represents a novel condition, which means that accumulation of mutations with conditional deleterious effects only at young age cannot be excluded. However, while the dip in care was larger in young (relative to old) L-females compared to the dip in old (relative to young) E-females, the E-regime had undergone more generations of (potential) accumulation of mutations with age-specific effect. Thus, the observed aging trajectories of germline maintenance are not fully compatible with the MA hypothesis.

347 Another (not mutually exclusive) explanation for the evolved aging trajectories of germline
348 maintenance and female care is antagonistic pleiotropy (AP) (Williams, 1957). Indeed,
349 previous findings have shown that aging trajectories of fertility and longevity in the E- and L
350 lines of *A. obtectus* seem to be a combined result of both MA and AP (Stojković et al., 2015).
351 Pleiotropic effects of genes improving germline maintenance at young age could lead to
352 reproductive disadvantages at later ages (Maklakov & Immler, 2016; Nussey et al., 2006)
353 and/or reduced survival and longevity via trade-offs between somatic and germline DNA-
354 maintenance (Chen et al., 2020; Maklakov & Immler, 2016), and hence, would be
355 disfavoured by selection in the L-regime. One likely molecular candidate linking
356 development, longevity, and reproduction in insects is juvenile growth hormone and its
357 variants, which, in fruit flies is known to increase sexual maturation, vitellogenesis, and
358 reproduction, while decreasing lifespan (Flatt et al., 2005). The pronounced dip in germline
359 maintenance in young L-females is also mirrored by common observations of reduced
360 fertility due to reproduction too early in life across animal taxa (Jones et al., 2014).
361
362 The potential for females to care over male ejaculates and offspring already at the very
363 onset of fertilization opens up interesting ecological scenarios and the potential for sexual
364 conflict over parental care, even in species traditionally assigned as having little to no care
365 (Koppik et al., 2023; Maklakov et al., 2012; Schärer et al., 2012; Servedio et al., 2019). For
366 example, in most species with internal fertilization, healthy females may efficiently
367 compensate for poor ejaculates of older males, while the converse seems unlikely. This
368 asymmetry should have implications for assortative mating by age. For example, the
369 baseline germline mutation rate is several times greater in males compared to females in
370 most animal taxa (Ellegren, 2007; Hurst & Ellegren, 1998; Sayres & Makova, 2011) and, at
371 least in well-studied groups like primates, this bias increases with age (Gao et al., 2019b;
372 Wang et al., 2020). Thus, mating decisions based on partner age are likely to have
373 considerable consequences, and the form of maternal care studied here should be
374 intertwined in those decisions.
375
376 Germline maintenance decisions can also depend on the overall condition of the individual
377 and state-dependent allocation decisions between competing life-history demands (Berger
378 et al., 2017; Maklakov & Immler, 2016; Monaghan & Metcalfe, 2019; Sharp & Agrawal,

2012), with implications for sexual selection theory and mate choice processes (Baur & Berger, 2020; Beck & Promislow, 2007; Koppik et al., 2023; Segami et al., 2021). Viewing germline maintenance through a resource acquisition and allocation lens (Jong & Noordwijk, 1992) suggests that optimal mate choice is contingent on not only choosers' inferences of genetic quality in offspring based on the phenotype of their mating partner, but also of how this phenotype influences changes to the genetic quality of its gametes. For example, in many polyandrous species, strong postcopulatory sexual selection (sperm competition) can favour compromised germline maintenance in males (Bartosch -Harlid et al., 2003; Baur & Berger, 2020; Blumenstiel, 2007; Koppik et al., 2023). This suggests that males that are most successful in reproductive competition may sometimes pass on the greatest load of mutations to their offspring, questioning why females should prefer them in the first place (Baur & Berger, 2020; Beck & Promislow, 2007; Segami et al., 2021)? Interestingly, however, optimality theory predicts that females my tailor care depending on the assessed state of male gametes (Servedio et al., 2019), with the result that a male's mutation rate is not independent of his female mate and her potential compensation for his short-comings, which can complicate interpretations of both age- and sex-biases in mutation rate (Gao et al., 2019a).

Can mechanisms that protect the germline evolve fast enough to keep up with rapid environmental and demographic change? This question is of importance for understanding long-term evolutionary responses that depend on both demographic parameters and the germline mutation rate. It is also a question tied to ongoing debates in human reproductive biology against the background of the increasing age at first reproduction in western civilisations (Khandwala et al., 2017b; Mills et al., 2011b). While assisted reproduction technologies may help to mitigate immediate age-related fertility declines associated with such changes, less attention is allotted to the evolutionary implications of ever later reproduction in human populations. Our study provides compelling evidence that female care of male gametes can evolve in response to selection on reproductive schedules. The maintenance and repair capacity in oocytes peaked at the time of reproduction in E- and L-lines, and the observed evolution of aging trajectories is compatible with some role for antagonistic pleiotropy in the underlying genes. This suggest that balancing selection on concerted life-history syndromes might be responsible for the maintenance of genetic

variation in germline repair in the wild founding population, which likely contributed to the observed evolutionary response. Our study highlights the evolutionary importance of this, often underappreciated, type of maternal care and we hope to stimulate further research aimed at elucidating the processes that govern the coevolution of germline maintenance, mate choice, and other life-history traits.

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Author contributions

DB, JB and MK designed the study. JB and MK performed the study. JB and DB analyzed the data, and DB and JB wrote the paper. US, MD and BS maintained, provided and prepared the experimental evolution lines. All authors read and commented on drafts of the manuscript.

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