Coevolution of longevity and female germline maintenance

Keywords: Life-history, reproduction, parental care, mutation rate, sperm competition, offspring quality, conflict, trade-off, germline

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 accordance 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 and screening of male gametes in their reproductive tract and oocytes. Here, we revealed the ability of young and aged 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 that germline maintenance in E-females peaks at young age and then declines, while the opposite is true for L-females, in accordance 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 (1,2). 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 (3–5). 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 (1,2,6).

Another, often less explicitly defined, dimension of life history optimization involves the allocation of resources to protect the germline (7–9). 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 (10–12). The costs of germline maintenance may be substantial (8), 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 (13). This sets the stage for trade-offs between the germline and other life history traits that are governed by related processes, such as somatic maintenance and repair affecting longevity (14,15), or germline replication rate affecting sperm competition success (16–18). Thus, 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 (7–9).

Theories of aging therefore do 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 (19), 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 (20), but also negatively affects the maintenance of the germline (9), shown, for example, by the fact that *de novo* germline mutation rates in primates seem to be foremost associated with advancing age (21–23). Ageing can also be driven by antagonistic pleiotropy (24), 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 (15), reproduction (14,16), 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 (25–31), or between individuals that differ in their overall energy budgets and allocation decisions (14,16,18,32,33). 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 (34–36). Additionally, females may also exert cryptic female choice of male sperm that compete for fertilization of eggs inside the female reproductive tract (37,38), which can serve as an additional barrier screening against male gametes of low genetic quality, even among sperm within an ejaculate from a single male (39,40). This process bares similarity to apoptosis (controlled cell death) of damaged germline cells, which limits the passing on of deleterious mutations to offspring (36,41–44). Here we consider all these related processes (cryptic female choice, apoptosis, antioxidant defence, and DNA repair) as alternative and non-mutually exclusive ways that females can exert germline maintenance in the broad sense. This female control over male gamete quality 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 (45–47).

Even though there now is a burgeoning 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 (48), pharmaceutical (49) and genetic (50) 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 century (51,52). However, the long-term

genetic consequences of lifespan extension depend on whether selection on reproductive schedules are coupled with concomitant changes in germline repair and maintenance, which remain unexplored. Here we harnessed the power of long-term experimental evolution to investigate how the evolution of lifespan affects age-dependent germline maintenance in the seed beetle, *Acanthoscelides obtectus.* Using a set of lines that were established in 1989 and selected for early or late reproduction for more than 350 and 240 generations, respectively, we examined germline maintenance in young and aged females receiving male ejaculates with artificially inflated levels of free radicals and DNA and protein damage (Fig. 1). This allowed us to explore i) whether female germline maintenance evolves in concerted fashion with reproductive schedules and other life history traits, and ii) whether the AP (24) or the MA (19) theory of ageing best explains the evolutionary patterns observed.

Methods

Study species

The seed beetle, *Acanthoscelides obtectus* (Coleoptera; Chrysomelidae; Bruchidae) is a pest of stored legumes, foremost, the common bean, *Phaseolus vulgaris* (53). Females lay eggs either directly under or in close proximity of host seeds and the hatched larvae burrow into a seed where juvenile development takes place (54). After pupation, adults emerge from the seed and typically become reproductively active within 24 hours. Adult beetles are aphageous, meaning they don´t need nutrition or water to be able to reproduce and finish their lifecycle (53,55).

Experimental populations

The original base population, from which the experimental evolution lines were derived, was created by mass-mating wild-caught beetles from three locations in the area of Belgrade, Serbia, in 1983 (54). Subsequently, the population was maintained on *P. vulgaris* in the laboratory for 3 years (27 generations) at large population size (ca. 5000 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 adult beetles.

122 During experimental evolution, one line was maintained under the same conditions as the
123 base population (from here on referred to as the "base" line); after 40 days of development,
124 400-500 adult beetles were chosen at random and provided with fresh host seeds and
125 allowed to reproduce for the next generation. Each of four replicate lines selected for early
126 reproduction (E-lines) was created by taking 400 adults at random on the peak of adult
127 emergence. To propagate the next generation, these adults were transferred to a new
128 bottle containing host seeds *ad libitum* and were there allowed to reproduce and lay eggs
129 for 48h, after which all adults were removed. Another four replicate lines were selected for
130 late reproduction (L-lines) by taking 100 newly emerged (0-24h) adults every day for up to
131 10 days. Each of these subgroups was kept in a vial without seeds for 10 days. On the 10th
132 day of each subgroup all the surviving beetles of a given replicate line were added to a
133 single bottle containing fresh host seeds to propagate the next generation (Fig. 1A).
134
135 Development time of E-lines has evolved to be 29 days and the average virgin lifespan
136 around 13 days in aphagous conditions, while L-lines emerge after 32 days and virgin
137 beetles live on average 26 days for females and 22 days for males (56). The regimes have
138 also diverged in other traits tied to rate-dependent life-history, such as the day of first
139 oviposition (E: 1 day after adult eclosion, L: 4 days after adult eclosion) (57), metabolic rate,
140 and body size (58). Care was taken not to select directly on development time (by collecting
141 adult beetles around the peak of emergence in each generation) or body size (by providing
142 host seeds ad libitum. It can thus be expected that life history evolution in the lines is a
143 result of correlated responses to the applied selection on reproductive schedules. For more
144 information on the maintenance of the experimental evolution lines, see (54,55).
145
146 *Experimental design*
147 The experiment was started in 2020, after 240 (L-lines) and 350 (E-lines) generations of
148 experimental evolution. To estimate the evolution of age-dependent germline maintenance
149 we compared females of all lines mated to males when aged 1-3 days (young treatment) or
150 6-8 days (aged treatment) (Fig. 1B). Because we wanted to make inferences about evolved
151 differences in biological aging and correlated life history between the regimes, we
152 compared E and L females at the same chronological age. We did not pick an older age than
153 8 days since E females age rapidly and we wanted to avoid any bias in form of selective

154 disappearance (59) of low quality individuals in the E-lines. We note, however, that 6-8 days
155 is not truly an old age for L-lines, but rather represents a relatively young reproductive age
156 (54,56). The comparison between E and L females of the same chronological age should thus
157 be interpreted in relative terms.
158
159 All lines were reared in staggered fashion by setting up several rearing containers of each
160 line in the two generations prior to the F0 generation of the experiment, so that emergence
161 of females would happen continuously during the experiment and females assigned to the
162 two age treatments could be assayed simultaneously and mated to standard males from the
163 same cohort (see below). We collected females on the day of their emergence, randomly
164 assigned them to the young or aged treatment, and then isolated them in perforated 0.5ml
165 Eppendorf tubes and left them age. Females of both age treatments were then mated to
166 young (1-2 days) virgin males from the base population in 60 mm petri-dishes placed on
167 heating plates kept at 30°C. Couples were observed to avoid multiple mating. Males were
168 removed after a mating behaviour had been observed and females were allowed to lay eggs
169 in a new 60 mm petri-dish containing host seeds *ad libitum* for 48h after the mating.
170
171 The base males were either untreated (controls) or had been exposed to a 10 Gy dose of γ-
172 radiation, which causes substantial cellular damage to the male germline (43) and impairs
173 male fertility in seed beetles (14,16,18). The dose of 10 Gy was chosen based on our
174 previous studies using similar experimental designs in the related seed beetle
175 *Callosobruchus maculatus* (16,18,60), as well as another study on *A. obtectus* (61). Hence,
176 the female repair, maintenance, and potential screening of damaged male gametes in each
177 line could be assessed by comparing the offspring production in lineages stemming from
178 females mated to control and irradiated males. γ-radiation produces free radicals with
179 mutagenic properties and causes direct damage to DNA within sperm cells (43) and to
180 proteins within the ejaculate (62). Hence, our protocol captures a multitude of effects that
181 occur naturally in male ejaculates (8,63), but that will have been greatly amplified by the
182 irradiation treatment.
183
184 As our interest lies in capturing effects of variation in female germline maintenance that
185 transcend generations, we scored the fitness consequences of variation in female

186 maintenance by counting the number of emerging F2 adults produced by all descending
187 lineages. To achieve this, F1 males and females stemming from the same treatment group
188 and line were paired (while avoiding sib-mating) and allowed to mate and produce F2
189 offspring in petri-dishes with ad libitum host seeds (Fig. 1C). The emerging F2 adult offspring
190 were counted to estimate each couple's fecundity, which was used to estimate germline
191 maintenance of their F0 grandmothers (see below: "Statistical analysis").
192
193 About half of the F0 couples produced no offspring (Supplementary Table 1a). This was
194 likely due to two reasons. First, the mating latency of *A. obtectus* can be relatively long, and
195 because the time in between male irradiation and mating needed to be standardized
196 (maximum of 30 minutes), many couples did not mate in this time. Secondly, even when a
197 mating was thought to have been observed, several couples did still not produce any
198 offspring. Using a binomial model, we tested whether the incidence of zero fertility was
199 related to any of the factors of interest in our experiment (i.e., irradiation treatment,
200 selection regime, and age treatment). However, despite large sample size, no factor had a
201 significant effect on infertility. Indeed, all effects including male irradiation treatment were
202 very weak and non-significant (all $P > 0.3$), and the proportion of infertile couples was
203 relatively equal across treatments (E: young ctrl = 0.43; young irr = 0.48; old ctrl = 0.59; old
204 irr = 0.61. L: young ctrl = 0.50; young irr = 0.39; old ctrl = 0.48; old irr = 0.50). Hence, we
205 carried out our downstream analyses based on the remaining data from the 830 F0 couples
206 that produced offspring. Each line and age-class was represented by 10-20 females mated to
207 control males, and 20-30 females mated to irradiated males. More couples were set up for
208 the irradiated treatment group since the induced damage to male ejaculates was expected
209 to generate more variance in offspring quality among lineages, requiring a larger sample size
210 to result in the same precision of estimates as for the control lineages.
211
212 To reduce the effect of selection against epigenetic and DNA mutations induced by the
213 irradiation we applied a Middle-Class Neighbourhood (MCN) design (64) (Fig. 1C). The MCN
214 design restricts each F0 couple to contribute the same number of F1 offspring for future
215 study, irrespective of their overall productivity. We note, however, that F0 couples that
216 produced less than four F1 offspring were not represented in the final data, and hence,
217 selection on very strongly deleterious effects of irradiation could not be excluded by the

MCN approach. For those F0 couples that did produce offspring, we aimed at propagating a single male and female from each couple, so that each F0 couple would replace itself. However, due to variability in the timing of eclosion of F1 individuals, additional F0 couples fell out of the experiment as we did not want to age F1 beetles before setting them up in assays. These strict criteria resulted in 490 F1 couples being analysed for offspring production. Each line and age class was represented by ca 11-27 and 5-15 F1 couples that originated from irradiated and control lineages, respectively (Supplementary Table 1b).

The experiment was conducted in three temporal blocks, 3 days apart, corresponding to the three occasions on which we irradiated F0 males from the base population (Supplementary Table 1a). In the F1 generation, when offspring productivity assays were set up, there was considerable overlap between these blocks due to variable timing of egg laying and juvenile development time, so that combinations of F1 individuals stemming from all combinations of F0 dates were crossed. Hence, we did not include the effect of irradiation day in further analysis.

Statistical analysis

We analyzed data using generalized linear mixed effect models implemented in the package MCMCglmm (65) for R (66). 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 (67).

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 aged F0 females of all E- and L-lines over the 48h period, when mated to the standard base males belonging to the control (un-irradiated) treatment (N = 307). This model included female age treamtent (young vs. aged), evolution regime (E vs. L),

250 and their interaction, as fixed effects of interest. We also added the main effect of male age
251 (1 or 2 days old) as a blocking factor. Replicate line, crossed with age, were added as
252 random effects (model summary in Supplementary Table 2a).
253
254 To analyse differences in female germline maintenance we compared the F2 offspring
255 counts of control and irradiated lineages. This model included fully crossed fixed effects of
256 evolution regime (E vs. L), F0 female age treatment (young vs. aged) and male radiation
257 treatment (control vs. irradiated). Note that the three-way interaction between these
258 effects tests our focal hypothesis that the E- and L-regime have diverged in age-specific
259 female germline maintenance. Replicate line crossed with age treatment and radiation
260 treatment were included as random effects (model summary in Supplementary Table 2b).
261 To provide an intuitive measure of germline maintenance for reporting effect sizes, we also
262 calculated the relative reduction in fertility due to unattended damage in the male ejaculate
263 for each replicate line for young and aged females, according to: $\Delta\zeta = 1 - \zeta_{IRR,\,i} / \zeta_{CTRL,\,i}$, where
264 $\zeta_{IRR,\,i}$ and $\zeta_{CTRL,\,i}$ is the number of F2 offspring produced by lineages deriving from F0 females
265 of age treatment *i*, mated to irradiated and control males, respectively (see: Fig. 3B).
266

267 ## Results

268 *Evolution of age-specific reproductive schedules*

269 There was no difference in F1 offspring production between young E and L females mated to
270 control males in the F0 generation ($p_{MCMC} = 0.28$). However, there were clear differences in
271 aging. As expected, aged females originating from the E-regime showed a sharp decline in
272 offspring production ($p_{MCMC} < 0.001$), while no such effect was seen in the aged L-females. As
273 follows, the interaction between regime and female age treatment was highly significant
274 ($p_{MCMC} < 0.001$) and accorded with predictions (Fig. 2).
275

276 *Coevolution of reproductive schedules and female germline maintenance*

277 The irradiation treatment led to an overall decrease in F2 offspring numbers (effect of
278 irradiation in L-regime: $p_{MCMC} < 0.001$; effect of irradiation in E-regime: $p_{MCMC} = 0.002$). Taken
279 across both age treatments, there was no difference between evolution regimes in the effect
280 of irradiation on offspring production (regime*irradiation interaction: $p_{MCMC} = 0.74$),

281 providing no evidence for strong divergence in female germline maintenance overall.
282 However, in terms of the age-dependency, the two regimes seemed to behave very
283 differently (regime*age*irradiation interaction; p_{MCMC} = 0.008, Fig. 3). This difference was due
284 to that the effect of irradiation was stronger in young, compared to aged, L-females (p_{MCMC} =
285 0.004), while the opposite pattern was observed for E-females (although the effect of age was
286 not significant, p_{MCMC} = 0.35). These results confirm that female germline maintenance has
287 evolved age-specific optimization.
288

289 ## Discussion

290 Here we have shown that selection on reproductive schedules can lead to the evolution of
291 age-specific female germline maintenance that mitigates deleterious effects caused by low
292 quality male gametes. Such germline maintenance is a somewhat underestimated form of
293 parental care with genetic consequences that can transcend generations. Our findings echo
294 previous studies that have provided evidence that germline maintenance is costly and can
295 trade off with life-history traits (14–16,32,68), emphasizing that germline maintenance
296 should be considered a life-history trait in its own right (7,8,14,69–71).
297

298 *Did germline maintenance evolve via mutation accumulation or antagonistic pleiotropy?*
299 The observed evolution of aging follows the general expectation that repair and
300 maintenance in the female reproductive tract evolves to optimize function at peak
301 reproduction. The mutation accumulation (MA) theory of ageing (19) predicts that E-lines
302 should show efficient maintenance early in life, but a sharp decline in maintenance at old
303 age, as old E-beetles have not been exposed to selection for >350 generations. In L-lines,
304 efficient maintenance of both young and aged females is expected, given that mature
305 gametes have already formed at adult eclosion (61) and should need continuous
306 maintenance. Indeed, the observed reproductive schedules of young and aged E- and L-
307 females mated to control (unirradiated) males were qualitatively consistent with the MA
308 hypothesis (Fig. 2). However, for female germline maintenance, E-females showed only a
309 modest reduction with increasing age, and instead it was young L-females that showed the
310 lowest maintenance (Fig. 3). L- females have not been provided with egg-laying substrate at
311 young age for over 240 generations and egg-laying at this age thus represents a novel

312 condition, which means that accumulation of mutations with conditional deleterious effects
313 only at young age cannot be excluded. However, while the dip in germline maintenance was
314 larger in young (relative to aged) L-females compared to the dip in aged (relative to young)
315 E-females, the E-regime had undergone more generations of (potential) accumulation of
316 mutations with age-specific effect. Thus, the observed patterns of aging of germline
317 maintenance are not fully compatible with the MA hypothesis.
318
319 Another (not mutually exclusive) explanation for the evolved aging trajectories is
320 antagonistic pleiotropy (AP) (24). Indeed, previous findings have shown that age-related
321 changes in fertility and longevity in the E- and L lines of *A. obtectus* seem to be a combined
322 result of both MA and AP (72). Pleiotropic effects of genes improving germline maintenance
323 at young age could lead to reproductive disadvantages at later ages (8,73) and/or reduced
324 survival and longevity via trade-offs between somatic and germline DNA-maintenance
325 (8,15), and hence, would be disfavoured by selection in the L-regime. Interestingly, the
326 pronounced dip in germline maintenance in young L-females is also mirrored by common
327 observations of reduced fertility due to reproduction too early in life across animal taxa (74).
328 Here it is also worth underlining that the aged treatment in L-females actually represents a
329 young reproductive age in this evolution regime, that likely corresponds to the onset of
330 female reproductive investment during experimental evolution (54,55). Hence, the E-
331 females in the young treatment and L-females of the aged treatment should closely
332 correspond to each other in terms of their biological age, and indeed, also show similar
333 levels of reproductive output (Fig. 2) and germline maintenance (Fig. 3).
334
335 *Male-female coevolution*
336 The potential for females to care over male ejaculates already at the very onset of
337 fertilization opens up interesting eco-evolutionary scenarios and the potential for sexual
338 conflict over parental care, even in species traditionally assigned as having little to no care
339 (18,68,75,76). For example, in most species with internal fertilization, healthy females may
340 efficiently compensate for poor ejaculates of older males, while the converse seems
341 unlikely. This asymmetry should have implications for assortative mating by age. For
342 example, the baseline germline mutation rate is several times greater in males compared to
343 females in most animal taxa (71,77,78) and, at least in well-studied groups like primates, this

344 bias increases with age (79,80). Thus, mating decisions based on partner age are likely to
345 have considerable consequences, and the form of maternal care studied here should be
346 intertwined in those decisions.
347
348 Germline maintenance decisions can also depend on the overall condition of the individual
349 and state-dependent allocation decisions between competing life-history demands
350 (8,9,14,33), with implications for sexual selection theory and mate choice processes
351 (16,18,81,82). Viewing germline maintenance through a resource acquisition and allocation
352 lens (83) suggests that optimal mate choice is contingent on not only choosers' inferences of
353 genetic quality in offspring based on the phenotype of their mating partner, but also of how
354 this phenotype influences changes to the genetic quality of its gametes. For example, in
355 many polyandrous species, strong postcopulatory sexual selection (sperm competition) can
356 favour compromised germline maintenance in males (16–18,69). This suggests that males
357 that are most successful in reproductive competition may sometimes pass on the greatest
358 load of mutations to their offspring, questioning why females should prefer them in the first
359 place (16,81,82)? Interestingly, however, optimality theory predicts that females my tailor
360 care depending on the assessed state of male gametes (76), with the result that a male's
361 mutation rate is not independent of his female mate and her potential compensation for his
362 short-comings, which can complicate interpretations of both age- and sex-biases in
363 mutation rate (45).
364
365 Another way that females could influence the resulting quality of their offspring is via
366 haploid selection through cryptic female choice of male sperm (40). In this study, we
367 defined female germline maintenance broadly, including both care of male gametes in form
368 of DNA repair and antioxidant defence, as well as screening against damaged sperm via
369 cryptic female choice or damaged zygotes via apoptosis. Our study does not allow us to
370 discriminate among these hypotheses, that are not mutually exclusive (42–44). Yet, it is
371 interesting to note that, while all these processes equate to germline maintenance and
372 increased offspring quality from a female's perspective, only female care improves male
373 gamete quality, while female screening eliminates the male's gametes. Therefore, the type
374 of female maintenance may have different implications for male fitness, with the level of
375 sperm competition within and between ejaculates of different males a predicted driver of

these dynamics (84,85). We do not know the extent of sperm competition in the E- and L-regime, although it would seem likely that the number of different male mates per female might be higher in L-females due to their prolonged lifespan. Nevertheless, we found no obvious differences between the evolution regimes in germline maintenance overall, or at the peak of early reproduction (young E females versus aged L-females) (Fig. 3). Future research could investigate if females kept under high levels of sperm competition evolve increased cryptic female choice and if this is associated with correlated evolutionary responses in other aspects of germline maintenance.

In relation to male-female coevolution, we note that there is a possibility that our results could have been driven by an indirect effect of female reproductive aging. If the F0 reference males in our experiment could assess the reproductive status of females, it is possible that males may have preferred females at their reproductive peak, and allocated resources in their ejaculates accordingly, resulting in higher offspring quality in young E- and aged L-females. However, we find this hypothesis unlikely due to three reasons. First, in addition to requiring the ability of males to assess variation in female condition, there would also need to be sufficient fitness gains for this male allocation decision to evolve. We find this unlikely given the high-density scramble mating system under which the reference males have been evolving for at least 300 generations in the lab environment, and the relatively minor effects on female fertility caused by large variation in male ejaculate volume and quality across other species of seed beetle (86). Second, the allocation decision would have needed to be very fast as most F0 males mated within less than X minutes after have being introduced to the females, while processes that affect DNA repair and oxidant defence of male sperm typically occur over longer time frames (43), as does sperm maturation in the relative *C. maculatus* (16). Third, this effect would also need to take effect in irradiated and not control lineages to generate the observed pattern (Fig. 3). Nevertheless, our current data do not allow us to completely rule out that this effect could have contributed to our results.

Conclusions

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 (87,88). 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 and/or screening of male gametes can evolve in response to selection on reproductive schedules. Germline maintenance peaked at the time of reproduction in E- and L-lines, and the observed evolution of aging of the germline 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 maintenance 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 processes, and other life-history traits.

Data accessibility

All data and code accompanying this study is uploaded to Dryad […].

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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References

1. Roff D. Evolution Of Life Histories: Theory and Analysis. Springer Science & Business Media; 1993. 554 p.

2. Stearns SC. THE EVOLUTION OF LIFE HISTORIES.

3. Flatt T, Heyland A, editors. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford ; New York: Oxford University Press; 2011. 478 p. (Oxford biology).

4. Monaghan P, Charmantier A, Nussey DH, Ricklefs RE. The evolutionary ecology of senescence. Funct Ecol [Internet]. 2008 Jun [cited 2019 Jan 17];22(3):371–8. Available from: http://doi.wiley.com/10.1111/j.1365-2435.2008.01418.x

5. Promislow DEL, Tatar M. Mutation and senescence: where genetics and demography meet. Genetica [Internet]. 1998 Mar 1 [cited 2020 Oct 5];102(0):299–314. Available from: https://doi.org/10.1023/A:1017047212008

6. Lemaître JF, Moorad J, Gaillard JM, Maklakov AA, Nussey DH. A unified framework for evolutionary genetic and physiological theories of aging. PLOS Biol [Internet]. 2024 Feb 27 [cited 2024 Feb 29];22(2):e3002513. Available from: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3002513

7. Avila P, Lehmann L. Life history and deleterious mutation rate coevolution. J Theor Biol [Internet]. 2023 Sep 21 [cited 2024 Feb 5];573:111598. Available from: https://www.sciencedirect.com/science/article/pii/S0022519323001959

8. Maklakov AA, Immler S. The Expensive Germline and the Evolution of Ageing. Curr Biol [Internet]. 2016 Jul [cited 2019 Jan 17];26(13):R577–86. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960982216303347

9. Monaghan P, Metcalfe NB. The deteriorating soma and the indispensable germline: gamete senescence and offspring fitness. Proc R Soc B Biol Sci. 2019 Dec 18;286(1917):20192187.

10. Agrawal AF, Whitlock MC. Mutation Load: The Fitness of Individuals in Populations Where Deleterious Alleles Are Abundant. Annu Rev Ecol Evol Syst [Internet]. 2012 Dec [cited 2018 Feb 19];43(1):115–35. Available from: http://www.annualreviews.org/doi/10.1146/annurev-ecolsys-110411-160257

11. Lynch M. Evolution of the mutation rate. Trends Genet [Internet]. 2010 Aug 1 [cited 2018 Aug 4];26(8):345–52. Available from: https://www.cell.com/trends/genetics/abstract/S0168-9525(10)00103-4

12. Muller HJ. Our load of mutations. Am J Hum Genet [Internet]. 1950 Jun [cited 2021 Jan 28];2(2):111–76. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1716299/

13. Lynch M. The Cellular, Developmental and Population-Genetic Determinants of Mutation-Rate Evolution. Genetics [Internet]. 2008 Oct [cited 2022 Jan 31];180(2):933–43. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2567392/

14. Berger D, Stångberg J, Grieshop K, Martinossi-Allibert I, Arnqvist G. Temperature effects on life-history trade-offs, germline maintenance and mutation rate under simulated climate warming. Proc R Soc B [Internet]. 2017 Nov 15 [cited 2018 Sep 19];284(1866):20171721. Available from: http://rspb.royalsocietypublishing.org/content/284/1866/20171721

15. Chen H yen, Jolly C, Bublys K, Marcu D, Immler S. Trade-off between somatic and germline repair in a vertebrate supports the expensive germ line hypothesis. Proc Natl Acad Sci. 2020 Apr 21;117(16):8973–9.

16. Baur J, Berger D. Experimental evidence for effects of sexual selection on condition-dependent mutation rates. Nat Ecol Evol [Internet]. 2020 May [cited 2020 Jul 10];4(5):737–44. Available from: https://www.nature.com/articles/s41559-020-1140-7

17. Blumenstiel JP. Sperm competition can drive a male-biased mutation rate. J Theor Biol [Internet]. 2007 Dec [cited 2019 Jan 17];249(3):624–32. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022519307004109

18. Koppik M, Baur J, Berger D. Increased male investment in sperm competition results in reduced maintenance of gametes. PLOS Biol [Internet]. 2023 Apr 4 [cited 2023 Apr 5];21(4):e3002049. Available from: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3002049

19. Medawar PB. An unsolved problem of biology. Published for the College by H.K. Lewis; 1952.

20. Partridge L, Barton NH. On measuring the rate of ageing. Proc R Soc Lond B Biol Sci. 1996 Oct 22;263(1375):1365–71.

21. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, et al. Rate of de novo mutations and the importance of father's age to disease risk. Nature. 2012 Aug;488(7412):471–5.

22. Rahbari R, Wuster A, Lindsay SJ, Hardwick RJ, Alexandrov LB, Al Turki S, et al. Timing, rates and spectra of human germline mutation. Nat Genet. 2016 Feb;48(2):126–33.

23. Venn O, Turner I, Mathieson I, de Groot N, Bontrop R, McVean G. Strong male bias drives germline mutation in chimpanzees. Science [Internet]. 2014 Jun 13 [cited 2019 Jan 17];344(6189):1272–5. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.344.6189.1272

24. Williams GC. Pleiotropy, Natural Selection, and the Evolution of Senescence. Evolution [Internet]. 1957 [cited 2019 Mar 6];11(4):398–411. Available from: https://www.jstor.org/stable/2406060

25. Berger D, Stångberg J, Baur J, Walters RJ. Elevated temperature increases genome-wide selection on de novo mutations. Proc R Soc B Biol Sci [Internet]. 2021 Feb 10 [cited 2021 Feb 3];288(1944):20203094. Available from: https://royalsocietypublishing.org/doi/full/10.1098/rspb.2020.3094

26. Chu XL, Zhang BW, Zhang QG, Zhu BR, Lin K, Zhang DY. Temperature responses of mutation rate and mutational spectrum in an Escherichia coli strain and the correlation with metabolic rate.

517 BMC Evol Biol [Internet]. 2018 Aug 29 [cited 2018 Oct 29];18(1):126. Available from:
518 https://doi.org/10.1186/s12862-018-1252-8

519 27. Crean AJ, Immler S. Evolutionary consequences of environmental effects on gamete
520 performance. Philos Trans R Soc B Biol Sci [Internet]. 2021 Jun 7 [cited 2021 Jun
521 21];376(1826):20200122. Available from:
522 https://royalsocietypublishing.org/doi/abs/10.1098/rstb.2020.0122

523 28. Drake JW. Avoiding dangerous missense: thermophiles display especially low mutation rates.
524 PLoS Genet. 2009;5(6):e1000520.

525 29. Kurhanewicz NA, Dinwiddie D, Bush ZD, Libuda DE. Elevated Temperatures Cause Transposon-
526 Associated DNA Damage in C. elegans Spermatocytes. Curr Biol [Internet]. 2020 Dec 21 [cited
527 2023 Mar 3];30(24):5007-5017.e4. Available from:
528 https://www.sciencedirect.com/science/article/pii/S0960982220314202

529 30. Puurtinen M, Elo M, Jalasvuori M, Kahilainen A, Ketola T, Kotiaho JS, et al. Temperature-
530 dependent mutational robustness can explain faster molecular evolution at warm temperatures,
531 affecting speciation rate and global patterns of species diversity. Ecography [Internet]. 2016 Nov
532 [cited 2018 Jan 29];39(11):1025–33. Available from: http://doi.wiley.com/10.1111/ecog.01948

533 31. Svetec N, Cridland JM, Zhao L, Begun DJ. The Adaptive Significance of Natural Genetic Variation
534 in the DNA Damage Response of Drosophila melanogaster. PLOS Genet [Internet]. 2016 Mar
535 [cited 2018 Aug 4];12(3):e1005869. Available from:
536 http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1005869

537 32. Agrawal AF, Wang AD. Increased transmission of mutations by low-condition females: evidence
538 for condition-dependent DNA repair. PLoS Biol. 2008 Feb;6(2):e30.

539 33. Sharp NP, Agrawal AF. Evidence for elevated mutation rates in low-quality genotypes. Proc Natl
540 Acad Sci [Internet]. 2012 Apr 17 [cited 2019 Jan 17];109(16):6142–6. Available from:
541 http://www.pnas.org/cgi/doi/10.1073/pnas.1118918109

542 34. Horta F, Catt S, Ramachandran P, Vollenhoven B, Temple-Smith P. Female ageing affects the
543 DNA repair capacity of oocytes in IVF using a controlled model of sperm DNA damage in mice.
544 Hum Reprod [Internet]. 2020 Mar 27 [cited 2021 Jul 7];35(3):529–44. Available from:
545 https://doi.org/10.1093/humrep/dez308

546 35. Jaroudi S, Kakourou G, Cawood S, Doshi A, Ranieri DM, Serhal P, et al. Expression profiling of
547 DNA repair genes in human oocytes and blastocysts using microarrays. Hum Reprod. 2009 Oct
548 1;24(10):2649–55.

549 36. Stringer JM, Winship A, Liew SH, Hutt K. The capacity of oocytes for DNA repair. Cell Mol Life Sci.
550 2018 Aug 1;75(15):2777–92.

551 37. Firman RC, Gasparini C, Manier MK, Pizzari T. Postmating Female Control: 20 Years of Cryptic
552 Female Choice. Trends Ecol Evol [Internet]. 2017 May [cited 2021 Aug 11];32(5):368–82.
553 Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5511330/

554 38. Kustra MC, Alonzo SH. The coevolutionary dynamics of cryptic female choice. Evol Lett
555 [Internet]. 2023 Aug 1 [cited 2023 Oct 13];7(4):191–202. Available from:
556 https://doi.org/10.1093/evlett/qrad025

557 39. Alavioon G, Hotzy C, Nakhro K, Rudolf S, Scofield DG, Zajitschek S, et al. Haploid selection within
558 a single ejaculate increases offspring fitness. Proc Natl Acad Sci [Internet]. 2017 Jul 25 [cited
559 2021 Feb 4];114(30):8053–8. Available from: https://www.pnas.org/content/114/30/8053

560 40. Immler S, Otto SP. The Evolutionary Consequences of Selection at the Haploid Gametic Stage.
561 Am Nat [Internet]. 2018 Jun 8 [cited 2018 Sep 14];192(2):241–9. Available from:
562 https://www.journals.uchicago.edu/doi/abs/10.1086/698483

563 41. Bhagavan NV, Ha CE. Chapter 22 - DNA Replication, Repair, and Mutagenesis. In: Bhagavan NV,
564 Ha CE, editors. Essentials of Medical Biochemistry (Second Edition) [Internet]. San Diego:
565 Academic Press; 2015 [cited 2020 Dec 15]. p. 401–17. Available from:
566 http://www.sciencedirect.com/science/article/pii/B9780124166875000221

567 42. Borràs-Fresneda M, Barquinero JF, Gomolka M, Hornhardt S, Rössler U, Armengol G, et al.
568 Differences in DNA Repair Capacity, Cell Death and Transcriptional Response after Irradiation
569 between a Radiosensitive and a Radioresistant Cell Line. Sci Rep [Internet]. 2016 Jun 1 [cited
570 2019 Jun 4];6:27043. Available from: https://www.nature.com/articles/srep27043

571 43. Friedberg EC, Walker GC, Siede W, Wood RD. DNA Repair and Mutagenesis. American Society
572 for Microbiology Press; 2005. 2845 p.

573 44. González-Marín C, Gosálvez J, Roy R. Types, Causes, Detection and Repair of DNA Fragmentation
574 in Animal and Human Sperm Cells. Int J Mol Sci [Internet]. 2012 Oct 31 [cited 2019 Jan
575 17];13(12):14026–52. Available from: http://www.mdpi.com/1422-0067/13/11/14026

576 45. Gao Z, Moorjani P, Sasani TA, Pedersen BS, Quinlan AR, Jorde LB, et al. Overlooked roles of DNA
577 damage and maternal age in generating human germline mutations. Proc Natl Acad Sci
578 [Internet]. 2019 May 7 [cited 2019 Oct 2];116(19):9491–500. Available from:
579 https://www.pnas.org/content/116/19/9491

580 46. Rahbari R, Wuster A, Lindsay SJ, Hardwick RJ, Alexandrov LB, Al Turki S, et al. Timing, rates and
581 spectra of human germline mutation. Nat Genet [Internet]. 2016 Feb [cited 2022 Jan
582 17];48(2):126–33. Available from: https://www.nature.com/articles/ng.3469

583 47. Ségurel L, Wyman MJ, Przeworski M. Determinants of Mutation Rate Variation in the Human
584 Germline. Annu Rev Genomics Hum Genet [Internet]. 2014 [cited 2019 Jan 18];15(1):47–70.
585 Available from: https://doi.org/10.1146/annurev-genom-031714-125740

586 48. Klass MR. Aging in the nematode Caenorhabditis elegans: Major biological and environmental
587 factors influencing life span. Mech Ageing Dev. 1977 Jan 1;6:413–29.

588 49. Johnson SC, Rabinovitch PS, Kaeberlein M. mTOR is a key modulator of ageing and age-related
589 disease. Nature. 2013 Jan;493(7432):338–45.

590 50. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature. 2000
591 Nov;408(6809):255–62.

592 51. Khandwala YS, Zhang CA, Lu Y, Eisenberg ML. The age of fathers in the USA is rising: an analysis
593 of 168 867 480 births from 1972 to 2015. Hum Reprod [Internet]. 2017 Oct 1 [cited 2022 Nov
594 11];32(10):2110–6. Available from: https://doi.org/10.1093/humrep/dex267

595 52. Mills M, Rindfuss RR, McDonald P, te Velde E, on behalf of the ESHRE Reproduction and Society
596 Task Force. Why do people postpone parenthood? Reasons and social policy incentives. Hum

Reprod Update [Internet]. 2011 Nov 1 [cited 2022 Nov 11];17(6):848–60. Available from: https://doi.org/10.1093/humupd/dmr026

53. Thakur DR. Taxonomy, Distribution and Pest Status of Indian Biotypes of Acanthoscelides obtectus (Coleoptera: Chrysomelidae: Bruchinae) - A New Record. Pakistan J Zool. 2012;44(1):189–95.

54. Tucić N, Gliksman I, Šešlija D, Milanović D, Mikuljanac S, Stojković O. Laboratory evolution of longevity in the bean weevil (Acanthoscelides obtectus). J Evol Biol. 1996;9(4):485–503.

55. Tucić N, Stojković O, Gliksman I, Milanović D, Šešlija D. Laboratory Evolution of Life-History Traits in the Bean Weevil (Acanthoscelides obtectus): The Effects of Density-Dependent and Age-Specific Selection. Evolution. 1997;51(6):1896–909.

56. Đorđević M, Savković U, Lazarević J, Tucić N, Stojković B. Intergenomic Interactions in Hybrids Between Short-Lived and Long-Lived Lines of a Seed Beetle: Analyses of Life History Traits. Evol Biol [Internet]. 2015 Dec 1 [cited 2023 Nov 13];42(4):461–72. Available from: https://doi.org/10.1007/s11692-015-9340-9

57. Tucić N, Šešlija D, Stanković V. The short-term and long-term effects of parental age in the bean weevil (Acanthoscelides obtectus). Evol Ecol. 2004 Mar 1;18(2):187.

58. Arnqvist G, Stojković B, Rönn JL, Immonen E. The pace-of-life: A sex-specific link between metabolic rate and life history in bean beetles. Funct Ecol [Internet]. 2017 [cited 2019 Mar 6];31(12):2299–309. Available from: https://besjournals.onlinelibrary.wiley.com/doi/abs/10.1111/1365-2435.12927

59. van de Pol M, Verhulst S. Age-Dependent Traits: A New Statistical Model to Separate Within- and Between-Individual Effects. Am Nat. 2006 May 1;167(5):766–73.

60. Grieshop K, Stångberg J, Martinossi-Allibert I, Arnqvist G, Berger D. Strong sexual selection in males against a mutation load that reduces offspring production in seed beetles. J Evol Biol [Internet]. 2016 Jun [cited 2019 Jan 17];29(6):1201–10. Available from: http://doi.wiley.com/10.1111/jeb.12862

61. Biemont JC. Effects of the irradiations x on the reproductive power of Acanthoscelides obtectus Say (coleoptera: bruchidae). Ann Zool Ecol Anim V 5 No 4 Pp 581-591 [Internet]. 1973 Jan 1 [cited 2022 Feb 24]; Available from: https://www.osti.gov/biblio/4119273-effects-irradiations-reproductive-power-acanthoscelides-obtectus-say-coleoptera-bruchidae

62. Kempner ES. Effects of high-energy electrons and gamma rays directly on protein molecules. J Pharm Sci. 2001 Oct;90(10):1637–46.

63. Dowling DK, Simmons LW. Reactive oxygen species as universal constraints in life-history evolution. Proc R Soc B Biol Sci [Internet]. 2009 May 22 [cited 2019 Jan 17];276(1663):1737–45. Available from: http://www.royalsocietypublishing.org/doi/10.1098/rspb.2008.1791

64. Shabalina SA, Yampolsky LY, Kondrashov AS. Rapid decline of fitness in panmictic populations of Drosophila melanogaster maintained under relaxed natural selection. Proc Natl Acad Sci [Internet]. 1997 Nov 25 [cited 2018 Feb 19];94(24):13034–9. Available from: http://www.pnas.org/content/94/24/13034

636 65. Hadfield JD. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The
637 MCMCglmm R Package. J Stat Softw. 2010;33(2):1–22.

638 66. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. Vienna,
639 Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.R-
640 project.org/

641 67. Wickham H. ggplot2: Elegant Graphics for Data Analysis [Internet]. Springer-Verlag New York;
642 2016. Available from: https://ggplot2.tidyverse.org

643 68. Maklakov AA, Immler S, Lovlie H, Flis I, Friberg U. The effect of sexual harassment on lethal
644 mutation rate in female Drosophila melanogaster. Proc R Soc B Biol Sci [Internet]. 2012 Nov 21
645 [cited 2019 Jan 17];280(1750):20121874–20121874. Available from:
646 http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2012.1874

647 69. Bartosch -Harlid A, Berlin S, Smith NGC, Mosller AP, Ellegren H. Life History and the Male
648 Mutation Bias. Evolution [Internet]. 2003 Oct 1 [cited 2018 Dec 11];57(10):2398–406. Available
649 from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.0014-3820.2003.tb00251.x

650 70. Bromham L. The genome as a life-history character: why rate of molecular evolution varies
651 between mammal species. Philos Trans R Soc B Biol Sci [Internet]. 2011 Sep 12 [cited 2019 Jan
652 17];366(1577):2503–13. Available from:
653 http://rstb.royalsocietypublishing.org/cgi/doi/10.1098/rstb.2011.0014

654 71. Ellegren H. Characteristics, causes and evolutionary consequences of male-biased mutation.
655 Proc R Soc B Biol Sci [Internet]. 2007 Jan 7 [cited 2022 Feb 6];274(1606):1–10. Available from:
656 https://royalsocietypublishing.org/doi/10.1098/rspb.2006.3720

657 72. Stojković B, Đorđević M, Janković J, Savković U, Tucić N. Heterosis in age-specific selected
658 populations of a seed beetle: sex differences in longevity and reproductive behavior. Insect Sci.
659 2015 Apr;22(2):295–309.

660 73. Nussey DH, Kruuk LEB, Donald A, Fowlie M, Clutton-Brock TH. The rate of senescence in
661 maternal performance increases with early-life fecundity in red deer. Ecol Lett.
662 2006;9(12):1342–50.

663 74. Jones OR, Scheuerlein A, Salguero-Gómez R, Camarda CG, Schaible R, Casper BB, et al. Diversity
664 of ageing across the tree of life. Nature [Internet]. 2014 Jan [cited 2023 Oct 8];505(7482):169–
665 73. Available from: https://www.nature.com/articles/nature12789

666 75. Schärer L, Rowe L, Arnqvist G. Anisogamy, chance and the evolution of sex roles. Trends Ecol
667 Evol [Internet]. 2012 May [cited 2019 Jan 17];27(5):260–4. Available from:
668 https://linkinghub.elsevier.com/retrieve/pii/S0169534712000043

669 76. Servedio MR, Powers JM, Lande R, Price TD. Evolution of sexual cooperation from sexual
670 conflict. Proc Natl Acad Sci [Internet]. 2019 Nov 12 [cited 2020 Feb 13];116(46):23225–31.
671 Available from: https://www.pnas.org/content/116/46/23225

672 77. Hurst LD, Ellegren H. Sex biases in the mutation rate. Trends Genet. 1998 Nov 1;14(11):446–52.

673 78. Sayres MAW, Makova KD. Genome analyses substantiate male mutation bias in many species.
674 BioEssays [Internet]. 2011 [cited 2019 Jan 18];33(12):938–45. Available from:
675 https://onlinelibrary.wiley.com/doi/abs/10.1002/bies.201100091

79. Gao Z, Moorjani P, Sasani TA, Pedersen BS, Quinlan AR, Jorde LB, et al. Overlooked roles of DNA damage and maternal age in generating human germline mutations. Proc Natl Acad Sci. 2019 May 7;116(19):9491–500.

80. Wang RJ, Thomas GWC, Raveendran M, Harris RA, Doddapaneni H, Muzny DM, et al. Paternal age in rhesus macaques is positively associated with germline mutation accumulation but not with measures of offspring sociability. Genome Res. 2020 Jan 6;30(6):826–34.

81. Beck CW, Promislow DEL. Evolution of Female Preference for Younger Males. PLoS ONE [Internet]. 2007 Sep 26 [cited 2019 Jun 3];2(9). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1976549/

82. Segami JC, Lind MI, Qvarnström A. Should females prefer old males? Evol Lett [Internet]. 2021 [cited 2022 Feb 6];5(5):507–20. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/evl3.250

83. Jong G de, Noordwijk AJ van. Acquisition and Allocation of Resources: Genetic (CO) Variances, Selection, and Life Histories. Am Nat [Internet]. 1992;139(4):749–70. Available from: http://www.jstor.org/stable/2462620

84. Immler S. Haploid Selection in “Diploid” Organisms. Annu Rev Ecol Evol Syst [Internet]. 2019 [cited 2021 Jun 21];50(1):219–36. Available from: https://doi.org/10.1146/annurev-ecolsys-110218-024709

85. Sutter A, Immler S. Within-ejaculate sperm competition. Philos Trans R Soc B Biol Sci [Internet]. 2020 Dec 7 [cited 2021 Jun 21];375(1813):20200066. Available from: https://royalsocietypublishing.org/doi/full/10.1098/rstb.2020.0066

86. Rönn JL, Katvala M, Arnqvist G. Interspecific variation in ejaculate allocation and associated effects on female fitness in seed beetles. J Evol Biol [Internet]. 2008 [cited 2022 Sep 15];21(2):461–70. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1420-9101.2007.01493.x

87. Khandwala YS, Zhang CA, Lu Y, Eisenberg ML. The age of fathers in the USA is rising: an analysis of 168 867 480 births from 1972 to 2015. Hum Reprod. 2017 Oct 1;32(10):2110–6.

88. Mills M, Rindfuss RR, McDonald P, te Velde E, on behalf of the ESHRE Reproduction and Society Task Force. Why do people postpone parenthood? Reasons and social policy incentives. Hum Reprod Update. 2011 Nov 1;17(6):848–60.

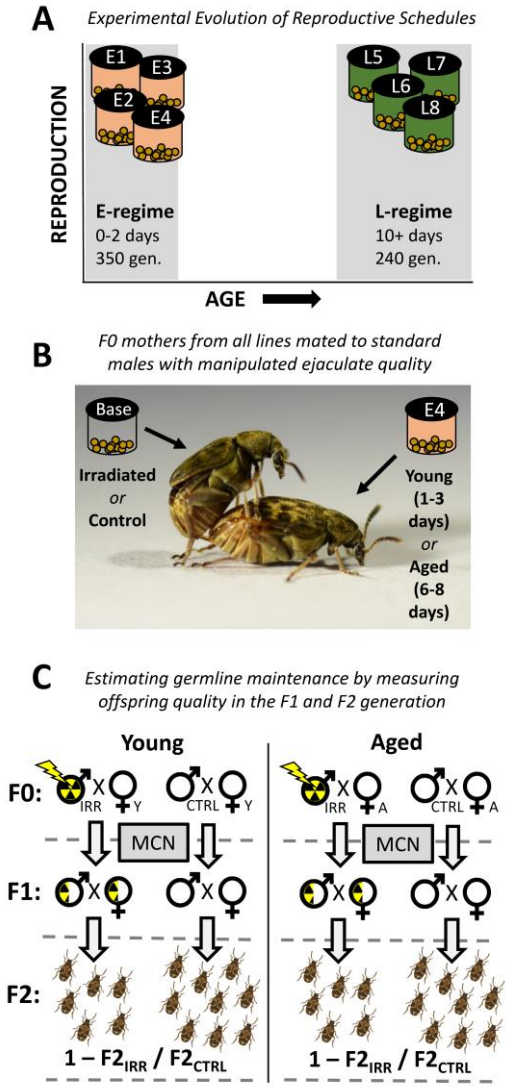


Figure 1: Experimental design.

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 aged (6-8 days), were mated to standard males from the base population and allowed to lay eggs for 48h. The 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. **C)** F1 offspring were crossed within line and treatment group (while avoiding inbreeding) using a middle-class neighbourhood (MCN) design to relax selection against induced (epi)mutations. Counts of emerging F2 adult offspring were used to assess age-specific female germline maintenance by comparing the proportional reduction in offspring quality in lineages deriving from either young and aged F0 females, mated with either irradiated or control males. Photo credit for panel B: Mareike Koppik. Beetles in panel C were generated using BioRender.

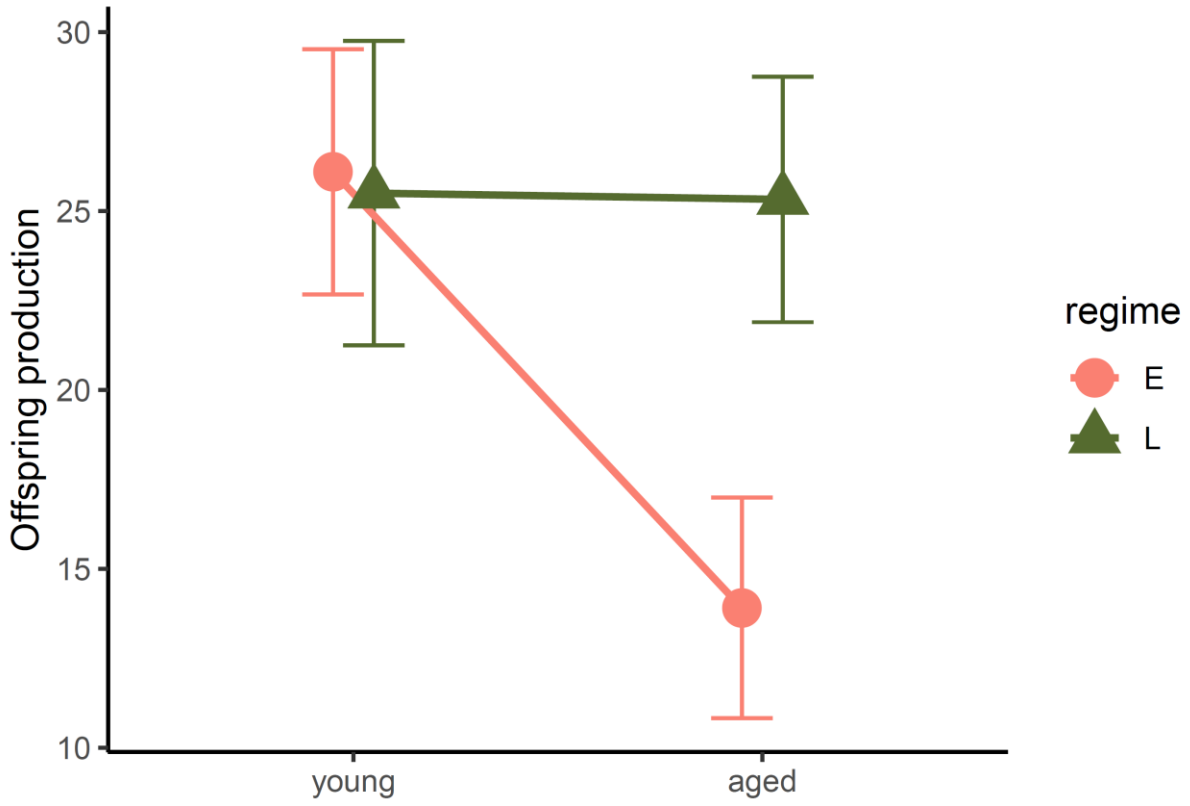


Figure 2: **Evolution of female reproductive schedules.**

The number of F1 offspring produced by young and aged F0 females of the E (salmon) and L (green) regime, during the 48h of egg laying following mating to standard control males from the base line. Shown are means and 95% confidence limits.

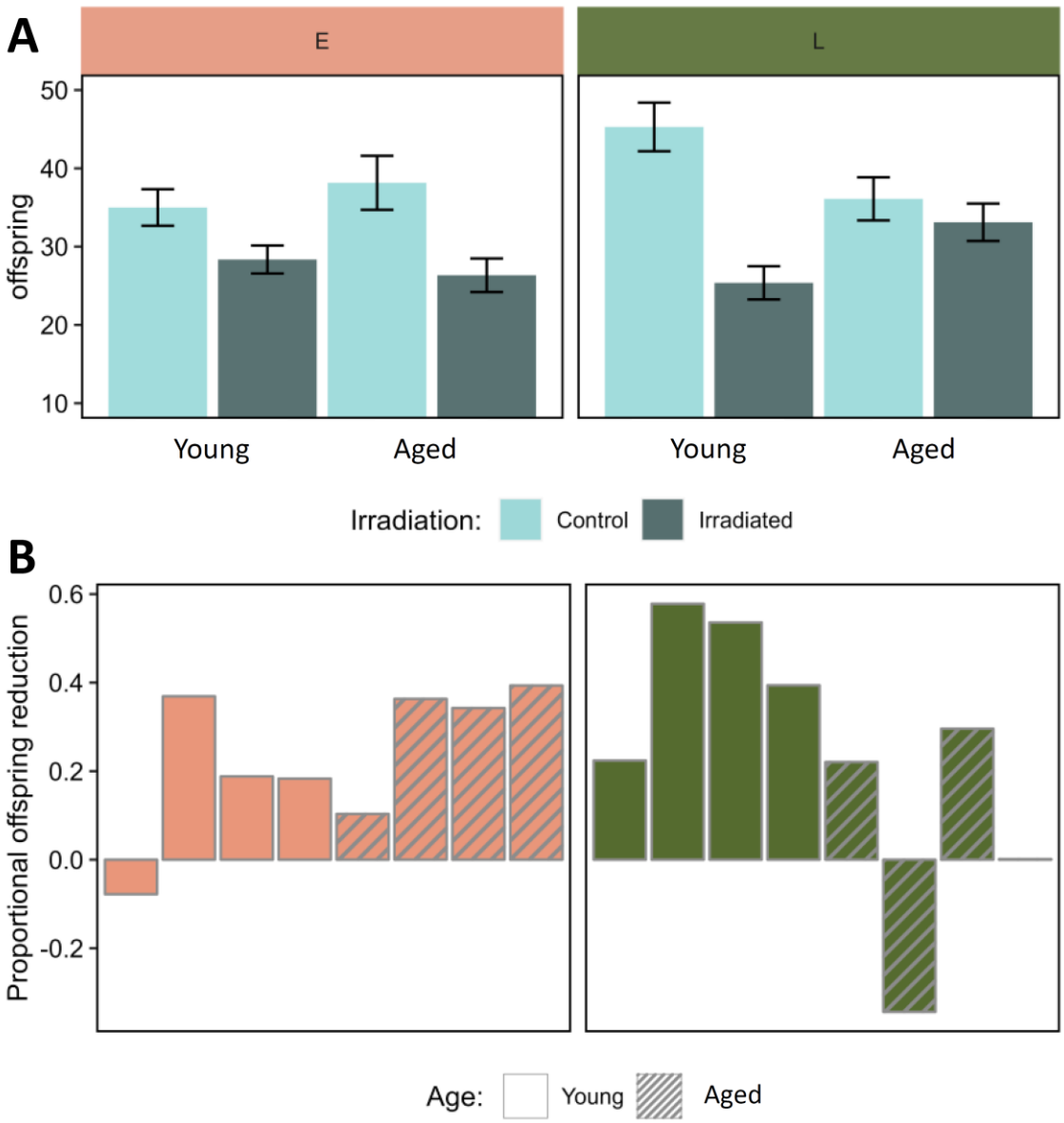
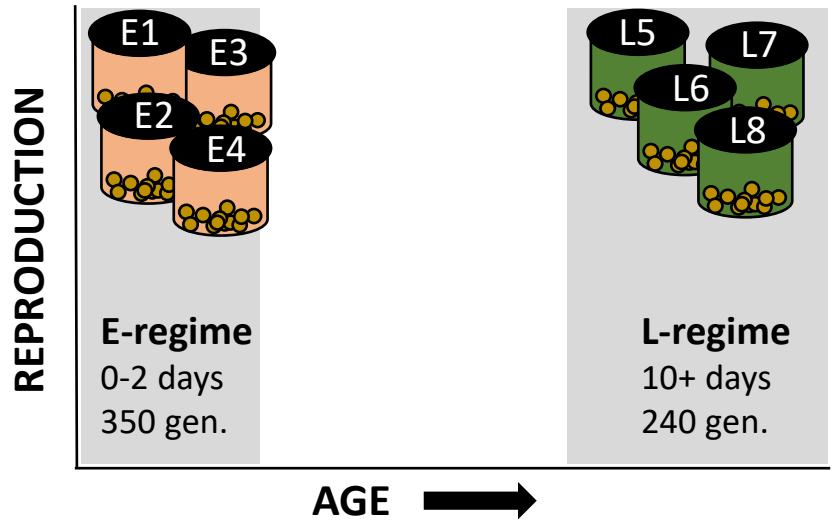


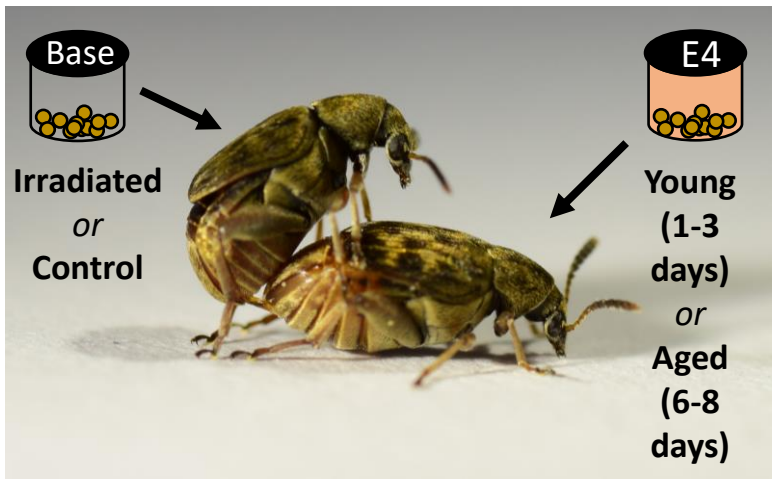
Figure 3: **Evolution of age-dependent female germline maintenance.**

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 aged (6-8 days) at mating, and belonged to either the early reproducing (E: salmon, left panel) or late reproducing (L: green, right panel) evolution 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 from the base population, 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 maintenance in F0 mothers. Plain and striped bars represent lineages originating from young and aged F0 grandmothers, respectively.

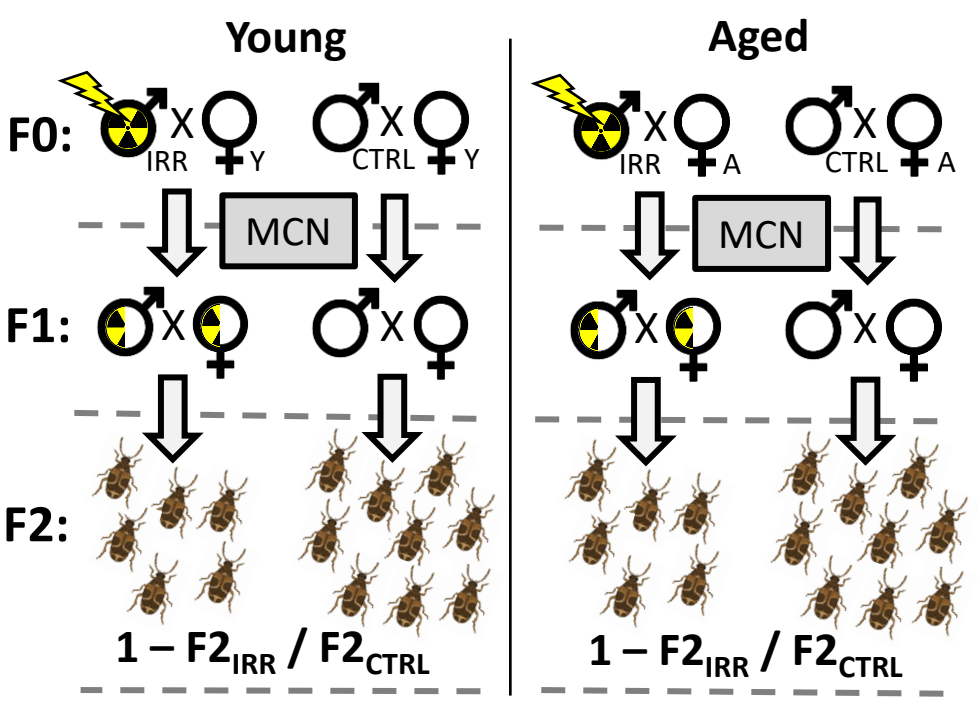
A *Experimental Evolution of Reproductive Schedules*

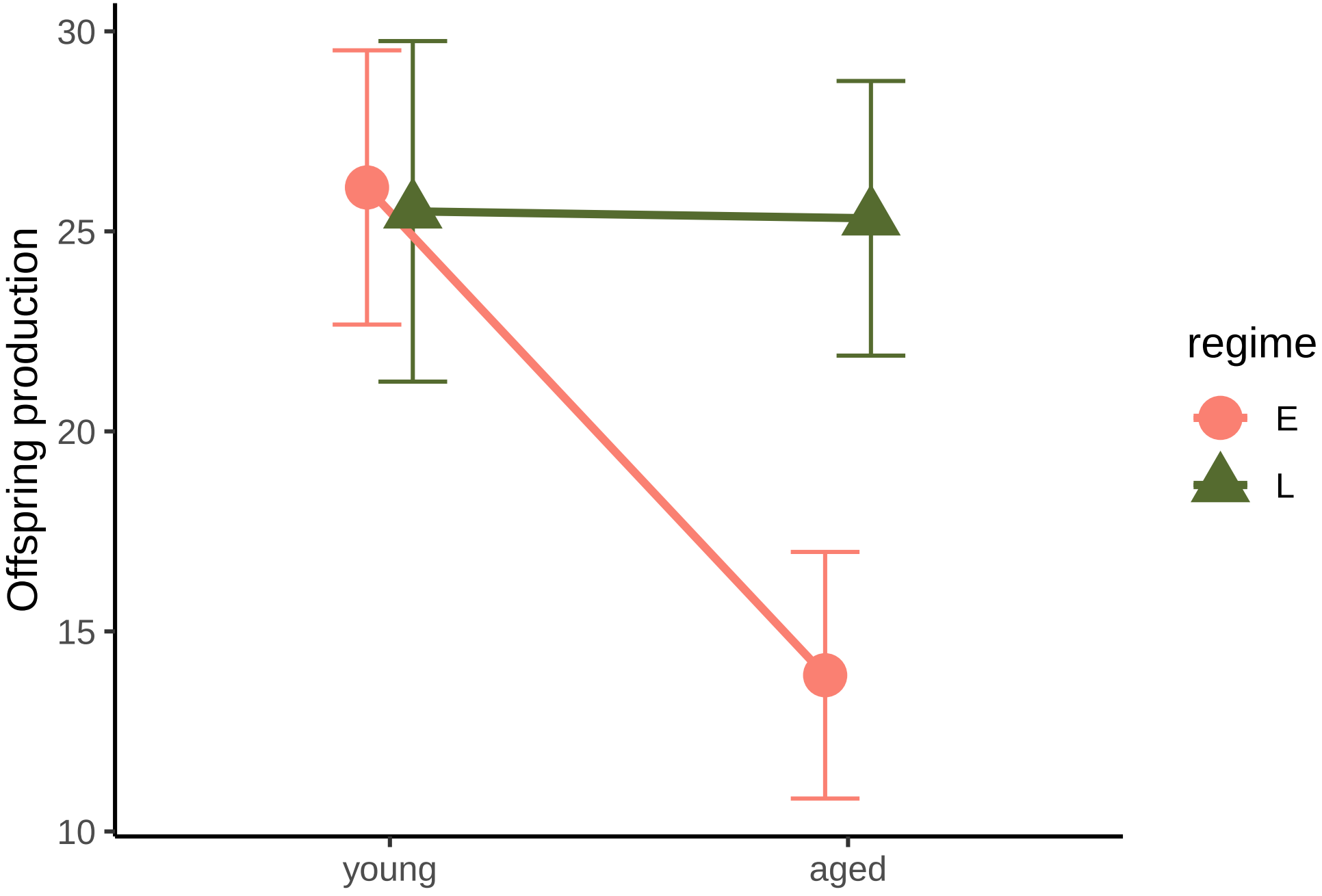


B *F0 mothers from all lines mated to standard males with manipulated ejaculate quality*



C *Estimating germline maintenance by measuring offspring quality in the F1 and F2 generation*




Offspring production
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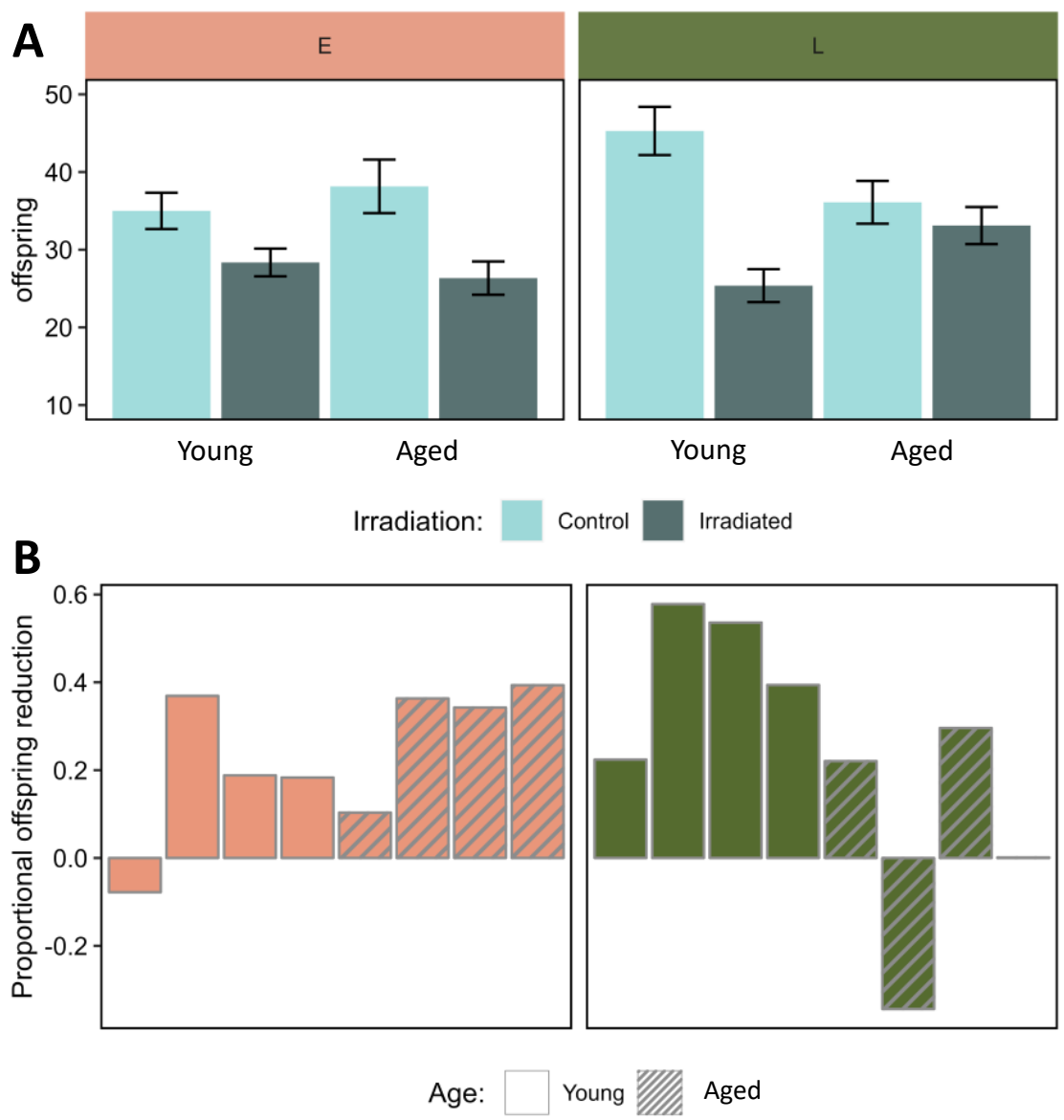
E
L


A
E
L
offspring
50
40
30
20
10
Young
Aged
Young
Aged
Irradiation:
Control
Irradiated
B
Proportional offspring reduction
0.6
0.4
0.2
0.0
-0.2
Age:
Young
Aged