Evolution of increased early-life fecundity but faster reproductive senescence in *Drosophila melanogaster* females through adaptation to chronic live-yeast deprivation

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Abstract

Dietary restriction is a common ecological challenge that limits reproduction. Yet only a few studies have explored adaptation under chronic protein deprivation. We subjected four replicate laboratory-adapted populations (YLB) of *Drosophila melanogaster* to a complete deprivation of live-yeast to mimic diet restricted ecology. In this insect, live-yeast is a critical source of protein that strongly affect reproductive output, especially in females. Following 24 generations of experimental evolution, compared to their matched controls (BL), females from YLB populations showed increase in reproductive output early in life, both in presence and absence of live-yeast. The observed increase in reproductive output was not associated with any accommodating alteration in average egg size; and development time, pre-adult survivorship, and body mass at eclosion of the progeny. Interestingly, adult lifespan was also found to be unaffected. However, YLB females were found to have a significantly faster rate of reproductive senescence albeit without any change in a measure of lifetime reproductive output. Taken together, adaptation to LYD ecology shows that reproductive output can evolve without affecting lifespan, suggesting that widely observed reproduction-survival trade-off is not universal. Populations can optimize fitness by fine tuning the scheduling of reproduction even when lifetime reproductive output is constrained.

1. Introduction

The ability of an organism to cope with a nutritional challenge is essential to adaptation in a given ecology (Kolss et al 2009, Zhang et al. 2019). Beyond providing the essential energy to sustain life, diet determines survival by affecting resistance to stress and other ecological challenges, including pathogens (Chippindale 1993, Field et al. 2002, Andersen et al. 2010, Sisodia and Singh 2012, Miller and Cotter 2017, Weldon et al. 2019). In addition, it also affects reproductive output and is central to a variety of trade-offs between components of life history (Chippindale 1993, Tatar & Carey 1995, Chapman & Partridge 1996, Marden et al. 2003, Adler & Bonduriansky 2014). Adaptation to even the simplest of nutritional challenges is expected to be complex in nature, involving a network of life history, physiological and behavioural traits. In response to one form of nutritional challenge, often referred to as dietary restriction (DR), organisms ranging from yeast to mammals have been found to elicit a complex physiological response that culminates in the partial or complete shutdown of reproduction, and extension of lifespan (Nakagawa et al. 2012, Fontana & Partridge 2015). Though the physiology and molecular genetic basis of DR response are well understood (Fontana et al. 2010, Fontana & Partridge 2015), there is considerable debate on the evolutionary significance of such a short term response, and the long term consequence of a chronic DR and other forms of nutritional challenges (Adler & Bonduriansky 2014). Further, little is known about quantitative genetic variance and covariance for traits that determine the DR response (but see Rikke et al. 2010, Jin et al. 2020).

Dietary restriction of one or more essential nutrients without causing malnutrition, has been widely reported to trigger an extension of lifespan in *D. melanogaster,* possibly through lowered reproduction (Mair et al. 2005, Partridge et al. 2005). This plastic response of life history to DR has been investigated in *D. melanogaster* by limiting yeast supplement on food

(Partridge et al. 1987, Chippindale et al.1993) or by dilution of nutrients in food (Chapman and Partridge, 1996). Mair et al. (2005) showed that simultaneous dilution of sugar and heat-killed yeast in their food resulted in lifespan extension in laboratory populations. Moreover, the lifespan extension effect was more prominent in response to yeast restriction than an equivalent reduction in sucrose, emphasizing the importance of yeast in generating DR response in flies (Mair et al. 2005). Apart from such DR, other forms of nutritional challenges, including extreme starvation, have also been studied using the fruit fly system (Hoffmann & Harshman 1999, Parkash & Munjal 2000, Partridge et al. 2005, Rion & Kawecki 2007). Similar to DR, both evolutionary and plastic responses to starvation (with some exceptions) involve a physiological shift to 'survival mode' marked by greater response to stress and longer life-span at the cost of reduced fecundity (reviewed in Rion & Kawecki 2007).

Resource re-allocation hypothesis, a theory aimed at explaining the plasticity of life history in response to DR (simply, DR response hereon), proposes that such a response is an adaptive diversion of resources from reproduction to somatic maintenance to maximise the chance of survival under a period of temporary resource scarcity (Shanley & Kirkwood 1999, Moatt et al. 2020). Such resource re-allocation, however, can only lead to higher fitness when DR condition prevails for a shorter duration (Zajitschek et al. 2016 & 2019, Moatt et al. 2020). In face of chronic DR, continuing for several generations, natural selection should favour a higher investment in reproduction at the cost of survival (Zajitschek et al. 2016, 2019). There have been a few recent investigations on adaptation to chronic DR using *D. melanogaster* (Kolss et al. 2009, Vijendravarma & Kawecki 2013, Zajitschek et al. 2016, 2019). Kolss et al. (2009) found that after 29-64 generations of selection under larval DR, the populations had evolved better egg-adult survivorship and faster development in DR food

94 indicating adaptation to the novel diet. Zajitschek et al. (2016, 2019) investigated adaptation
95 to prolonged DR in adults, and contrary to the prediction from re-allocation theory, they
96 found that males from the DR selected populations had higher reproductive output on a DR
97 food without any effect on longevity even after 25 generations of selection (Zajitschek,
98 2016). The females on the other hand, after 50 generations of selection, had reduced
99 longevity without any effect on fecundity (Zajitschek, 2019).
100
101 Both laboratory cultures and wild populations of fruit flies, often need live-yeast as a dietary
102 protein source for reproduction (Begon 1982, Starmer 1982, Chippindale et al., 1993;
103 Anagnostou, Dorsch & Rohlfs 2010). Importantly, besides affecting female reproductive
104 output, such yeast supplement impacts adult lifespan and starvation resistance (Chippindale et
105 al. 1993), oviposition site preference (Becher et al 2012), and may even affect offspring
106 survival and fitness components (Rohlfs & Hoffmeister 2005, Anagnostou, Dorsch & Rohlfs
107 2010). Given the strong fitness consequence of feeding on live-yeast, the lack of
108 experimental data on adaptation to live-yeast deprivation (LYD) in the available literature is
109 rather surprising. Interestingly, because live-yeast acts as dietary protein supplement
110 primarily for females (Chippindale et al. 1993, Nandy et al. 2012), it is possible for LYD to
111 represent a sex-specific selective environment.
112
113 Here, we subjected four replicate populations of *D. melanogaster* to chronic LYD in order to
114 investigate the life-history and behavioural adaptations to such ecology. After 23 generations
115 of selection, we addressed the following questions to assess adaptation to such an ecology:
116 (a) Considering female fecundity to be the prime target of selection under LYD, do evolved
117 females show a clear sign of adaptation to this new maintenance regime? (b) Given that
118 female reproductive output in *D. melanogaster* is often influenced by their mates in addition

119 to their own physiology and/or behaviour, does male effect on female fecundity evolve in
120 response to LYD? (c) If adaptation to LYD involves changes in reproductive output, what
121 consequences do such adaptation have on life-history traits besides fecundity?
122
123 **2. Materials and methods**
124 Study populations and selection regime
125 Four new experimental populations (referred to as YLB_{1-4} here on) were derived from four
126 replicate baseline populations BL_{1-4} (Figure S1). The BL populations (Nandy et al. 2016) are
127 outbred, wild type populations of *D. melanogaster*. They are maintained under a 14-day
128 discrete generation cycle, under 24-hour light, at 25 °C (±1) ambient temperature, and on a
129 standard banana-sugarcane jaggery-yeast medium. Flies are grown in standard vials (25 mm
130 diameter × 96 mm height) for 12 days. Larval density is controlled at ~70 per vial with 8 ml
131 food. On day 12 of the generation cycle, adults are transferred to plexiglass cages (23cm ×
132 20cm × 15 cm) with a food plate smeared with an *ad-lib* quantity of live yeast (a paste made
133 with water). On the day 14, the flies are given fresh food for oviposition and eggs laid during
134 an 18-hour window (fitness window) are collected to start the next generation. BL
135 populations were maintained in the laboratory under this regime for almost five years prior to
136 the derivation of the experimental YLB populations. The maintenance of the YLB
137 populations is identical to that of the BL except for the absence of live-yeast supplement in
138 the YLB ecology. All assays reported here were conducted after 23-25 generations of
139 experimental evolution. To equalize any potential non-genetic parental effect, all populations
140 were passed through one generation of common rearing ecology (Rose 1984). During this, a
141 subset of the experimental populations was generated by collecting eggs from the stock BL_x
142 and YLB_x populations. These populations were maintained under a regime that exactly
143 matched that of the BL-regime. Experimental flies were generated by collecting eggs from

144 these subset populations. All fly handling, including the collection of virgin flies, wherever
145 necessary, was done under light CO_2 anaesthesia.
146
147 Experiment 1: Juvenile traits and early-life fecundity in females
148 After 23 generations of experimental evolution, we assessed whether the YLB populations
149 have adaptively diverged from the ancestral BL populations. Our initial observation
150 suggested fecundity to be the most likely target trait of selection (data not shown). Therefore,
151 as the first set of assays, we quantified female early-life fecundity under a range of yeasting
152 conditions. In addition, since fecundity is often a function of body size and related juvenile
153 traits, we also investigated these traits in a parallel assay.
154
155 Female early-life fecundity and a three important juvenile traits – pre-adult (egg-to-adult)
156 development time, juvenile (egg-to-adult) survivorship and dry weight at eclosion were
157 measured between 23-25 generations. Following one generation of common rearing (see
158 above), eggs were collected from the population cages in exact numbers. We allowed an
159 oviposition window of about one hour. Such a short window of oviposition ensured the
160 developmental synchrony of the eggs collected for the experiment. Eggs were transferred
161 from the oviposition plate to an egg-collection surface made of solidified agar gel (1% Agar-
162 agar solution) using a fine brush. Eggs were carefully counted and a small piece of Agar gel
163 containing 70 eggs was cut out and transferred to a fresh food vial having 6-8ml food. Ten
164 such vials were set up for each population. Each vial was labelled individually and placed
165 into the incubator. As an added precaution, the vials in the incubator were shuffled twice
166 every day to randomize any positional effect. The vials were monitored carefully for the signs
167 of eclosion. Beginning from the eclosion of the first individual, the eclosing adults were
168 counted and sexed every 6 hours until all the pupae eclosed. This data was used to derive the

169 average measure of egg-to-adult development time (i.e., pre-adult development time) for
170 males and females in each vial. Out of the 70 eggs initially cultured, the proportion that
171 finally eclosed from a vial was considered as the measure of egg-to-adult survivorship (i.e.,
172 juvenile survivorship). Three vials (one from the YLB_1; two from BL_2) were excluded from
173 the analysis due to mishandling. For each population (BL_x and YLB_x), 50 males and 50
174 females were collected for dry whole body weight measurement. They were collected within
175 6-hours of eclosion, and were immediately frozen at -20 °C. They were later dried at 60°C for
176 48 hr and weighed in groups of five using Shimadzu AUW220D to the nearest 0.01 mg. The
177 mean body weight of each group was calculated and taken as a unit of analysis.
178
179 For early-life fecundity assay, flies were raised and collected as virgins as mentioned above.
180 Virgin flies were held in same-sex vials in groups of 12 in each vial. On the day 12 post egg-
181 collection (i.e., corresponding to 2-3 day post-eclosion), one vial each of males and females
182 (i.e., 12 males and 12 females) belonging to the same regime, were transferred to a fresh food
183 vial assigned to one of the three live-yeast supplementation treatments - No yeast
184 (N), Limiting yeast (L: 4.5 mg yeast) and High yeast (H: 9 mg yeast). Twelve such vials were
185 set up per live-yeast supplementation treatment for a population. Live-yeast was dissolved in
186 tap water to make a suspension, which was then dispensed on the top of the food in a vial.
187 The suspension was allowed to air-dry before the introduction of the experimental flies. The
188 males and the females were allowed to interact in these vials for 48 hours. Following this, on
189 day 4-5 post-eclosion flies in each treatment vial were anaesthetized under light CO_2 - six
190 females were randomly separated and transferred to a vial for egg size measurement, while
191 the other six were individually transferred to six oviposition tubes (12 × 75 mm) for fecundity
192 measurement. For the former, females were allowed to oviposit for 16 hours in a vial,
193 following which they were discarded. The vial with eggs was frozen at -20 °C for a minimum

194 of one day. For egg size measurement, eggs were mounted on a glass slide on their dorsal
195 side and photographed using a stereozoom microscope and the area of the two-dimensional
196 elliptical outline of the eggs were measured in Image J software. This area was taken as a
197 proxy for the size of each egg. A given egg was measured three times and the average of
198 these three measurements was taken as the unit of analysis. Females were allowed to oviposit
199 in these tubes for 16 hours. Females from the oviposition tubes were then discarded and
200 number of eggs was counted. We removed a few vials from the analysis due to a variety of
201 handling errors, including escaped flies and broken tube/vial, during the assay. The final
202 number of vials used for the analysis ranged from 9-12 vials per live-yeast treatment for a
203 population.
204
205 Experiment 2: Female × male effect on female fitness
206 To further compare the evolution of female fitness, we quantified female early-life
207 reproductive output (progeny count = number of surviving adult offspring produced by a
208 female) during a 16-hour window on the day 4-5 post-eclosion. This window corresponded to
209 the 18-hour fitness window on the 14th day of the generation cycle and therefore, is a good
210 measure of Darwinian fitness in this system. Importantly, female fitness, especially in *D.*
211 *melanogaster*, is affected by both female fecundity and male factors such as seminal fluid
212 composition (Chapman et al. 1995; Arnqvist & Nilsson 2000; Wolfner 2009). Therefore, it is
213 important to assess male and female specific effect of selection on female fitness. To test this
214 effect, we combined YLB and BL males and females in a full factorial design and measured
215 early-life reproductive output of females in the above-mentioned protocol. These assays were
216 carried out after 26-29 generations of selection. Experimental flies were generated following
217 a protocol similar to that stated above. Males and females were collected as virgins and held
218 in single-sex vials in groups of six in each vial until day 12 post-egg collection. On the day

219 12 of the generation cycle (i.e., 2-3 days post-eclosion), sexes were combined to set up adult
220 interaction vials having six individuals of each sex in a vial. Four types of combinations (viz.,
221 female×male: BL×YLB; BL×BL; YLB×YLB; YLB×BL), each having 15 replicate vials,
222 were set up. A few flies escaped during the setting up of one vial in the third block with
223 $YLB_3 \times YLB_3$ combination and hence, it was removed from the experiment. These adult
224 interaction vials were left undisturbed for 48 hours. On the day 14, females from each of
225 these vials were sorted under light CO_2 anaesthesia, and individually transferred to
226 oviposition test tubes (one female per tube) where they were allowed to oviposit for 17 hours.
227 After 17 hours, females from the oviposition tubes were discarded and the tubes were kept in
228 the incubator for the development and emergence of the progeny. After the emergence of all
229 the progeny, the test tubes were frozen at -20 °C for the final count.
230
231 Experiment 3: Longevity and age-specific fecundity
232 To test reproduction vs. lifespan trade-off theory, longevity and fecundity of the experimental
233 females were measured after 26 generations of selection. To assess age related changes in
234 fecundity, we measured fecundity of the test females in regular intervals. Similar to the other
235 assays described above, all populations were passed through one generation of common
236 rearing. The eggs were collected from the common rearing cages and cultured under standard
237 density of ~70 per 6-8ml food in a vial. Adult flies were collected as virgins within six hours
238 of eclosion and held in single-sex vials in groups of 12 in standard food vials. These vials
239 were left undisturbed for two days. On the day 3 post eclosion (i.e., on the day 12 post egg
240 collection), experimental vials were set up by transferring the flies into fresh food vials. Two
241 sets of vials were set up for each population – (a) Continuously exposed (CE): 12 males and
242 12 females placed (without anaesthesia) in a vial and (b) Virgin females (VF): 12 virgin
243 females placed in a vial. For a population, 12 vials were set up for each of CE and VF. Except

244 for the fecundity count days (see below), flies were transferred into fresh food vials every
245 alternate day without anaesthesia. Dead flies were sexed and counted during every transfer.
246 In the course of the repeated vial-to-vial transfers, a few flies escaped at various time points.
247 These flies were right censored for the survivorship analysis. For analysis of mean longevity,
248 vials where more than four flies escaped were removed from the final analysis. Thus mean
249 longevity analysis included data from 8-12 vials per treatment for each population. In this
250 study, we have focused on the response to selection on female longevity only.
251
252 Fecundity of the CE females were measured at regular intervals. Starting from day 5 post-
253 eclosion (i.e. day 14 post-egg collection), every fifth day, the flies were transferred into fresh
254 food vials (fecundity vials), where they were allowed a window of 17-hours before being
255 transferred to fresh food vials again. The number of eggs in these fecundity vials was
256 counted. This was done at day 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 (by this time-
257 point all females had died). For each time point, the number of eggs in a vial was divided by
258 the number of surviving females to calculate per female fecundity. This was taken as the unit
259 of analysis. However, the final analysis of age-specific fecundity was done excluding the last
260 few age points (post day 40) to avoid an unbalanced design arising due to the death of most
261 individuals towards the later age points. Additionally, the cumulative fecundity of females
262 was also quantified across all the above-mentioned age points till death.
263
264 Data analysis:
265 The entire study used a total of eight populations. Populations with replicate identity (for
266 example, BL_1 and YLB_1) were phylogenetically closer (see Figure S1) compared to those
267 with different replicate identity. In addition, during an assay a given replicate set (for
268 example, BL_1 and YLB_1) was handled together whereas different replicate sets were handled

independently, often on different days. Thus data from replicate sets were treated as statistical blocks and was modelled as a random effect in all the analyses. The entire study, thus involved four statistical blocks. Within each block, vials were the unit of replication and vial values / means were used as the unit of analysis / response variable. Except for adult survivorship and age-specific fecundity, results of all assays were analysed using mixed-model analysis of variance (ANOVA). In case of the analysis of juvenile traits, selection regime and sex (wherever applicable) were treated as fixed effects. For the early-life female fecundity assay and egg size measurement, selection regime and live-yeast concentration treatment were considered as fixed effects. For the analysis of the Experiment 2 early-life progeny count data, male (selection) regime and female (selection) regime were used as fixed effects. For analysing female mean longevity data from Experiment 3, selection regime and mating status were treated as fixed effects. Cumulative life-time fecundity data was analysed using selection regime as fixed factor. These analyses were performed on Statistica (version 10, Statsoft, Tulsa, OK, USA).

For female survivorship analysis, we applied a mixed Cox's Proportional Hazard models, using the `coxme` package in R version 4.0.2 (Gupta et al. 2013, Therneau et al. 2015). The effects of regime, mating status and their interaction on survivorship was analysed and block was fitted as a random intercept. Cox partial likelihood estimates were compared across treatment and selection regime.

Age-specific fecundity was analysed as linear mixed-effects models with the `lme4` package in R version 4.0.2 (Bates et al. 2014). Further, to obtain the p-values for the `lmer` models with degrees of freedom based on the Satterthwaite approximation, we used the R package `lmerTest` (Kuznetsova et al. 2017). Regime and time-point (age) were fitted as categorical

294 fixed effects. Block as well as all interactions involving it were fitted as random effects. In
295 addition, vial ID (replicate vial identity) was nested within block and was fitted as a random
296 effect. The nested random effect accounts for the fact that subsequent egg counts from the
297 same group of flies within a particular vial are not independent (Harrison et al. 2018). Post-
298 hoc analysis was done using Tukey's HSD with the `emmeans` package (Lenth 2018). To
299 measure reproductive senescence, rates at which fecundity declined with age, after reaching a
300 peak, were quantified for both regimes. Rate of decline in fecundity was quantified across
301 seven time-points (τ), starting from the peak, i.e. day 11 post-eclosion until day 41. For a
302 particular age-point, the mean of per-capita fecundity (ε) of a population was calculated using
303 the observed fecundity across all the vials within that population. A linear model ($\varepsilon \sim b_p\tau +$
304 c_p, where b_p is population specific slope and c_p is population specific intercept) was fitted to
305 these data using the `lm` function in R to obtain a b-coefficient (i.e., slope) for each of the
306 eight populations (Fig. 3b). The slope of the regression line served as a proxy for rate of
307 reproductive senescence. Finally, these b-coefficients, without the sign, were analysed using
308 mixed model ANOVA in Statistica, taking selection regime as a fixed effect and block as a
309 random effect.
310

311 **3. Results**

312 Experiment 1: Juvenile traits and female fecundity

313 We did not find a significant effect of selection regime on juvenile traits including
314 development time, pre-adult (egg-to-adult) survivorship and dry body weight at the time of
315 eclosion (Table-S1 and S2). Analysis of the early-life fecundity data indicated significant
316 effects of the selection regime and yeast concentration, however, the effect of the selection
317 regime × yeast concentration interaction was not significant (Table 1). Compared to the BL
318 females, YLB females had 8.7-22.89 % higher mean per-capita fecundity across all live-yeast

concentrations in the assay (Fig.1). Moreover, the higher early-life fecundity output of YLB females was consistent across a gradient of live-yeast concentration (Fig.1). The effect of selection regime on egg size was non-significant (Table S1 and S2). Hence, there was no evidence of differential provisioning in eggs produced by the experimental females.

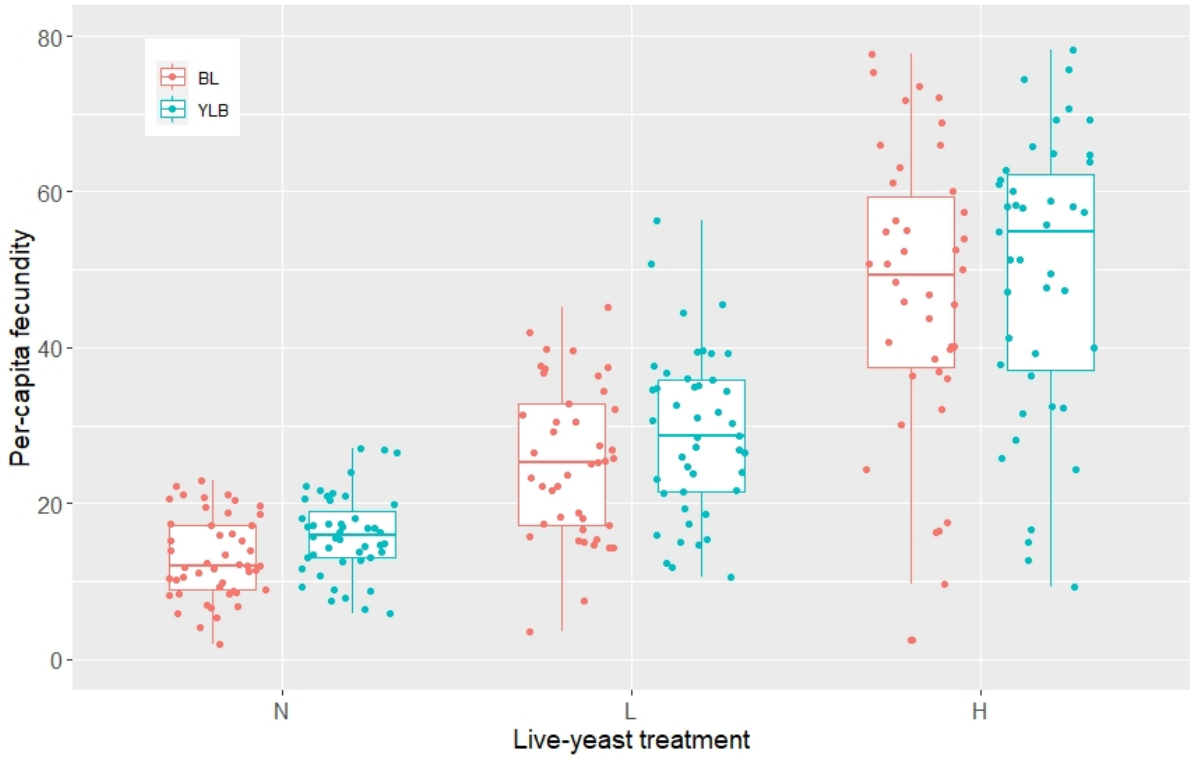


Figure 1: Female early-life fecundity from Experiment 1. Number of eggs laid during a 16-hour assay window by 4-5 day old (post-eclosion) females following the 48 hour live-yeast treatment was measured. This was done under three live-yeast conditions - no yeast (N), limiting yeast (L) and high yeast (H). Data points represent mean per-capita fecundity of selected (YLB) and the ancestral (BL) females. Six of the twelve test females in a vial were used for fecundity measurement. Mean per-capita fecundity in a replicate vial was calculated using these six females. Three factor mixed model ANOVA indicated significant effects of selection regime and live-yeast treatment. However, the effect of selection regime × live-yeast treatment interaction was not significant.

Experiment 2: Female × male effect on female fitness

The analysis of the early-life progeny output data from Experiment 2 indicated a significant effect female selection regime. However, neither the male selection regime nor the interaction between these two fixed factors were found to have significant effects on progeny count

(Table 1, Fig. 2). YLB females produced significantly more progeny irrespective of the regime of the males they interacted with (Fig. 2). Thus, the higher early-life reproductive output in YLB's seems to be independent of any male influence.

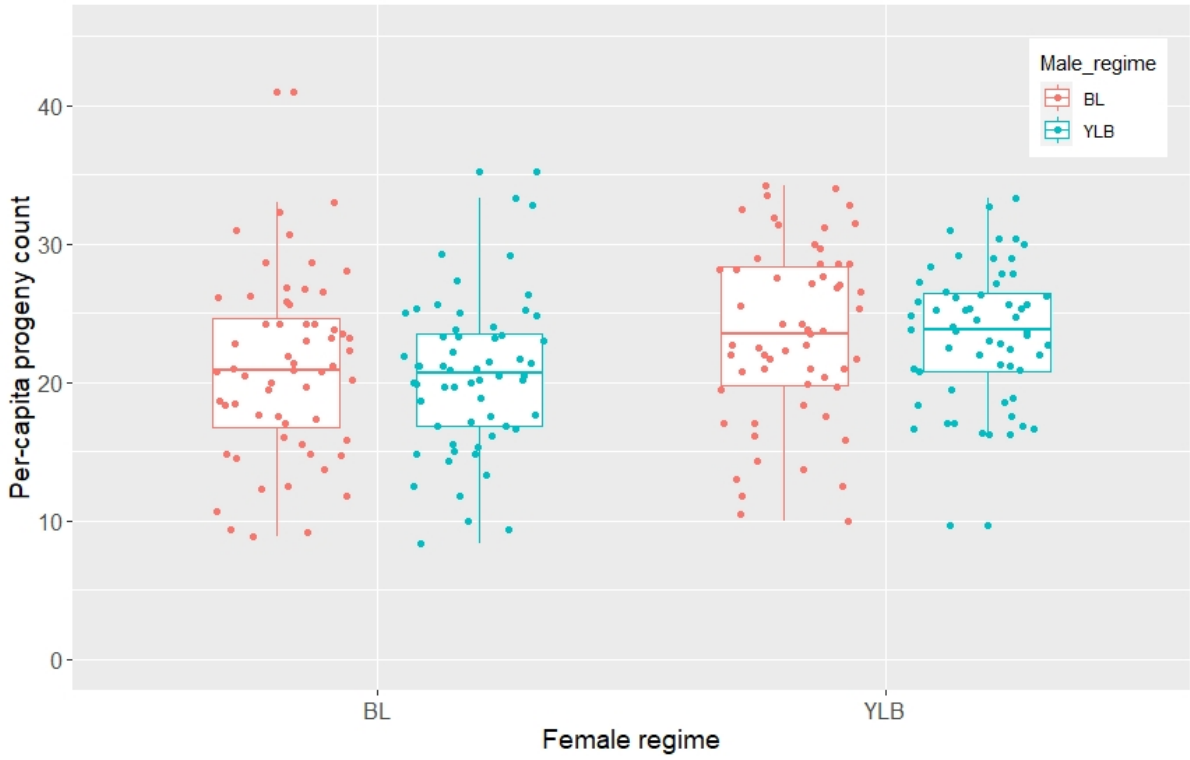


Figure 2: Female × male effect on early-life reproductive output in females as measured in Experiment 2. Number of progeny produced during a 17-hour assay window by 4-5 day old (post-eclosion) BL and YLB females, mated and held with BL and YLB males (full factorial combination), was measured. Data points represent per capita progeny output from selected (YLB) and ancestral (BL) females. All six females in each replicate vial were allowed to produce progeny. Vials mean, which was calculated over these six test females, was used as the unit of analysis. Two factor mixed model ANOVA indicated a significant effect of female regime, but effect of male regime on progeny count was not significant, nor was that of the male regime × female regime interaction.

Traits	Effects	SS	DF	MS	DEN DF	DEN MS	F	p
Early life fecundity	Selection Regime (SR)	947.8	1	947.8	3.01	7.47	126.83	**<0.01**
	Live-yeast concentration (YC)	51192.3	2	25596.2	6.00	1043.57	24.52	**<0.01**
	Block	19814.0	3	6604.7	5.59	1008.10	6.55	**0.03**
	SR × YC	23.0	2	11.5	6.01	42.40	0.27	0.77
	SR × Block	22.4	3	7.5	6.02	42.41	0.17	0.90
	YC × Block	6266.1	6	1044.4	6.00	42.39	24.63	**<0.01**
	SR × YC × Block	254.3	6	42.4	247.00	59.14	0.71	0.64
Progeny count	Female regime	411.13	1	411.14	3.00	7.40	55.55	**<0.01**
	Male regime	0.99	1	0.99	3.00	36.84	0.03	0.88
	Block	3149.10	3	1049.70	0.64	20.48	51.26	0.26
	Female regime × Male regime	7.18	1	7.18	3.00	23.76	0.30	0.62
	Female regime × Block	22.20	3	7.40	3	23.76	0.31	0.82
	Male regime × Block	110.51	3	36.84	3	23.76	1.55	0.36
	Fem. reg. × Male reg. × Block	71.28	3	23.76	222	20.71	1.15	0.33

Table 1: Results of the analyses on early-life fecundity (from experiment 1) and progeny output (from experiment 2) in females. Fecundity data was analysed using three factor mixed-model ANOVA with selection regime and live-yeast concentration as fixed factors, and block as random factor. Progeny count data from experiment 2 was analysed using three factor mixed-model ANOVA with female regime and male regime as fixed factors, and block as random factor. Statistically significant p-values are highlighted in boldface.

361 Experiment 3: Longevity and age-specific fecundity

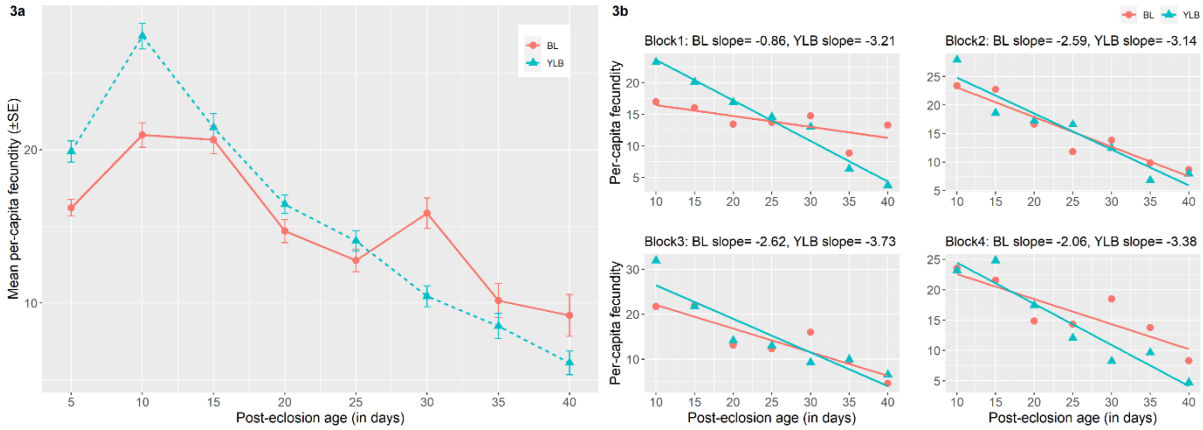
362 We did not find a significant effect of selection regime on survival probability (Table S3) and
363 mean longevity (Table S4) of females. Mating status, however, had a significant impact.
364 Virgin females had a better survival rate compared to the mated females (Fig. S2).
365 Unsurprisingly, analysis of the mean longevity indicated that mating status had a significant
366 effect on mean longevity (Table S4). Virgin females were found to have 67.58 % longer
367 mean longevity compared to the mated females for both BL and YLB selection regimes (Fig.
368 S3,Table S4). Results of these analyses can be found in the supplementary information.

369

370 Analysis of the cumulative fecundity of females quantified across different age points till
371 death revealed that there was no significant effect of selection regime on per-capita fecundity,
372 however, the effect of the selection regime × time point interaction was found to be
373 significant (Table 3a, Fig.3a). Tukey's adjusted p-value obtained by pairwise comparisons
374 suggested that, as compared to the ancestors, YLBs have significantly higher fecundity at the
375 age of 6th and 11th day PE. Interestingly, there was a clear trend of YLBs having higher mean
376 per-capita fecundity in initial age points compared to BLs (through age- 6, 11, 16, 21 and 26
377 days PE). The trend, however, was found to be reversed at the later time points, with BLs
378 showing comparatively higher fecundity (through age- 31, 36 and 41 days PE). The detailed
379 outcome of the post-hoc analysis is mentioned in the supplementary information
380 (Supplementary sheet, Table- S5). Further, a comparison of the slopes (b-coefficients) of the
381 linear regression fit of age-specific fecundity on time for the two regimes suggested a faster
382 rate of decline in fecundity in YLBs compared to that of the BLs (mean ±SE: b_{BL}= -2.03
383 ±0.41; b_{YLB} = -3.36 ±0.13, Fig. 3b). Two factor mixed model ANOVA on the b-coefficient
384 indicated a significant effect of selection regime (Table 3b). Considering the b-coefficient to
385 be a measure of reproductive senescence, YLB females showed faster reproductive

386 senescence compared to that of the BL females. The cumulative fecundity of females,
387 quantified across all the above-mentioned age points, did not differ between the two regimes
388 (Table 3c, mean ±SE; BL: 129.22 ±4.52; YLB: 127.91 ±4.37).

389



390

391

392 **Figure 3: Age-specific fecundity of females measured in Experiment 3.** Number of eggs laid during 17 hour windows on
393 day 5, 10, 15, 20, 25, 30, 35, 40 were measured during the longevity assay. Only CE (continuously exposed) vials were used
394 for this purpose. Per capita fecundity was calculated for a vial by diving the fecundity count by the number of surviving
395 females, which was twelve to start with. (a) Mean age-specific per capita fecundity analysis indicated a significant effect of
396 selection regime × age interaction. (b) Slope (b-coefficient) of the linear decline in per capita fecundity with age following
397 the peak fecundity was computed for each population by fitting a linear model. The two factor mixed model ANOVA on the
398 b-coefficients indicated a significant effect of selection regime.

399

Trait	Effects	SS	DF	MS	Denominator DF	F	p
(a) Age-specific fecundity	Selection Regime (SR)	8.2	1	8.18	59.03	0.39	0.53
	Time point	6531.9	7	933.13	22.27	44.44	**< 0.01**
	SR × Time point	986.2	7	140.88	22.41	6.71	**<0.01**
(b) b-coefficient	Selection Regime	4.1	1	4.1	3	13.68	**0.03**
	Block	1.4	3	0.47	3	1.54	0.37
(c) Cumulative Fecundity	Selection Regime (SR)	35	1	35	3.01	0.06	0.82
	Block	4089	3	1363	3	2.26	0.26
	SR × Block	1806	3	602	76	0.73	0.53

400

Table 3: Results of the analyses of age-specific fecundity measured in Experiment 2. (a) Age-specific fecundity count was analysed using linear mixed-effect model, where selection regime and time point (age) were fitted as categorical fixed effects, and block as random effect. (b) For each population, age-specific fecundity data was fitted in a linear model ($\varepsilon \sim b_p\tau + c_p$, where b_p is population specific slope and c_p is population specific intercept) to compute b-coefficient (slope). These b-coefficient values (i.e., slope values without the sign) were analysed using two factor mixed model ANOVA with selection regime and block as fixed and random factors respectively. (c) Cumulative fecundity, i.e., fecundity count summed over all age points, were analysed using a two factor mixed model ANOVA with selection regime and block as fixed and random factors respectively. Statistically significant p-values are mentioned in bold face.

4. Discussion

In *D. melanogaster,* live-yeast is a strong stimulus for female fecundity both in wild and laboratory-adapted *D. melanogaster* (Begon 1982, Stramer 1982, Chippindale et al. 1993, Simmons & Bradley 1997, Anagnostou, Dorsch & Rohlfs 2010). In absence of live-yeast supplement females usually produce far fewer eggs. Therefore, in absence of live-yeast fecundity selection is expected to work on unstimulated fecundity of females. Selection imposed by such an ecology is expected to be female-specific at least to some extent. This is because adult females forage extensively on live-yeast, whereas males do not (Simmons & Bradley 1997). After 23 generations of laboratory evolution, we found clear evidence of increased early-life female fecundity. The results thus indicate the presence of substantial standing genetic variation in traits that determine unstimulated early-life fecundity in females. Several hundreds of generations of laboratory domestication, thus, have not eroded such variation. What maintains such standing genetic variation is thus an open question.

Interestingly, higher early-life fecundity of the evolved females was observed under unstimulated assay condition (i.e., in absence of yeast supplement) as well as low and high concentrations of yeast supplement. We also showed that this increased early-life fecundity is reflected on the reproductive output (i.e., progeny number) of the females. Taken together,

428 these results suggest that the YLB females have adapted to the live-yeast supplement
429 deprived ecology. Though males in *D. melanogaster* are known to affect female fecundity
430 (Wolfner et al. 2009), the results of experiment-3 rule out this possibility, further suggesting
431 that this to be a female adaptation. There are several non-mutually exclusive ways in which
432 YLB females could achieve higher early-life fecundity. In the following section, we explore
433 these different possibilities.
434
435 In principle, females can adapt to such nutritional deprivation either by increasing acquisition
436 and/or utilization of dietary protein. The selection did not affect body mass at eclosion and
437 juvenile traits, such as development time and egg-to-adult survivorship. Thus, there was no
438 indication of a change in juvenile stage resource acquisition. Alternatively, a change in the
439 relative abundance of different macronutrients, especially total protein and/or lipid content, in
440 the adult female body may also explain our results. This idea, however, remains to be tested
441 with additional experimental data. Theoretically, higher fecundity can be achieved by trading
442 off with per-capita provisioning, i.e., egg size (Hein et al. 2018, also see Vijendravarma et al.
443 2010). We, however, did not find any evidence supporting this number vs. size trade-off as
444 there was no effect of selection on egg size. This is perhaps not surprising as previous
445 investigations on *D. melanogaster* found egg size and number to be genetically uncorrelated
446 (Schwarzkopf et al. 1999).
447
448 Surprisingly, we did not find a negative impact of the increased early-life fecundity on
449 longevity, as predicted by the trade-off theory. A trade-off between reproduction and lifespan
450 is pervasive in nature and has been widely reported even in laboratory systems (Rose 1984,
451 Chippindale et al. 1993, Zwaan et al. 1995, Maklakov et al. 2017, Flatt 2011, Hensen et al.
452 2013). Several attempts to test this prediction, however, have been uninformative (Dudash &

453 Fenster 1997, Kotiaho & Simmons 2003). More recently, laboratory adaptation to chronic
454 dietary yeast deprivation resulted in *D. melanogaster* females with reduced lifespan without a
455 corresponding increase in reproductive output (Zajitschek et al. 2019). Thus, the lack of
456 evidence of reproduction-longevity trade-off in our populations is an addition to a growing
457 list of evidence challenging the generality of this theory (Le Bourg and Minois 1996,
458 Partridge et al. 2005, Grandison et al. 2009, Flatt 2011, Zajitschek et al. 2016, 2019).
459 Importantly, it is possible that this trade-off is more sensitive to total reproductive allocation.
460 If YLB females have evolved age-specific reproductive allocation without any change in total
461 lifetime reproductive investment, the expected decline in longevity may not evolve. This is
462 particularly important because there is no fitness consequence of altered longevity in
463 YLB/BL ecology and therefore, longevity is free to evolve only as a correlated response. On
464 the other hand, early life fecundity selection is a prime target of the YLB selection,
465 irrespective of the correlated consequences expressed later in life. Therefore, it is not
466 unreasonable to expect age-specific changes in fecundity to evolve in our selection regime.
467 We will return to this theory later in this section.
468
469 In addition to adult lifespan, changes in several adult traits may also result in increased
470 fecundity in females. There are at least four such possibilities, which are not mutually
471 exclusive. First, the simplest possibility is that an increased foraging behaviour can also result
472 in more resources being available for egg production (McCaffery 1975, Ives 1981, Barnes et
473 al. 2008). Secondly, a reduction in unnecessary wastage of resources may make more
474 resources available for egg production. For example, *D. melanogaster* females seem to
475 achieve higher fecundity by showing lower spontaneous locomotion (Long & Rice 2006).
476 Third, better resource recycling or reduced physiological wastage can potentially explain
477 increased fecundity (Adler & Bonduriansky 2014). For example, certain kinds of nutritional

478 deprivation seem to influence autophagy and scavenging of molecules – processes that may
479 be viewed as the physiology of resource recycling (Scott et al. 2004, Barth et al. 2011,
480 Cuervo & Macian 2012, Mason et al. 2018). More data are needed to test these theories. A
481 fourth way by which YLB females could have achieved higher fecundity is through an
482 alteration in the reproductive schedule without altering the overall lifetime reproductive
483 output – an idea we explore next.
484
485 The evolution of early life fitness components at the cost of late-life fitness has been one of
486 the central issues in life-history theory (Stearns 1992). In the laboratory ecology of 14-day
487 discrete generation, the YLB populations are selected for early-life fitness (see Promislow
488 and Tatar 1998, Houle and Rowe 2003). Hence, it is not surprising to find the evolution of
489 early-life fecundity in YLB females. The associated observation of a faster decrease in
490 fecundity without a significant change in mean lifetime egg output is somewhat surprising,
491 but it seems to fit with the antagonistic pleiotropy (AP) theory of ageing (Williams 1957).
492 Antagonistic pleiotropy theory has been in the centre stage especially due to its success in
493 explaining the evolution of organismal ageing. Early investigations
494 on *Drosophila* populations substantiated the theory (Rose 1984, Rauser et al. 2006), and have
495 been shown to explain both somatic as well as reproductive senescence. Multiple
496 investigations also reported a substantial genetic variation in age-specific fecundity in *D.*
497 *melanogaster* (for example, Tatar et al. 1996, Durham et al. 2014). More recently, using *C.*
498 *elegans,* Anderson et al. (2011) found that genetically heterogeneous populations selected for
499 early-life fecundity evolved increased early but reduced late-life reproductive output without
500 suffering a total fitness or lifespan cost. Thus, it is not unreasonable to suggest that adaptation
501 seen in our YLB populations involves the evolution of early-life beneficial alleles with
502 detrimental pleiotropic effects on late-life reproductive output.

5. Conclusion

Adaptive evolution of early-life fecundity in YLB females seems to support the hitherto reported phenomenon of the release of hidden genetic variation in novel ecology or stress (Badyaev 2005). Several past investigations have shown that novel environmental challenges or stresses (e.g., heat shock) can generate new trait variances or convert otherwise phenotypically neutral alleles to non-neutral (Hall 1990, Ratner et al. 1992, Kondrashov and Houle 1996, Wessler 1996, Leips & Mackay 2000, Queitsch et al. 2002, Dworkin et al. 2003, Nedelcu & Michod 2003, Ruden et al. 2003, Sollars et al. 2003). Such novel genetic variance can then be subjected to selective pressures leading to adaptation. It is possible that under LYD ecology, hidden variation in fecundity traits might have been expressed, followed by its directional selection in the YLB regime. Further, there is a growing literature on substantial G×E interaction on fitness-related traits, including fecundity (see Leroi et al. 1994, Fry et al. 1998, Burns et al. 2012, Pallares et al. 2021). Pallares et al. (2021) reported that dietary sugar enrichment has a profound effect on the fitness landscape of the fecundity and longevity traits in *D. melanogaster*. Fitness consequences of many fitness affecting alleles were found to be much stronger in the altered diet compared to the standard diet (Pallares et al. 2021). Our results could also be affected by a similar diet dependence of fitness consequence of fecundity traits in this system. Selectively neutral alleles that affect unstimulated (by live-yeast) fecundity could have strong fitness effects under LYD ecology and hence, be subjected to directional selection, at least in the initial generations of the YLB history. Many of these theories will need further investigation with genomic and transcriptomic data.

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