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

indicating adaptation to the novel diet. Zajitschek et al. (2016, 2019) investigated adaptation to prolonged DR in adults, and contrary to the prediction from re-allocation theory, they found that males from the DR selected populations had higher reproductive output on a DR food without any effect on longevity even after 25 generations of selection (Zajitschek, 2016). The females on the other hand, after 50 generations of selection, had reduced longevity without any effect on fecundity (Zajitschek, 2019).

Both laboratory cultures and wild populations of fruit flies, often need live-yeast as a dietary protein source for reproduction (Begon 1982, Starmer 1982, Chippindale et al., 1993; Anagnostou, Dorsch & Rohlf 2010). Importantly, besides affecting female reproductive output, such yeast supplement impacts adult lifespan and starvation resistance (Chippindale et al. 1993), oviposition site preference (Becher et al 2012), and may even affect offspring survival and fitness components (Rohlf & Hoffmeister 2005, Anagnostou, Dorsch & Rohlf 2010). Given the strong fitness consequence of feeding on live-yeast, the lack of experimental data on adaptation to live-yeast deprivation (LYD) in the available literature is rather surprising. Interestingly, because live-yeast acts as dietary protein supplement primarily for females (Chippindale et al. 1993, Nandy et al. 2012), it is possible for LYD to represent a sex-specific selective environment.

Here, we subjected four replicate populations of *D. melanogaster* to chronic LYD in order to investigate the life-history and behavioural adaptations to such ecology. After 23 generations of selection, we addressed the following questions to assess adaptation to such an ecology: (a) Considering female fecundity to be the prime target of selection under LYD, do evolved females show a clear sign of adaptation to this new maintenance regime? (b) Given that female reproductive output in *D. melanogaster* is often influenced by their mates in addition

to their own physiology and/or behaviour, does male effect on female fecundity evolve in response to LYD? (c) If adaptation to LYD involves changes in reproductive output, what consequences do such adaptation have on life-history traits besides fecundity?

2. Materials and methods

Study populations and selection regime

Four new experimental populations (referred to as YLB₁₋₄ here on) were derived from four replicate baseline populations BL₁₋₄ (Figure S1). The BL populations (Nandy et al. 2016) are outbred, wild type populations of *D. melanogaster*. They are maintained under a 14-day discrete generation cycle, under 24-hour light, at 25 °C (±1) ambient temperature, and on a standard banana-sugarcane jaggery-yeast medium. Flies are grown in standard vials (25 mm diameter × 96 mm height) for 12 days. Larval density is controlled at ~70 per vial with 8 ml food. On day 12 of the generation cycle, adults are transferred to plexiglass cages (23cm × 20cm × 15 cm) with a food plate smeared with an *ad-lib* quantity of live yeast (a paste made with water). On the day 14, the flies are given fresh food for oviposition and eggs laid during an 18-hour window (fitness window) are collected to start the next generation. BL populations were maintained in the laboratory under this regime for almost five years prior to the derivation of the experimental YLB populations. The maintenance of the YLB populations is identical to that of the BL except for the absence of live-yeast supplement in the YLB ecology. All assays reported here were conducted after 23-25 generations of experimental evolution. To equalize any potential non-genetic parental effect, all populations were passed through one generation of common rearing ecology (Rose 1984). During this, a subset of the experimental populations was generated by collecting eggs from the stock BL_x and YLB_x populations. These populations were maintained under a regime that exactly matched that of the BL-regime. Experimental flies were generated by collecting eggs from

these subset populations. All fly handling, including the collection of virgin flies, wherever necessary, was done under light CO₂ anaesthesia.

Experiment 1: Juvenile traits and early-life fecundity in females

After 23 generations of experimental evolution, we assessed whether the YLB populations have adaptively diverged from the ancestral BL populations. Our initial observation suggested fecundity to be the most likely target trait of selection (data not shown). Therefore, as the first set of assays, we quantified female early-life fecundity under a range of yeasting conditions. In addition, since fecundity is often a function of body size and related juvenile traits, we also investigated these traits in a parallel assay.

Female early-life fecundity and a three important juvenile traits – pre-adult (egg-to-adult) development time, juvenile (egg-to-adult) survivorship and dry weight at eclosion were measured between 23-25 generations. Following one generation of common rearing (see above), eggs were collected from the population cages in exact numbers. We allowed an oviposition window of about one hour. Such a short window of oviposition ensured the developmental synchrony of the eggs collected for the experiment. Eggs were transferred from the oviposition plate to an egg-collection surface made of solidified agar gel (1% Agar-agar solution) using a fine brush. Eggs were carefully counted and a small piece of Agar gel containing 70 eggs was cut out and transferred to a fresh food vial having 6-8ml food. Ten such vials were set up for each population. Each vial was labelled individually and placed into the incubator. As an added precaution, the vials in the incubator were shuffled twice every day to randomize any positional effect. The vials were monitored carefully for the signs of eclosion. Beginning from the eclosion of the first individual, the eclosing adults were counted and sexed every 6 hours until all the pupae eclosed. This data was used to derive the

average measure of egg-to-adult development time (i.e., pre-adult development time) for males and females in each vial. Out of the 70 eggs initially cultured, the proportion that finally eclosed from a vial was considered as the measure of egg-to-adult survivorship (i.e., juvenile survivorship). Three vials (one from the YLB₁; two from BL₂) were excluded from the analysis due to mishandling. For each population (BL_x and YLB_x), 50 males and 50 females were collected for dry whole body weight measurement. They were collected within 6-hours of eclosion, and were immediately frozen at -20 °C. They were later dried at 60°C for 48 hr and weighed in groups of five using Shimadzu AUW220D to the nearest 0.01 mg. The mean body weight of each group was calculated and taken as a unit of analysis.

For early-life fecundity assay, flies were raised and collected as virgins as mentioned above. Virgin flies were held in same-sex vials in groups of 12 in each vial. On the day 12 post egg-collection (i.e., corresponding to 2-3 day post-eclosion), one vial each of males and females (i.e., 12 males and 12 females) belonging to the same regime, were transferred to a fresh food vial assigned to one of the three live-yeast supplementation treatments - No yeast (N), Limiting yeast (L: 4.5 mg yeast) and High yeast (H: 9 mg yeast). Twelve such vials were set up per live-yeast supplementation treatment for a population. Live-yeast was dissolved in tap water to make a suspension, which was then dispensed on the top of the food in a vial. The suspension was allowed to air-dry before the introduction of the experimental flies. The males and the females were allowed to interact in these vials for 48 hours. Following this, on day 4-5 post-eclosion flies in each treatment vial were anaesthetized under light CO₂ - six females were randomly separated and transferred to a vial for egg size measurement, while the other six were individually transferred to six oviposition tubes (12 × 75 mm) for fecundity measurement. For the former, females were allowed to oviposit for 16 hours in a vial, following which they were discarded. The vial with eggs was frozen at -20 °C for a minimum

of one day. For egg size measurement, eggs were mounted on a glass slide on their dorsal side and photographed using a stereozoom microscope and the area of the two-dimensional elliptical outline of the eggs were measured in Image J software. This area was taken as a proxy for the size of each egg. A given egg was measured three times and the average of these three measurements was taken as the unit of analysis. Females were allowed to oviposit in these tubes for 16 hours. Females from the oviposition tubes were then discarded and number of eggs was counted. We removed a few vials from the analysis due to a variety of handling errors, including escaped flies and broken tube/vial, during the assay. The final number of vials used for the analysis ranged from 9-12 vials per live-yeast treatment for a population.

Experiment 2: Female $\times$ male effect on female fitness

To further compare the evolution of female fitness, we quantified female early-life reproductive output (progeny count = number of surviving adult offspring produced by a female) during a 16-hour window on the day 4-5 post-eclosion. This window corresponded to the 18-hour fitness window on the 14th day of the generation cycle and therefore, is a good measure of Darwinian fitness in this system. Importantly, female fitness, especially in *D. melanogaster*, is affected by both female fecundity and male factors such as seminal fluid composition (Chapman et al. 1995; Arnqvist & Nilsson 2000; Wolfner 2009). Therefore, it is important to assess male and female specific effect of selection on female fitness. To test this effect, we combined YLB and BL males and females in a full factorial design and measured early-life reproductive output of females in the above-mentioned protocol. These assays were carried out after 26-29 generations of selection. Experimental flies were generated following a protocol similar to that stated above. Males and females were collected as virgins and held in single-sex vials in groups of six in each vial until day 12 post-egg collection. On the day

12 of the generation cycle (i.e., 2-3 days post-eclosion), sexes were combined to set up adult interaction vials having six individuals of each sex in a vial. Four types of combinations (viz., female×male: BL×YLB; BL×BL; YLB×YLB; YLB×BL), each having 15 replicate vials, were set up. A few flies escaped during the setting up of one vial in the third block with YLB₃×YLB₃ combination and hence, it was removed from the experiment. These adult interaction vials were left undisturbed for 48 hours. On the day 14, females from each of these vials were sorted under light CO₂ anaesthesia, and individually transferred to oviposition test tubes (one female per tube) where they were allowed to oviposit for 17 hours. After 17 hours, females from the oviposition tubes were discarded and the tubes were kept in the incubator for the development and emergence of the progeny. After the emergence of all the progeny, the test tubes were frozen at -20 °C for the final count.

Experiment 3: Longevity and age-specific fecundity

To test reproduction vs. lifespan trade-off theory, longevity and fecundity of the experimental females were measured after 26 generations of selection. To assess age related changes in fecundity, we measured fecundity of the test females in regular intervals. Similar to the other assays described above, all populations were passed through one generation of common rearing. The eggs were collected from the common rearing cages and cultured under standard density of ~70 per 6-8ml food in a vial. Adult flies were collected as virgins within six hours of eclosion and held in single-sex vials in groups of 12 in standard food vials. These vials were left undisturbed for two days. On the day 3 post eclosion (i.e., on the day 12 post egg collection), experimental vials were set up by transferring the flies into fresh food vials. Two sets of vials were set up for each population – (a) Continuously exposed (CE): 12 males and 12 females placed (without anaesthesia) in a vial and (b) Virgin females (VF): 12 virgin females placed in a vial. For a population, 12 vials were set up for each of CE and VF. Except

for the fecundity count days (see below), flies were transferred into fresh food vials every alternate day without anaesthesia. Dead flies were sexed and counted during every transfer. In the course of the repeated vial-to-vial transfers, a few flies escaped at various time points. These flies were right censored for the survivorship analysis. For analysis of mean longevity, vials where more than four flies escaped were removed from the final analysis. Thus mean longevity analysis included data from 8-12 vials per treatment for each population. In this study, we have focused on the response to selection on female longevity only.

Fecundity of the CE females were measured at regular intervals. Starting from day 5 post-eclosion (i.e. day 14 post-egg collection), every fifth day, the flies were transferred into fresh food vials (fecundity vials), where they were allowed a window of 17-hours before being transferred to fresh food vials again. The number of eggs in these fecundity vials was counted. This was done at day 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 (by this time-point all females had died). For each time point, the number of eggs in a vial was divided by the number of surviving females to calculate per female fecundity. This was taken as the unit of analysis. However, the final analysis of age-specific fecundity was done excluding the last few age points (post day 40) to avoid an unbalanced design arising due to the death of most individuals towards the later age points. Additionally, the cumulative fecundity of females was also quantified across all the above-mentioned age points till death.

Data analysis:

The entire study used a total of eight populations. Populations with replicate identity (for example, BL₁ and YLB₁) were phylogenetically closer (see Figure S1) compared to those with different replicate identity. In addition, during an assay a given replicate set (for example, BL₁ and YLB₁) 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

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

3. Results

Experiment 1: Juvenile traits and female fecundity

We did not find a significant effect of selection regime on juvenile traits including development time, pre-adult (egg-to-adult) survivorship and dry body weight at the time of eclosion (Table-S1 and S2). Analysis of the early-life fecundity data indicated significant effects of the selection regime and yeast concentration, however, the effect of the selection regime $\times$ yeast concentration interaction was not significant (Table 1). Compared to the BL 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 $\times$ live-yeast treatment interaction was not significant.

Experiment 2: Female $\times$ 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.

Experiment 3: Longevity and age-specific fecundity

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

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

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

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

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 $\times$ 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 $\times$ Block	1806	3	602	76	0.73	0.53

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 ($\epsilon \sim b_p \tau + c_p$, 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 & Rohlf 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,

these results suggest that the YLB females have adapted to the live-yeast supplement deprived ecology. Though males in *D. melanogaster* are known to affect female fecundity (Wolfner et al. 2009), the results of experiment-3 rule out this possibility, further suggesting that this to be a female adaptation. There are several non-mutually exclusive ways in which YLB females could achieve higher early-life fecundity. In the following section, we explore these different possibilities.

In principle, females can adapt to such nutritional deprivation either by increasing acquisition and/or utilization of dietary protein. The selection did not affect body mass at eclosion and juvenile traits, such as development time and egg-to-adult survivorship. Thus, there was no indication of a change in juvenile stage resource acquisition. Alternatively, a change in the relative abundance of different macronutrients, especially total protein and/or lipid content, in the adult female body may also explain our results. This idea, however, remains to be tested with additional experimental data. Theoretically, higher fecundity can be achieved by trading off with per-capita provisioning, i.e., egg size (Hein et al. 2018, also see Vijendravarma et al. 2010). We, however, did not find any evidence supporting this number vs. size trade-off as there was no effect of selection on egg size. This is perhaps not surprising as previous investigations on *D. melanogaster* found egg size and number to be genetically uncorrelated (Schwarzkopf et al. 1999).

Surprisingly, we did not find a negative impact of the increased early-life fecundity on longevity, as predicted by the trade-off theory. A trade-off between reproduction and lifespan is pervasive in nature and has been widely reported even in laboratory systems (Rose 1984, Chippindale et al. 1993, Zwaan et al. 1995, Maklakov et al. 2017, Flatt 2011, Hensen et al. 2013). Several attempts to test this prediction, however, have been uninformative (Dudash &

Fenster 1997, Kotiaho & Simmons 2003). More recently, laboratory adaptation to chronic dietary yeast deprivation resulted in *D. melanogaster* females with reduced lifespan without a corresponding increase in reproductive output (Zajitschek et al. 2019). Thus, the lack of evidence of reproduction-longevity trade-off in our populations is an addition to a growing list of evidence challenging the generality of this theory (Le Bourg and Minois 1996, Partridge et al. 2005, Grandison et al. 2009, Flatt 2011, Zajitschek et al. 2016, 2019). Importantly, it is possible that this trade-off is more sensitive to total reproductive allocation. If YLB females have evolved age-specific reproductive allocation without any change in total lifetime reproductive investment, the expected decline in longevity may not evolve. This is particularly important because there is no fitness consequence of altered longevity in YLB/BL ecology and therefore, longevity is free to evolve only as a correlated response. On the other hand, early life fecundity selection is a prime target of the YLB selection, irrespective of the correlated consequences expressed later in life. Therefore, it is not unreasonable to expect age-specific changes in fecundity to evolve in our selection regime. We will return to this theory later in this section.

In addition to adult lifespan, changes in several adult traits may also result in increased fecundity in females. There are at least four such possibilities, which are not mutually exclusive. First, the simplest possibility is that an increased foraging behaviour can also result in more resources being available for egg production (McCaffery 1975, Ives 1981, Barnes et al. 2008). Secondly, a reduction in unnecessary wastage of resources may make more resources available for egg production. For example, *D. melanogaster* females seem to achieve higher fecundity by showing lower spontaneous locomotion (Long & Rice 2006). Third, better resource recycling or reduced physiological wastage can potentially explain increased fecundity (Adler & Bonduriansky 2014). For example, certain kinds of nutritional

deprivation seem to influence autophagy and scavenging of molecules – processes that may be viewed as the physiology of resource recycling (Scott et al. 2004, Barth et al. 2011, Cuervo & Macian 2012, Mason et al. 2018). More data are needed to test these theories. A fourth way by which YLB females could have achieved higher fecundity is through an alteration in the reproductive schedule without altering the overall lifetime reproductive output – an idea we explore next.

The evolution of early life fitness components at the cost of late-life fitness has been one of the central issues in life-history theory (Stearns 1992). In the laboratory ecology of 14-day discrete generation, the YLB populations are selected for early-life fitness (see Promislow and Tatar 1998, Houle and Rowe 2003). Hence, it is not surprising to find the evolution of early-life fecundity in YLB females. The associated observation of a faster decrease in fecundity without a significant change in mean lifetime egg output is somewhat surprising, but it seems to fit with the antagonistic pleiotropy (AP) theory of ageing (Williams 1957). Antagonistic pleiotropy theory has been in the centre stage especially due to its success in explaining the evolution of organismal ageing. Early investigations on *Drosophila* populations substantiated the theory (Rose 1984, Rauser et al. 2006), and have been shown to explain both somatic as well as reproductive senescence. Multiple investigations also reported a substantial genetic variation in age-specific fecundity in *D. melanogaster* (for example, Tatar et al. 1996, Durham et al. 2014). More recently, using *C. elegans*, Anderson et al. (2011) found that genetically heterogeneous populations selected for early-life fecundity evolved increased early but reduced late-life reproductive output without suffering a total fitness or lifespan cost. Thus, it is not unreasonable to suggest that adaptation seen in our YLB populations involves the evolution of early-life beneficial alleles with 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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