Evolution under dietary restriction decouples survival from fecundity in *Drosophila melanogaster* females

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ABSTRACT

One of the key tenets of life-history theory is that reproduction and survival are linked and that they trade-off with each other. When dietary resources are limited, reduced reproduction with a concomitant increase in survival is commonly observed. It is often hypothesised that this dietary restriction (DR) effect results from strategically reduced investment in reproduction in favour of somatic maintenance in order to survive starvation periods until resources become plentiful again. We used experimental evolution to test this "waiting-for-the-good-times" hypothesis, which predicts that selection under sustained DR will favour increased investment in reproduction at the cost of survival because "good-times" never come. We assayed fecundity and survival of female *Drosophila melanogaster* fruit flies that had evolved for 50 generations on three different diets varying in protein content – low (classic DR diet), standard and high, in a full-factorial design. High-diet females evolved overall increased fecundity but showed reduced survival on low and standard diets. Low-diet females evolved reduced survival on low diet without corresponding increase in reproduction. In general, there was little correspondence between the evolution of survival and fecundity across all dietary regimes. Our results contradict the hypothesis that resource reallocation between fecundity and somatic maintenance underpins lifespan extension under DR.

Keywords: *Drosophila melanogaster*, nutrition, adaptation, DR, experimental evolution

INTRODUCTION

Understanding the relationship between nutrition, reproduction and survival, on the genetic and the phenotypic level, is thought to be essential for healthspan and lifespan extension (1). Research on genes involved in the modulation of these traits has revealed a network of nutrient and energy sensing signalling pathways that govern reproduction and survival (2), with substantial evolutionary conservation across the tree of life (3, 4). Lifespan extending effects of dietary restriction (DR) – the most successful intervention to prolong life to date (3) – is a case of phenotypic plastic response that generally not only increases survival, but also decreases reproduction (5). Evolutionary life-history theory and the antagonistic pleiotropy theory for the evolution of aging state that early and late life fitness components are generally trading off against each other, and that these negative correlations between traits are genetically based (6, 7). Within this framework, the plastic response to DR can be understood as the consequence of a shift in the energy trade-off between reproduction and survival.

The disposable soma theory of aging is built around this theoretical conjecture (8), and it states that resource requirements for reproduction directly compete with those required for somatic maintenance, and that this relationship should be observed both on the physiological and genetic level (see the distinction between 'physiological' and 'genetic' (evolutionary) trade-off, discussed in 9). Under the diposable soma theory, if the observed plasticity in this trade-off is adaptive, living longer and reproducing less under short term DR (within an individual's lifespan) should confer an evolutionary advantage (10, 11), and can be understood as a short-term emergency solution to cope with nutritional stress (12). This prediction was tested in a formal life-history DR model parameterized using house

mouse data by Shanley and Kirkwood (13), who found that under certain assumptions (i.e. an extra cost before successful reproduction and lower juvenile survival under DR), the classic DR response can evolve (discussed in 8). While there is suggestive evidence from a recent meta-analysis that DR might act differently on mortality rates in rodents, compared to *D. melanogaster* (14, but see 15), the main pathways leading to reduced aging seem to be evolutionary conserved between phyla (3). Nevertheless, one fundamental assumption of the disposable soma theory is that organisms can reallocate resources (mainly regarded in terms of energy units in this context) from reproduction to somatic maintenance and survival, and vice versa (16). While allocating more resources to survival, away from reproduction, is adaptive under short-term DR, this response should be maladaptive if resources are restricted permanently. If food shortage is permanent, spanning adult lifetimes over many generations, individuals that switch to a strategy of increased reproductive output at the cost of decreased survival, will have a selective advantage. One way this could happen is when the ability to respond plastically to DR erodes over evolutionary time (i.e. when the reaction norm for reproductive output across nutritional environments becomes less steep), or when either already segregating alleles or *de novo* mutations that confer higher reproductive output under DR are favoured (i.e. evolutionary adaptation).

If a negative genetic correlation *(evolutionary trade-off*) between reproduction (especially during early life) and survival exists, as has often been observed (17-23), higher levels of reproductive output under DR (regardless if short-term and transient, or evolved) should at the same time decrease lifespan, to an extent that depends on the strength of the correlation. On the other hand, even if reproduction and lifespan are decoupled, we would still expect an

increase in reproduction after sufficient numbers of generations under chronic DR, independent of a response in lifespan.

In the present study, we test this prediction using experimental evolution in *Drosophila melanogaster*, by manipulating adult dietary yeast levels and testing for an evolved response in female flies after approximately 50 generations. We previously found a response in male reproduction to this experimental evolution regime, with males evolved on DR having increased reproduction when tested on DR, standard or enriched diets, but no reduction in survival (24).

METHODS

Experimental design

Experimental flies (*Drosophila melanogaster*) originated from experimental evolution lines that evolved on three distinct diets with different yeast contents as adults (low diet (LD), standard diet (SD), high diet (HD); specific diet characteristics are given in supplementary table S1). Flies in the experimental evolution lines were kept in four replicate mixed-sex population cages per diet treatment, containing 150 adult males and 150 adult females each. All larvae were reared on standard diet, and only adults were exposed to the experimental evolution diets in the population cages. More specific details on the experimental setup of the lines can be found in Zajitschek et al. (24). In short, our experimental flies were derived from Dahomey, a large outbred laboratory population which originally was sampled in 1970 from the wild in Benin, West Africa. Ever since the population has been maintained in mixed-sex population cages with overlapping generations under constant environmental conditions (25°C, 60% humidity, 12:12 light-dark

cycle, on standard yeast-sugar diet). Recent studies on this population showed that it hosts substantial levels of genetic variation for lifespan (25, 26). We tested for an evolutionary response in females after approximately 50 generations of experimental evolution. Sample sizes are given in the Supplement (Table S2).

To remove any parental effects from the diet treatments before the start of the experiment, experimental flies were passed through two generations of common garden. To accomplish this females from the experimental population cages were allowed to lay eggs in wide plastic vials (28.5 mm × 95 mm used for all experimental work) with standard diet (SD) overnight. Eggs were trimmed to 100 eggs per vial, and eclosing adults were allowed to mate for the 2 days after eclosion before females were allowed to lay eggs in new SD vials for 2 hours. Eggs were again trimmed to 100 eggs per vial and eclosing adult females were used in assays. Each vial was populated with around 50 female flies.

Assay flies were provided with one of the three experimental evolution diets, with two replicate vials per cage and evolution diet × assay diet combination (total number of individual females per ED × AD treatment: N = 400). For weekly matings, females of each vial were transferred to new SD vials and given the matching number of 2 day old males that were bred in a separate stock sourced from the same population cage, once every week for 12 hours. Eggs laid during this period were counted. Total fecundity was calculated by summing eggs laid over all vials and weeks. Survival was checked every Monday, Wednesday and Friday until all flies had died.

We measured dry adult body mass of groups of 10 individual female flies, replicated 10 times per cage per evolutionary diet treatment (N = 400 per treatment). Prior to weighing, all flies were raised for 2 generations on SD medium, as described above.

Statistical analysis

To analyze survival, we used mixed Cox proportional hazard models (function coxme, R package coxme, 27). As the interaction term between assay diet and evolution diet was significant in a global analysis (χ^2= 104.63, df = 4, P < 0.001), we performed a) post-hoc analyses for assay diet effects within evolution diet groups, using Tukey's HSD method to adjust for multiple testing (function glht in R package multcomp, 28), and b) separate analyses for each assay diet, with evolution diet (ED) as a fixed effect and experimental vial, and population cage fitted as a random intercept. Models containing ED were compared to models that only contained an intercept, using log-likelihood ratio tests, with twice the difference in log-likelihoods of the models taken as chi-square distributed, and a 0.05 significance level. Untransformed lifespan and body mass were tested in linear mixed models (LMM, using maximum likelihood estimation), after testing residuals for normal distribution, with the same random effects as specified for Cox proportional hazards analyses (using function lmer in R package lme4, 29). We used the R package lmertest to calculate *p*-values for LMM, with degrees of freedom based on the Satterthwaite approximation (30), and performed post-hoc analyses as described above. To test for differences in hazard rates, we fitted exponential and Gompertz models, using Bayesian methods implemented in the R package BaSTA (31). The exponential model assumes a constant mortality rate at all ages, whereas the Gompertz model assumes an increase in mortality rate at later ages (i.e. aging):

$$\mu_x = b_0 e^{b_1 x}$$

with instantaneous mortality rate (hazard rate) at age x given by μ_x, parameter b_0 is the intercept and is interpreted as the initial or baseline mortality rate, parameter b_1 is the increase of mortality rate with advancing age (the *aging* parameter). We compared exponential and Gompertz model

fits using the deviance information criterion, DIC (32). For all reported analyses, diet was treated as a categorical variable. Lifespan summary statistics and sample sizes are given in Table S2, median lifespan is plotted in Figure S3.

Female reproductive fitness was estimated as the sum of all weekly fecundity measurements of each population of females in a vial, scaled by the initial number of females in a vial. Total fecundity was analyzed in linear mixed effects models following the same process as in the analysis for survival and lifespan, with population cage fitted as a random intercept. To specifically compare early, mid and late life fecundity, we also tested effects on mean fecundity in age classes (early life fecundity = fecundity in week 1, mid life fecundity = fecundity in weeks 2 and 3, late life fecundity = fecundity in week 4 and later). Post-hoc tests were conducted using function difflsmeans in R package lmerTest. Evolution diet effects on age-dependent fecundity trajectories across lifespan were tested in general additive mixed models (GAMM) to account for non-linear relationships, with vial fitted as a random effect, and correcting for initial number of females in a vial by including it as a fixed effect. We used a tensor product smooth function of age at measuring fecundity (weekly), and thin plate regression splines. Effects of evolution diet within assay diet were tested by comparing a model fitting separate curves to evolution diet groups, with a model without accounting for evolution diet, using Akaike's Information Criterion (AIC). All models were fitted and predicted trajectories visualized in R package mgcv (33). All analyses were run in the software R, version 3.3.1 or higher (34).

RESULTS

Survival

We report effects of long-term experimental evolution under low, standard, and high yeast adult diets, on survival and reproduction of *D. melanogaster* females that were mated once

every week. In contrast to male flies which were tested previously (24), female survival responded to the experimental evolution regimes. The effect of assay diet on survival rates and mean lifespan was dependent on evolution diet (survival: χ^2= 104.63, df = 4, P < 0.001; lifespan: $F_{4,2941}$ = 21.20, P < 0.001; Figures 1, 2).

Evolution diet regime affected survival and lifespan when tested on LD (survival: χ^2= 110.89, df = 2, P < 0.001; lifespan: χ^2= 131.57, df = 2, P < 0.001) and SD (survival:χ^2= 32.15, df = 2, P < 0.001; lifespan: χ^2= 43.93, df = 2, P < 0.001), but not on HD (survival:χ^2= 0.43, df = 2, P = 0.808; lifespan: χ^2= 0.84, df = 2, P = 0.658). On LD assay diet, SD evolution diet group survival and mean lifespan was higher than that of LD evolution diet (survival: z = 4.34, P < 0.001; Fig 2; mean lifespan: z = −4.76, P < 0.001; Fig 1), and of flies evolved on HD evolution diet (survival: z = 10.57, P < 0.001; Fig 2; mean lifespan: z = −11.78, P <0.001; Fig 1). When tested on LD, flies evolved on SD lived on average 6.5 days longer than flies evolved on LD, and 14.5 days longer than flies evolved on HD (Table S2). On SD assay diet, LD and SD evolution diet group survival and mean lifespan were not different (survival: z = 1.99, P = 0.116; Fig 2; mean lifespan: z = −1.38, P = 0.352; Fig 1), and both higher than that of flies on HD evolution diet (LD vs. HD: survival: z = 3.95, P < 0.001; Fig 2; mean lifespan: z = −5.11, P <0.001; Fig 1; SD vs. HD: survival: z = 5.65, P < 0.001; Fig 2; mean lifespan: z = −6.37, P <0.001; Fig 1).

Our control treatment females (evolution diet SD) showed the classic dietary restriction lifespan extension effect when assayed on low diet, with females on low assay diet living on average 5 days longer than females on standard diet (survival: z = 7.55, P < 0.001; Fig 2; lifespan: z = −3.93, P = 0.003; Fig 1, Table S2). This DR effect was not observed in females evolved on low diet, where no significant difference in lifespan

between standard and restricted assay diet was found ($z = 0.72$, $P = 0.999$; shape of survival curves did marginally not differ: $z = 3.05$, $P < 0.057$; Fig 2), neither in females evolved on high protein diet (lifespan: $z = -0.84$, $P = 0.996$; survival: $z = 3.02$, $P = 0.064$).

All groups showed an exponential increase in hazard rate – a signature of aging (see Table S3; Fig S2). Differences between evolution diet regimes in age-dependent hazard rate occurred when tested on LD, with SD evolution regime flies having the lowest baseline hazard rate, and the highest aging rate, compared to LD and HD evolution regimes (Table S3; Fig S2). When tested on SD, the lower lifespan of HD evolution regime flies was caused by a higher baseline hazard rate, compared to LD and SD evolution regime flies, despite a lower aging rate (Table S3). While the DR lifespan extension effect that was observed only in SD evolution diet flies was based on a decrease in baseline hazard rate, aging rate was decreased and baseline hazard rate increased in LD and HD evolution diet flies tested on LD, compared to when tested on SD (Table S3).

Reproduction

Effects of evolution diet and assay diet on reproduction, but not their interaction were significant (ED: $F_{2,71} = 4.29$, $P = 0.017$; AD: $F_{2,71} = 319.36$, $P < 0.001$; AD × ED: $F_{4,71} = 1.23$, $P = 0.305$), with richer assay diet having a positive effect on fecundity (Fig 3). In separate analyses for each assay diet, the effect of evolution diet was not significant (LD: $F_{2,9} = 1.28$, $P = 0.324$; SD: $F_{2,20} = 1.83$, $P = 0.187$; HD: $F_{2,21} = 2.08$, $P = 0.150$).

Testing age-dependent (vial-based) fecundity trajectories, we found an overall difference between evolution diet regimes when tested on LD ($\Delta AIC = 11.38$; Fig S1) and SD ($\Delta AIC = 15.81$; Fig S1), but not on HD assay diet ($\Delta AIC = 7.73$; Fig S1). Visual

inspection of fitted splines suggest lower early life fecundity of LD evolution flies tested on LD, compared to SD and HD evolution diet flies (Fig S1), lower early life fecundity of SD evolution flies on SD assay diet when compared to LD and HD evolution diet, and no difference due to evolution diet when tested on HD. Analysis of age classes (week 1, weeks 2 and 3, older than 3 weeks (week 4 up); see Methods) showed that ED affected age classed fecundity in females tested on LD diet (age class × ED: $F_{4,73.3} = 2.92$, P = 0.027), but not on SD (age class × ED: $F_{4,32.7} = 2.39$, P = 0.071) and HD assay diet (age class × ED: $F_{4,31.1} = 2.35$, P = 0.077). The effect on LD assay diet was driven by lower initial fecundity of flies evolved on LD (Fig S1), compared to flies evolved on SD (week1: $t_{23} = -3.16$, P = 0.004) and HD (week1: $t_{24.3} = -2.35$, P = 0.027). This supports the visual difference in spline shapes on low evolution diet, but not on standard evolution diet.

Body mass

Female body mass did not differ between evolution diet regimes ($F_{2,2.53} = 5.77$, P = 0.114).

DISCUSSION

The lifespan extending effect of dietary restriction is often explained as an adaptive plastic response, which reallocates energy from reproduction to somatic maintenance to survive temporary periods of food shortage (16). When DR becomes chronic, such strategy becomes maladaptive, and selection is predicted to favour reproduction over somatic maintenance and longevity. In accordance with this prediction, we found decreased lifespan of females that evolved on low diet, compared to females evolved on standard diet, when populations from both evolutionary regimes were tested on low assay diet. However, the evolution of shorter lifespan under low diet was not accompanied by the evolution of increased

246 reproduction, as predicted by the disposable soma hypothesis. On the contrary, early
247 fecundity was reduced in lines that evolved on the low diet and were tested on the low diet,
248 compared to the standard diet.
249
250 We previously tested this prediction in males, using the same experimental evolution
251 lines as in the present study (24). In contrast to females, male reproduction increased when
252 evolving on low protein diet. However, we did not observe a simultaneous decrease in
253 survival, as would be expected from a negative correlation between reproduction and
254 survival. Together, our results from this long-term DR experiment show that while both
255 sexes evolved in response to different dietary regimes, there was no detectable correlated
256 response between reproduction and survival in either sex. The evolutionary response of the
257 sexes to dietary regimes differed considerably, but the lack of genetic correlation between
258 survival and reproduction across populations was, perhaps, one unifying feature. A previous
259 experimental evolution study that manipulated larval diet, instead of adult diet as in the
260 present study, found a negative effect of low nutrient food (restricted in protein and
261 carbohydrates) on adult body mass (35). However, there is no indication that our results
262 were affected by differences in female body mass, since we observed no evolutionary
263 response of body mass in either of our dietary regimes.
264
265 While empirical studies often support a trade-off between reproduction and survival
266 – the so-called cost of reproduction (6, 36, 37) – including in *D. melanogaster* females (5,
267 36, 38), there are many studies in which no trade-off has been detected (reviewed for
268 example in 36, 37). For example, recent studies show that ratios of dietary amino acids can

be manipulated to produce the standard DR lifespan extension, without any reduction in reproductive output (39, 40). This reveals that survival and reproduction can be uncoupled to a substantial extent. In Grandison et al.'s study (39), the level of only one amino acid, methionine, was increased in a DR diet to result in the apparent resolution of a potential trade-off between reproduction and lifespan. Another line of evidence for a substantially decoupled effect of DR on reproduction and survival comes from studies that show a DR-induced increase in lifespan when reproduction is experimentally inhibited (41, 42). It is important to recognize that if no trade-off is detected, there is still a possibility that trade-offs are manifest only with other fitness components, such as immune response, which can have a weak undetectable correlation with fitness under the specific experimental conditions and might not even be measured.

Discussing our previous results in males, we invoked IIS/TOR signalling dependent autophagy (43). This process is upregulated in low dietary resource environments (44), and could be a potential mechanism to explain higher reproduction without lowered survival in males, which has been previously suggested as a general explanation for DR effects on lifespan (45). We hypothesized that a sexually antagonistic effect, for example through the p53 pathway (46) that is involved in regulating autophagy, might explain the positive effect on reproduction in males, trading-off with fitness effects in females. If this would be the case, evolving under chronic DR would be expected to have negative effects in females, presumably in reproductive traits, as a more efficient re-use of internal resources through increased autophagy (organelles and long-lived proteins, 47) might also negatively affect processes related to egg production under DR. A certain level of autophagy and apoptosis, targeted at somatic nurse cells and germline follicle cells that are essential during oocyte

development, is part of the normal process of oogenesis (48). While extreme nutrient depletion increases the level of autophagy in ovaries (49, 50), it is not clear at this stage whether restricted nutrient regimes have a less pronounced but similar effect on egg production. We did not find a strong effect of multigenerational chronic DR on female reproduction: evolving on low diet decreased early female fecundity, with no significant effect on total reproduction. Females evolved under DR had lower survival compared to females evolved on standard diet. Together, these responses can be cautiously interpreted as negative effects of multigenerational chronic DR on females, compared to positive effect on male fitness, and thus putatively support a role for sexual antagonistic genetic variation in the observed qualitative sex differences in response to chronic DR. Genetically based metabolic and physiological constraints that are genotype (female/male) and environment (protein-rich/protein-poor) specific might also constrain the evolution of similar phenotypes in females, compared to males.

When tested on low diet, flies evolved on standard diet had a lower baseline hazard rate and therefore lived longer than flies evolved on low or high diet, as observed in other studies (51-53). Flies evolved on low diet and tested on low diet showed slower actuarial aging, compared to flies evolved on standard diet. It, therefore, seems that evolution under DR not only removes any lifespan extension observed in female flies evolved on standard diet, but is also characterized by an earlier onset of aging. Evolution in a rich resource environment (high diet) resulted in low lifespan when tested on DR, but also when assayed on standard diet. The fact that females evolved on high diet and tested on DR had very low survival, but did not show a simultaneous increase in reproduction also does not support a direct reallocation between reproduction and survival. However, the disposable soma theory

315 is generally not very suitable to explain phenotypes in resource-rich environments, as one of
316 its fundamental assumption is that resources are limited. The negative effect on lifespan
317 caused by evolving on high-protein diet points to a specific loss of plasticity in the ability to
318 adjust lifespan to nutrition and to survive longer when assayed in nutritionally less rich
319 environments.

320 Measuring tradeoffs is always a difficult endeavour, even in the established model species
321 like *D. melanogaster*. We used female fecundity, measured as the number of eggs laid, as
322 our fitness measure. Negative fitness effects could potentially manifest in the quality of the
323 offspring, for example through egg viability, hatching success, and condition of eclosed
324 offspring, which we did not capture in our assay. Another caveat that concerns all
325 experimental evolution and artificial selection studies is the possibility of parental effects
326 through non-genetic transgenerational inheritance. To lower these effects, we allowed one
327 generation of relaxed selection on standard diet, before assessing treatment effects.

328 In summary, our findings do not support the leading hypothesis that lifespan
329 extension under dietary restriction results from the strategic reallocation of resources from
330 reproduction to survival in order to survive a temporary famine. It is possible that dietary
331 restriction is reducing superfluous nutrient-sensing signalling in late-life, as suggested by
332 the hyperfunction theory of aging (54, 55). Future studies should aim to test the whole range
333 of new theoretical approaches to solve the paradox of cost-free lifespan extension.

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335 **Authors' contributions**

FZ designed the study, carried out the lab work, analysed the data, and prepared the manuscript; GG, AV, ME, SRK carried out lab work and prepared the manuscript; UF and AAM designed the study and prepared the manuscript.

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Conflict of interest

None

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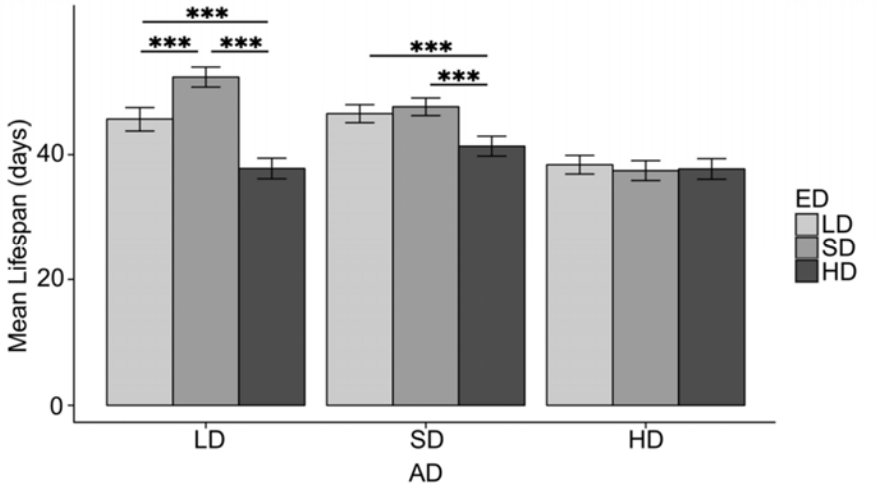

Figure 1. Female fruit fly mean lifespan. Each graph shows mean lifespan for assay diet groups. Error bars show ± 2 S.E. Asterisks indicate the statistical significance of differences between groups: *** ($P<0.001$)

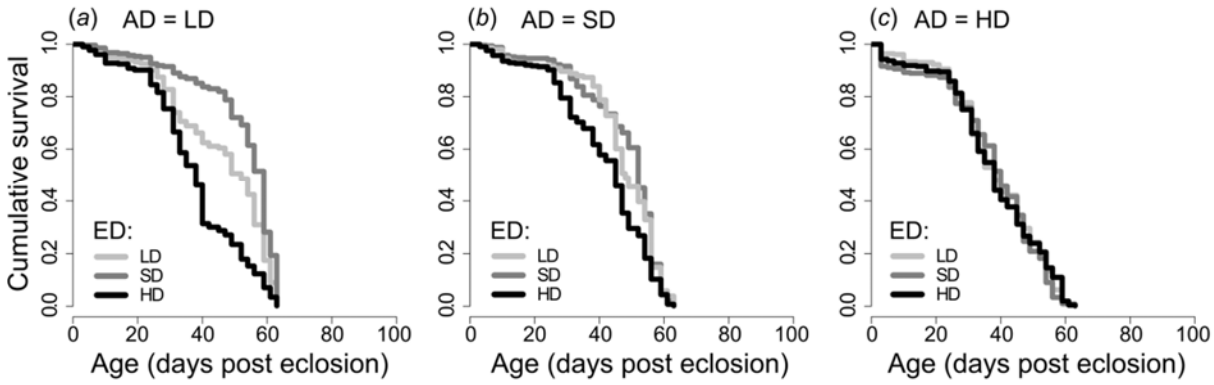

Figure 2. Female fruit fly survivorship. Each panel shows Kaplan-Meier survival curves for assay diet treatment groups. Separate curves depict survivorship of evolution diet populations, tested on different assay diets.

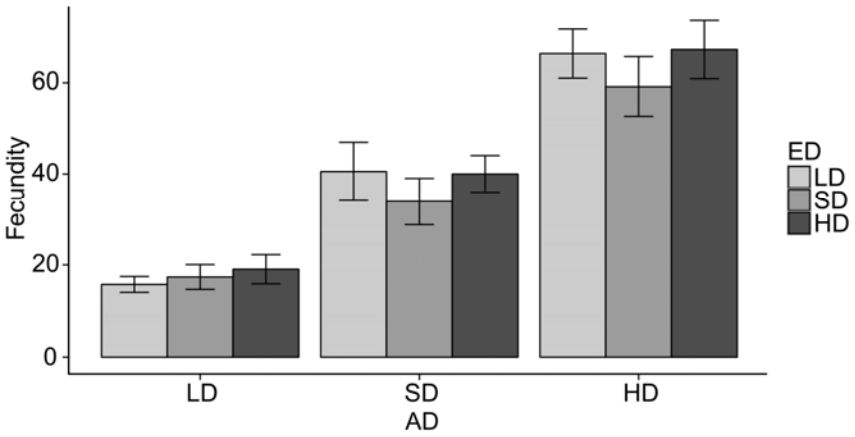

Figure 3. Female fruit fly fecundity, compared between evolution diet populations. Bars show fecundity as total egg numbers (sum of weekly counts, scaled by initial number of flies in each vial), averaged across vials in each treatment. Error bars show ± 2 S.E.