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**Evolution Under Dietary Restriction Decouples Survival From Fecundity in *Drosophila melanogaster* Females**

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### Article

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

5 Felix Zajitschek, PhD,<sup>1,2</sup> Grigorios Georgolopoulos, MSc,<sup>2</sup> Anna Vourlou<sup>2</sup>, Maja Ericsson<sup>2</sup>,  
6 Susanne R.K. Zajitschek, PhD,<sup>1,3,4</sup> Urban Friberg, PhD,<sup>4,5</sup> and Alexei A. Maklakov, PhD<sup>2</sup>

7 1. Department of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia.

8 2. Department of Animal Ecology, Evolutionary Biology Centre, Uppsala University, Uppsala,  
9 752 36, Sweden.

10 3. Doñana Biological Station, EBD-CSIC, Seville, Spain.

11 4. Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University,  
12 Uppsala, 752 36, Sweden.

13 5. IFM Biology, AVIAN Behavioural, Genomics and Physiology Group, Linköping University,  
14 Linköping, 581 83, Sweden.

15 Corresponding author:

16 Felix Zajitschek

17 Email: felix@zajitschek.net

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19

20 **ABSTRACT**

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

38

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

40

## 41 INTRODUCTION

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

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

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

81

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

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

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

95

## 96 **METHODS**

### 97 **Experimental design**

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

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

113 To remove any parental effects from the diet treatments before the start of the experiment,  
114 experimental flies were passed through two generations of common garden. To accomplish this  
115 females from the experimental population cages were allowed to lay eggs in wide plastic vials  
116 (28.5 mm × 95 mm used for all experimental work) with standard diet (SD) overnight. Eggs were  
117 trimmed to 100 eggs per vial, and eclosing adults were allowed to mate for the 2 days after  
118 eclosion before females were allowed to lay eggs in new SD vials for 2 hours. Eggs were again  
119 trimmed to 100 eggs per vial and eclosing adult females were used in assays. Each vial was  
120 populated with around 50 female flies.

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

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

## 131 **Statistical analysis**

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

$$150 \mu_x = b_0 e^{b_1 x}$$

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



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

157 Female reproductive fitness was estimated as the sum of all weekly fecundity measurements of  
158 each population of females in a vial, scaled by the initial number of females in a vial. Total  
159 fecundity was analyzed in linear mixed effects models following the same process as in the  
160 analysis for survival and lifespan, with population cage fitted as a random intercept. To  
161 specifically compare early, mid and late life fecundity, we also tested effects on mean fecundity  
162 in age classes (early life fecundity = fecundity in week 1, mid life fecundity = fecundity in weeks  
163 2 and 3, late life fecundity = fecundity in week 4 and later). Post-hoc tests were conducted using  
164 function `diffsmeans` in R package `lmerTest`. Evolution diet effects on age-dependent fecundity  
165 trajectories across lifespan were tested in general additive mixed models (GAMM) to account for  
166 non-linear relationships, with vial fitted as a random effect, and correcting for initial number of  
167 females in a vial by including it as a fixed effect. We used a tensor product smooth function of  
168 age at measuring fecundity (weekly), and thin plate regression splines. Effects of evolution diet  
169 within assay diet were tested by comparing a model fitting separate curves to evolution diet  
170 groups, with a model without accounting for evolution diet, using Akaike's Information Criterion  
171 (AIC). All models were fitted and predicted trajectories visualized in R package `mgcv` (33). All  
172 analyses were run in the software R, version 3.3.1 or higher (34).

## 173 RESULTS

### 174 Survival

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

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

181 Evolution diet regime affected survival and lifespan when tested on LD (survival:  
182  $\chi^2 = 110.89$ ,  $df = 2$ ,  $P < 0.001$ ; lifespan:  $\chi^2 = 131.57$ ,  $df = 2$ ,  $P < 0.001$ ) and SD (survival:  $\chi^2 =$   
183  $32.15$ ,  $df = 2$ ,  $P < 0.001$ ; lifespan:  $\chi^2 = 43.93$ ,  $df = 2$ ,  $P < 0.001$ ), but not on HD (survival:  $\chi^2 =$   
184  $0.43$ ,  $df = 2$ ,  $P = 0.808$ ; lifespan:  $\chi^2 = 0.84$ ,  $df = 2$ ,  $P = 0.658$ ). On LD assay diet, SD  
185 evolution diet group survival and mean lifespan was higher than that of LD evolution diet  
186 (survival:  $z = 4.34$ ,  $P < 0.001$ ; Fig 2; mean lifespan:  $z = -4.76$ ,  $P < 0.001$ ; Fig 1), and of  
187 flies evolved on HD evolution diet (survival:  $z = 10.57$ ,  $P < 0.001$ ; Fig 2; mean lifespan:  $z =$   
188  $-11.78$ ,  $P < 0.001$ ; Fig 1). When tested on LD, flies evolved on SD lived on average 6.5 days  
189 longer than flies evolved on LD, and 14.5 days longer than flies evolved on HD (Table S2).  
190 On SD assay diet, LD and SD evolution diet group survival and mean lifespan were not  
191 different (survival:  $z = 1.99$ ,  $P = 0.116$ ; Fig 2; mean lifespan:  $z = -1.38$ ,  $P = 0.352$ ; Fig 1),  
192 and both higher than that of flies on HD evolution diet (LD vs. HD: survival:  $z = 3.95$ ,  $P <$   
193  $0.001$ ; Fig 2; mean lifespan:  $z = -5.11$ ,  $P < 0.001$ ; Fig 1; SD vs. HD: survival:  $z = 5.65$ ,  $P <$   
194  $0.001$ ; Fig 2; mean lifespan:  $z = -6.37$ ,  $P < 0.001$ ; Fig 1).

195 Our control treatment females (evolution diet SD) showed the classic dietary  
196 restriction lifespan extension effect when assayed on low diet, with females on low assay  
197 diet living on average 5 days longer than females on standard diet (survival:  $z = 7.55$ ,  $P <$   
198  $0.001$ ; Fig 2; lifespan:  $z = -3.93$ ,  $P = 0.003$ ; Fig 1, Table S2). This DR effect was not  
199 observed in females evolved on low diet, where no significant difference in lifespan

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

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

213

#### 214 Reproduction

215 Effects of evolution diet and assay diet on reproduction, but not their interaction were  
216 significant (ED:  $F_{2,71} = 4.29$ ,  $P = 0.017$ ; AD:  $F_{2,71} = 319.36$ ,  $P < 0.001$ ; AD  $\times$  ED:  $F_{4,71} =$   
217  $1.23$ ,  $P = 0.305$ ), with richer assay diet having a positive effect on fecundity (Fig 3). In  
218 separate analyses for each assay diet, the effect of evolution diet was not significant (LD:  
219  $F_{2,9} = 1.28$ ,  $P = 0.324$ ; SD:  $F_{2,20} = 1.83$ ,  $P = 0.187$ ; HD:  $F_{2,21} = 2.08$ ,  $P = 0.150$ ).

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

223 inspection of fitted splines suggest lower early life fecundity of LD evolution flies tested on  
224 LD, compared to SD and HD evolution diet flies (Fig S1), lower early life fecundity of SD  
225 evolution flies on SD assay diet when compared to LD and HD evolution diet, and no  
226 difference due to evolution diet when tested on HD. Analysis of age classes (week 1, weeks  
227 2 and 3, older than 3 weeks (week 4 up); see Methods) showed that ED affected age classed  
228 fecundity in females tested on LD diet (age class  $\times$  ED:  $F_{4,73.3} = 2.92$ ,  $P = 0.027$ ), but not on  
229 SD (age class  $\times$  ED:  $F_{4,32.7} = 2.39$ ,  $P = 0.071$ ) and HD assay diet (age class  $\times$  ED:  $F_{4,31.1} =$   
230  $2.35$ ,  $P = 0.077$ ). The effect on LD assay diet was driven by lower initial fecundity of flies  
231 evolved on LD (Fig S1), compared to flies evolved on SD (week1:  $t_{23} = -3.16$ ,  $P = 0.004$ )  
232 and HD (week1:  $t_{24.3} = -2.35$ ,  $P = 0.027$ ). This supports the visual difference in spline  
233 shapes on low evolution diet, but not on standard evolution diet.

#### 234 Body mass

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

236

## 237 **DISCUSSION**

238 The lifespan extending effect of dietary restriction is often explained as an adaptive plastic  
239 response, which reallocates energy from reproduction to somatic maintenance to survive  
240 temporary periods of food shortage (16). When DR becomes chronic, such strategy becomes  
241 maladaptive, and selection is predicted to favour reproduction over somatic maintenance  
242 and longevity. In accordance with this prediction, we found decreased lifespan of females  
243 that evolved on low diet, compared to females evolved on standard diet, when populations  
244 from both evolutionary regimes were tested on low assay diet. However, the evolution of  
245 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

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

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

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

305         When tested on low diet, flies evolved on standard diet had a lower baseline hazard  
306 rate and therefore lived longer than flies evolved on low or high diet, as observed in other  
307 studies (51-53). Flies evolved on low diet and tested on low diet showed slower actuarial  
308 aging, compared to flies evolved on standard diet. It, therefore, seems that evolution under  
309 DR not only removes any lifespan extension observed in female flies evolved on standard  
310 diet, but is also characterized by an earlier onset of aging. Evolution in a rich resource  
311 environment (high diet) resulted in low lifespan when tested on DR, but also when assayed  
312 on standard diet. The fact that females evolved on high diet and tested on DR had very low  
313 survival, but did not show a simultaneous increase in reproduction also does not support a  
314 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.

334

335 **Authors' contributions**



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

339

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342 Council grant to UF, and a European Research Council Starting Grant (AGINGSEXDIFF) and  
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344

#### 345 **Conflict of interest**

346 None

347

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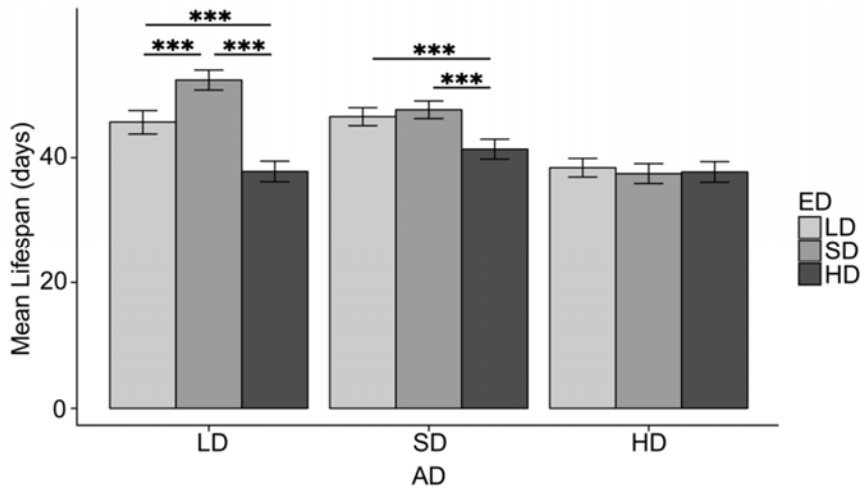
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471 Figure 1. Female fruit fly mean lifespan. Each graph shows mean lifespan for assay diet groups.

472 Error bars show  $\pm 2$  S.E. Asterisks indicate the statistical significance of differences between

473 groups: \*\*\* (P<0.001)

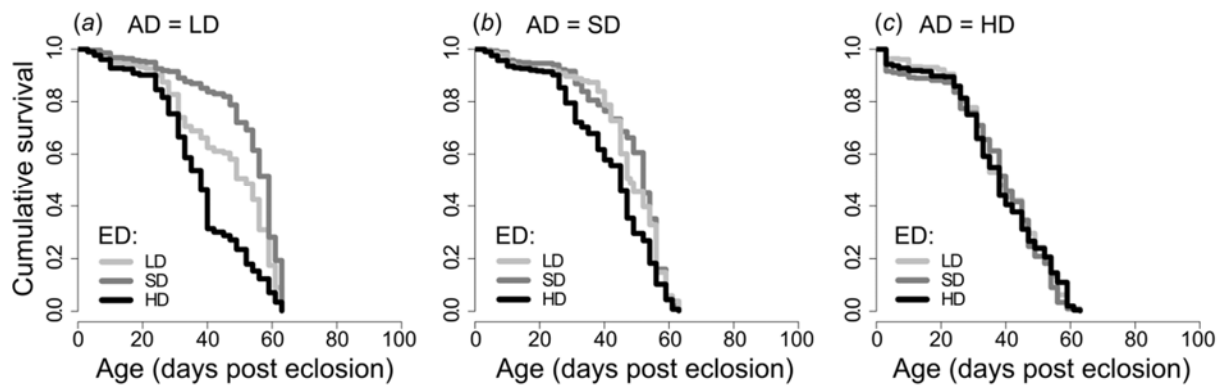
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481 Figure 2. Female fruit fly survivorship. Each panel shows Kaplan-Meier survival curves for

482 assay diet treatment groups. Separate curves depict survivorship of evolution diet populations,

483 tested on different assay diets.

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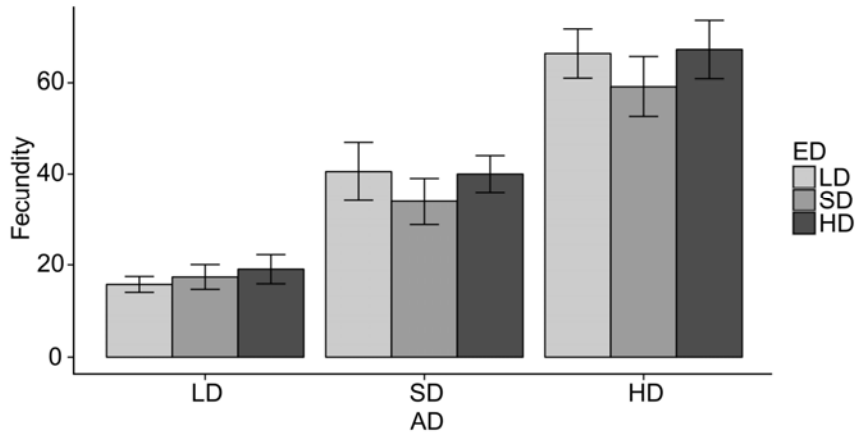
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493 Figure 3. Female fruit fly fecundity, compared between evolution diet populations. Bars show  
494 fecundity as total egg numbers (sum of weekly counts, scaled by initial number of flies in each  
495 vial), averaged across vials in each treatment.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

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