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The plastic fly: the effect of sustained fluctuations in adult food supply on life-history traits

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Abstract

Many adult traits in *Drosophila melanogaster* show phenotypic plasticity, and the effects of diet on traits such as lifespan and reproduction are well explored. Although plasticity in response to food is still present in older flies, it is unknown how sustained environmental variation affects life-history traits. Here, we explore how such life-long fluctuations of food supply affect weight and survival in groups of flies and affect weight, survival and reproduction in individual flies. In both experiments, we kept adults on constant high or low food and compared these to flies that experienced fluctuations of food either once or twice a week. For these 'yoyo' groups, the initial food level and the duration of the dietary variation differed during adulthood, creating four 'yoyo' fly groups. In groups of flies, survival and weight were affected by adult food. However, for individuals, survival and reproduction, but not weight, were affected by adult food, indicating that single and group housing of female flies affects life-history trajectories. Remarkably, both the manner and extent to which life-history traits varied in relation to food depended on whether flies initially experienced high or low food after eclosion. We therefore conclude that the expression of life-history traits in adult life is affected not only by adult plasticity, but also by early adult life experiences. This is an important but often overlooked factor in studies of life-history evolution and may explain variation in life-history experiments.

Introduction

Phenotypic plasticity is the ability of a genotype to express different phenotypes in response to environmental variation (Schlichting & Pagliucci, 1998; West-Eberhard, 2003). Some plastic traits such as wing coloration in butterflies or horn length in beetles are fixed at a specific developmental stage and cannot be changed once the phenotypes have been expressed. Such developmental plasticity may be maladaptive if the environment changes in an unexpected way after a phenotype is fixed (Ghalambor *et al.*, 2007; Reed *et al.*, 2010). Other traits such as metabolism and metabolic rate remain phenotypically plastic, for instance in response to food availability (Karowe & Martin, 1989; Compher *et al.*, 2006; Jobling, 2006).

Many adult traits of the fruit fly *Drosophila melanogaster* are plastic in response to different adult environments. Lifespan has been shown in many studies to vary with food availability and temperature (Miquel *et al.*, 1976; Chippindale *et al.*, 1993; Partridge *et al.*, 1995; Pletcher *et al.*, 2002; Mair *et al.*, 2003; Doroszuk *et al.*, 2012). Amounts of protein and fatty acids and other traits such as reproduction covary with lifespan between different types of food (Lee *et al.*, 2008; Skorupa *et al.*, 2008). When flies are transferred once between different types of food in later stages of adult life, lifespan and reproduction can still be affected (Carey *et al.*, 1998; Mair *et al.*, 2003). Nevertheless, it

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remains unknown to what age and to what extent fruit flies can respond plastically when environments change multiple times in a lifetime, and how variation in early life traits relates to variation in traits later in life.

In this study, we manipulate the environment of adult fruit flies using the nutritional level of food as the main treatment. We compare flies living in constant environments with flies that received fluctuations of food throughout adult life. Four 'yoyo' treatment groups were designed along two variables in a full-factorial design. The first variable relates to the frequency of the nutritional fluctuations; flies were transferred either once or twice a week between high and low food. The second variable constitutes the early life experience; at eclosion, flies either initially received high or low food. In a first experiment, we measured survival and weight of female flies that lived in vials at a density of five individuals (Exp #1). To enable us to follow the response in life-history traits on an individual based level, we repeated the experiment with individually housed flies (Exp #2) and also monitored egg production at every transfer. These experiments were designed to reveal whether sustained fluctuations of food would have an effect on survival and to quantify the degree of plasticity in weight and reproduction in response to food. This study aims to enhance the understanding of how life histories are shaped in a variable environment.

Materials and methods

Food

Three food levels were used in this experiment, indicated by 1× (low), 2× (intermediate) and 5× (high) medium. These food levels vary in amounts of sugar (50, 100 and 250 g L^{-1} in 1×, 2× and 5× medium, respectively) and yeast (35, 70, 175 g L^{-1} in 1×, 2× and 5× medium, respectively). The food contains agar (20 g L^{-1}), nipagine (15 mL of 100 g 4-methyl hydroxy benzoate per litre alcohol) and propionic acid (3 mL L^{-1}).

Flies

Flies (*D. melanogaster*) were wild-caught from six different populations along a transect between Vienna and Athens in the summer of 2008. Once established in the laboratory, they were crossed in a scheme that ensures a balanced contribution of each source population to the newly established outbred population. This latter population was reared in half-pint bottles for 50 generations with at least 300 individuals per generation on 1× medium before the experiments were started. These populations were originally established for the purpose of starting experimental evolution lines, and the choice of keeping them on 1× medium was made earlier and unconnected to the present study. Rather, we used these flies because they were genetically diverse, and therefore, the results are expected to be relatively 'public' and more widely relevant. The experimental media were 1× and 5×, and therefore, in addition, to avoid trans-generational effects on adults, flies were reared for at least three generations on 2× medium prior to the experiment. This means that the flies are possibly adapted to one of the food types (1×) and that the data might be affected by this. Because we did not rear flies under 5× medium, we cannot control for this. If adults clearly perform better for all traits on 1× medium, this might be an effect of the short prior period of evolution in the laboratory to this medium. The larvae were reared in vials with 6 mL of intermediate food, with a density of 50 eggs per vial. After eclosion, the sex of the flies was determined, and unmated female flies were distributed over experimental vials in experiment 1 (Exp #1) using ice as anaesthesia, whereas in experiment 2 (Exp #2), we randomly put flies in either a low food vial (6 mL of food throughout the experiment) or a high food vial (6 mL of food throughout the experiment) without using anaesthesia.

The singly housed flies were all checked for mating and possible fertilized eggs in the first 3 days, and fertilized females were removed from the experiments. All reported results in this study come thus from *virgin female* flies. We used virgins because fecundity in once-mated flies is strongly affected by sperm depletion during the first weeks of life. Life history of females (lifespan and fecundity) is affected by mating frequency, and this additional component of variation is also avoided in our study using virgin females.

Adult food treatment

In both Exp #1 and Exp #2, six food treatments were used. We compared flies living in constant environments of high (CH) and low food (CL) with flies that received fluctuations of food throughout adult life ('yoyo' treatment). These latter flies also received different treatments with groups that were transferred either once a week (slow yoyo) or twice a week (fast yoyo) between high and low food. Furthermore, we controlled for the first adult food vial experienced by separating both the slow and fast yoyo cohorts between flies that were initially on high food or low food. This resulted in four different yoyo fly groups: slow yoyo, high start (SYH); slow yoyo, low start (SYL); fast yoyo, high start (FYH); and fast yoyo, low start (FYL).

All flies from different treatments were transferred on the same day, even if nutrient levels did not change. Furthermore, the vial transfers were performed in such a way that, in total, the flies of the slow and fast yoyo groups fed for similar number of days on low or high food (namely always 7 days on low and 7 days on high medium per 2 weeks). In Exp #1, flies were kept in

densities of five flies per vial. Flies were redistributed between vials when flies had died so that the density remained five for most vials. In Exp #2, flies were kept individually. In Exp #1, we started with 25 vials of flies that we weighed (125 individuals per food treatment), and a similar number of flies that were not weighed. In Exp #2, we started with 65 individuals per food treatment.

Trait measurements

In both experiments, flies were weighed before transfer. Weight was measured to the nearest 0.01 mg (Sartorius). Survival was checked daily and escaped or accidentally crushed flies on vial transfer were right-censored in the analyses. In Exp #1, a control group of flies was not weighed to examine the effect of anaesthesia on survival. In Exp #2, we counted the number of eggs in every vial after flies were transferred.

Statistics

The program R was used for all statistics (R Development Core Team, 2011). We used chi-square tests to determine heterogeneous survival within the first 4 days of Exp #1. For other survival analyses, we fitted a Cox proportional hazard test (Cox, 1972). For weight measurements in Exp #1, we fitted an ANOVA model with age (as a polynomial covariate), food level (high or low), yoyo treatment (constant, slow, fast), initial food (high or low) and possible interactions. For Exp #2, we included individual as a random effect, therefore fitting a repeated measures ANOVA with a similar model to Exp #1. In both experiments residuals fitted well with a normal distribution and variances were not unequal, and we thus fitted the data using a Gaussian error distribution. For weight data, we simplified the inference by performing type II Wald test implemented in the car package (Fox & Weisberg, 2011). With egg production, we started with a generalized linear model (GLM) with similar factor as with weight, but with a Poisson error distribution. Because egg production showed a complex relationship with age, we fitted several GLM models, differing in the exponent used for the polynomial relationship between age and egg production, using Akaike's information criterion (AIC) to identify the best model. The analysis was continued including individual as a random effect [generalized linear mixed model (GLMM)], but this still lead to a polynomial with high exponent number and, therefore, many terms. We then fitted a generalized additive model (GAM) that uses smoothing functions (Zuur *et al.*, 2009). Because the fit and residual variation (mean and variances) were not equal, a negative binomial error distribution fitted the data better than a Poisson distribution. We used the mgcv package in R that automatically fits a smoothing function without a user-biased degree of smoothing. It does so by penalized regression splines which maximize the explained variance taking into account the smoothness, and where a penalty of a narrower window is applied to less smoothing. The advantage is that users do not choose a specific degree of smoothness, but the smoothness is determined by an objective algorithm, and given that data are similar, fits should be similar for different users (Wood, 2006). For pairwise testing of differences in weight and number of eggs between short- and long-lived cohorts of flies, *t*-tests were used. The relationship between weight and egg number was performed using an ANOVA and GLM with age and food as factors using a Gaussian and Poisson error distribution, respectively.

Results

Experiment 1: five flies per vial

Survival

A higher proportion of flies that were weighed died in the first 4 days of the experiment, whereas this did not happen for the group of flies that were not weighed (256 of the 609, 42.0% of the weighed flies, 61 of the 638, 9.6% of the unweighed flies, $\chi^2_{\text{d.f.}=1} = 173.32$, $P < 0.001$, see Fig. S1, Table S1). We tested whether the number of deaths was distributed heterogeneously over the food treatment groups. This was not the case ($\chi^2_{\text{d.f.}=5} = 2.42$, $P = 0.79$ for unweighed flies, $\chi^2_{\text{d.f.}=5} = 7.25$, $P = 0.20$ for weighed flies), and therefore, the analysis was conducted by removing the data from the first 4 days to improve the fit of the Cox proportional hazard tests. The survival analysis using food treatment and weighing treatment as explanatory variables indicated that the two-way interaction between food and weighing, and weighing as a main effect were not significant ($Z = 0.956$, $P = 0.34$, for the latter). The survival curves (Fig. 1) and hazard ratios per term (Table 1) indicate that the survival of the CL flies is significantly lower than all flies in all other treatments. Although the slow yoyo flies that started high did not have a higher survival compared to the constant high flies, they did have an improved survival compared to all the other groups (Table 1). All other groups of flies, besides the CL flies, were not significantly different in survival compared to the CH flies. Therefore, flies that received sustained fluctuations had an intermediate survival, but significantly higher than the constant low flies.

Weight

In this experiment, we weighed all individuals in groups of flies from one vial before they were transferred to a new vial. Because we redistributed the flies to maintain the number of flies per vial as close as possible to 5, we could not perform a statistical analysis with individual or vial number as a random variable

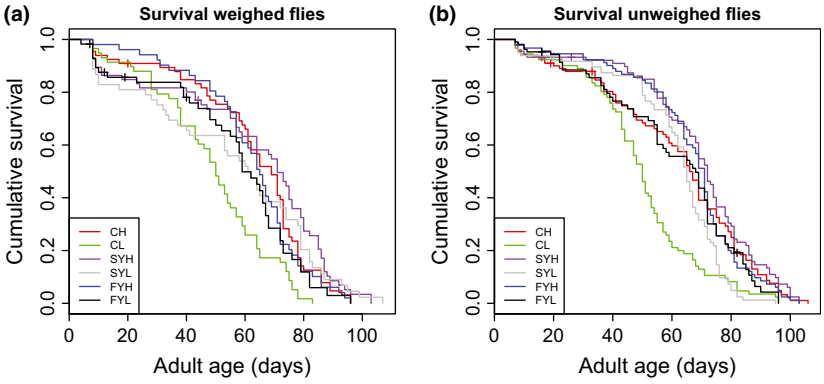

Fig. 1 (a) Survival of flies weighed with a lifespan longer than 4 days and (b) survival of flies not weighed with a lifespan longer than 4 days. Food treatments are indicated by lines with different colours.

Table 1 Statstics of the survival analysis of experiment 1.

Comparison	Hazard ratio	Con. Int. h. r.*	*Z* test statistic	*P* value
CL vs. CH	2.155	1.715–2.708	6.59	< 0.001
SYH vs. CH	0.800	0.637–1.004	−1.93	0.0539
SYL vs. CH	1.227	0.974–1.547	1.74	0.0822
FYH vs. CH	1.025	0.815–1.288	0.21	0.8349
FYL vs. CH	1.187	0.930–1.516	1.38	0.1683
Weighing	1.105	0.961–1.271	1.40	0.1680

CH, constant environments of high food; CL, constant environments of low food; FYH, fast yoyo, high start; FYL, fast yoyo, low start; SYH, slow yoyo, high start; SYL, slow yoyo, low start.
*95% confidence interval hazard ratio.

(e.g. repeated measures ANOVA). However, there remained a considerable number of measurements taken for flies in a vial with 1, 2, 3, 4 or 6 individuals, which allowed us to include number of flies in a vial in the statistical model. We only tested for treatment effects on weight until measurement 23 (84 days), because the number of replicate vials then fell below 5 for some treatments. A polynomial linear model was fitted because the effect of age was not linear with respect to weight. In the model, the effect of yoyo mode (constant, slow yoyo, fast yoyo) was separated from the nutritional value of the food in the first vial after eclosion. These two are fitted as a crossed design, together with food level, time (polynomial), and number of flies in a vial.

The food effect on weight of flies in the different food treatments is shown in Fig. 2. Food level ($F_{1,1652}$ = 228.03.14, $P < 0.0001$) was highly significant, whereas the effect of yoyo treatment less so ($F_{2,1652} = 4.17$, $P = 0.016$); flies were heavier when they were on high food. Interestingly, flies that began adult life on high food were on average heavier ($F_{1,1652} = 101.07$, $P < 0.0001$), but also maintained higher weights throughout life ($F_{1,1652} = 46.21$, $P < 0.0001$). Age of the flies had a large effect on weight ($F_{1,1652} = 381.28$, $P < 0.0001$, $F_{1,1652} = 196.23$, $P < 0.0001$, for terms with exponent of 1 and 2, respectively). The interaction of age and food level in the initial vial significantly affected weight ($F_{1,1652} = 46.21$, $P < 0.0001$), but also the three-way interactions with yoyo treatment ($F_{1,1652} = 9.41$, $P < 0.0001$), and to a smaller degree the number of flies ($F_{1,1652} = 2.054$, $P = 0.0252$). Unexpectedly, the number of flies in a vial as a main effect was also significant ($F_{5,1652} = 2.497$, $P = 0.029$). Lastly, the interaction between age and yoyo treatment was significant ($F_{2,1652} = 6.020$, $P = 0.0025$).

The effect of initial vial could be largely dependent on the effect of the constant lines, where the initial vial is similar to the food level throughout life. Therefore, a similar analysis was performed but only for the slow and fast yoyo lines. Both these models confirm that age, food, initial vial, and the interaction between age and initial food vial are significantly affecting weight. Therefore, the effect of initial vial was not due to the effect of the constant lines and also present when only data were taken from either the slow or either the fast yoyo lines.

Figure 2 suggests that the effect of food for the slow yoyo lines differs depending on whether flies are moved from low to high food or from a high to low food vial. To study this further, we assigned the weight on the first high food vial as period H1, the second as period H2, the first on low food as period L1, and the second as period L2. The effect of this can then be tested for both the high and low slow yoyo lines, although they are never on the same food at the same time. Figure 3 shows, and Table S2 lists, the average and standard errors per line, per period for the first 16 measurements. The flies from the SYH treatment lost weight between the high and low food vial ($t_{99.69} = 3.84$, $P < 0.001$), but then gained weight again between the low and high food vial ($t_{122.37} = -6.32$, $P < 0.001$, Fig. 3). In contrast, SYL treatment flies lost weight during the low food period ($t_{109.66} = 2.88$, $P < 0.005$), between the first and second low food vial, and then gained weight between the low and high food vials ($t_{94.68} = -2.75$, < 0.005). Remarkably, the difference in how food affects weight between SYH and

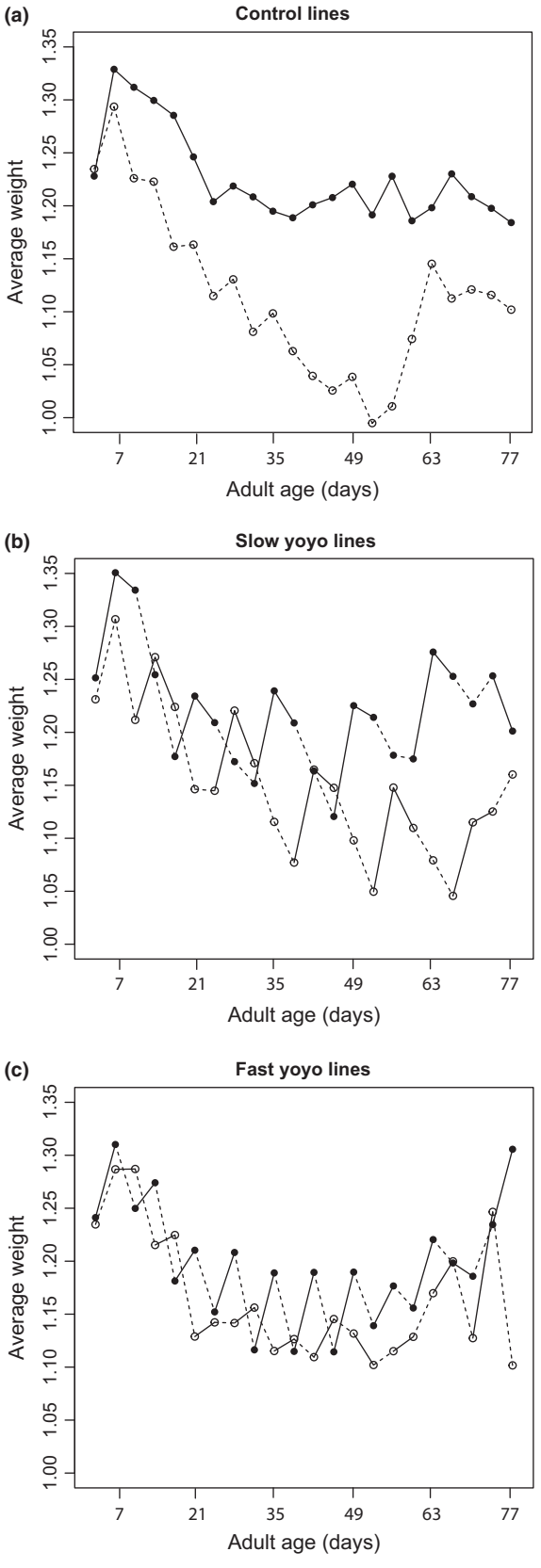

Fig. 2 Average weight of groups of flies for constant treatments (a), slow yoyo treatment (b) and fast yoyo treatment flies (c). Filled points indicate the flies that started high, open points those that started low. Dashed lines connect two consecutive data points with low food, solid lines with high food.

SYL flies is only caused by the food level in the first week of adult life.

Experiment 2: one fly per vial

In Exp #2, we monitored the dynamics of adult weight using single virgin female flies, in addition to counting the number of eggs laid. Because there was a large effect of the weighing treatment in Exp #1 (probably due to the use of anaesthesia during sexing of the flies), we distributed flies in vials without sedating them in Exp #2.

Survival

The hazard ratio for mortality was the highest for the CL flies, whereas it was the lowest for the CH flies (Table 2, Fig. 4; $Z = 5.62$, $P < 0.001$). The fast yoyo treatment flies tended to have a lower hazard ratio compared to the slow yoyo treatment, which was significant when the FYL flies were compared to the SYL flies (Table 2, $Z = -2.55$, $P < 0.05$). The FYH ($Z = 2.15$, $P < 0.05$) and SYH flies ($Z = 2.678$, $P < 0.01$) had significantly lower survival rates compared to the CH, but significantly higher than the CL. Thus, these flies had a significant and intermediate survival compared to the controls, whereas those started on low food were only significantly different compared to one of the controls (Table 2). These results are in line with the intermediate survival rates for 'yoyo' flies in Exp #1, including the higher resemblance to the CH flies.

Weight

In contrast to Exp #1, the weight of the individuals was not affected by food ($\chi^2_{\text{d.f.}=1} = 0.24$, $P = 0.62$) although flies with initial high food were lighter ($\chi^2_{\text{d.f.}=1} = 7.04$, $P < 0.01$) and lost weight faster ($\chi^2_{\text{d.f.}=1} = 9.52$, $P < 0.005$). In general, flies lost weight with age ($\chi^2_{\text{d.f.}=1} = 773.21$, $P < 0.0001$). Lastly, the interaction between food level and initial food was significant ($\chi^2_{\text{d.f.}=1} = 6.53$, $P < 0.05$). Flies that began life on low food were heavier on low food, whereas flies that began life on high food were heavier on high food. To test whether the large effect of initial food level was due to the constant food level treatments, we inspected similar statistical models per yoyo treatment. In the separate data sets, age was significant in all three yoyo treatments, and only the interaction between age and food in the constant food treatment and the interaction between age and initial food in the slow yoyo treatment were

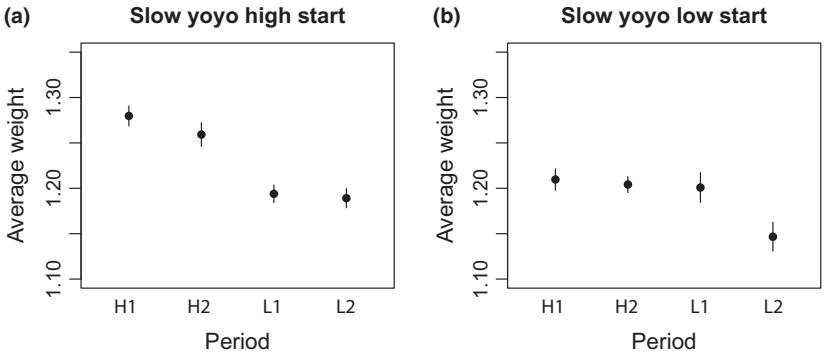

Fig. 3 Average weight per period, as explained in the text, for the slow yoyo line started on high food (a) and on low food (b). Error bars indicates 95% confidence intervals of the mean. The *x* axis gives the period where H1 and H2 are the first and second high food vial, and L1 and L2 are the first and second low food vial. Please note that as these are the slow yoyo lines, the slow yoyo, high start (SYH) lines first experienced two periods high food (H1 & H2) and then two periods low food (L1 & L2), whereas the slow yoyo, low start (SYL) first experienced two low food periods (L1 & L2) and thereafter two high food periods (H1 & H2).

Table 2 Statstics of the survival analysis of experiment 2.

Comparison	Hazard ratio	Con. Int. h. r.*	*Z* test statistic	*P* value
CL vs. CH	2.9543	2.024–4.311	5.617	< 0.0001
SYH vs. CH	1.6525	1.143–2.390	2.668	< 0.01
SYL vs. CH	2.0975	1.435–3.065	3.828	< 0.001
FYH vs. CH	1.4956	1.037–2.158	2.151	< 0.05
FYL vs. CH	1.3053	0.905–1.882	1.427	0.15

CH, constant environments of high food; CL, constant environments of low food; FYH, fast yoyo, high start; FYL, fast yoyo, low start; SYH, slow yoyo, high start; SYL, slow yoyo, low start
*95% confidence interval hazard ratio.

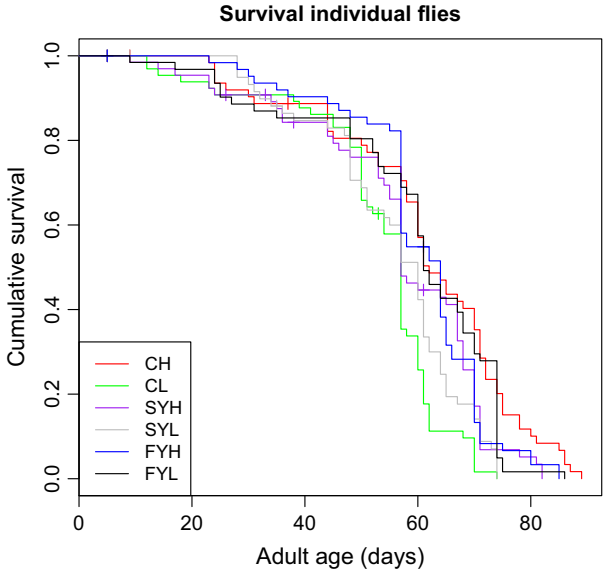

Fig. 4 Survival curves for individual flies for the six food treatments, indicated by lines with different colours.

significant. Therefore, the weight of flies was affected by age and initial food level, which reaches very high significant levels when all the data are pooled.

In Exp #1, the effect of food on weight was dependent both on the type of food and on how long a fly remained on the food. In Exp #2, weight is similar between the first and second time on high food for both the SYH and SYL flies (Fig. 5, Table S3). The SYH flies lost weight after transfer to the first low food vial and then gained weight again. The SYL flies have higher weights than the SYH flies in period 1, but lost weight in the second low vial. This difference in the first and second low food vial features is paralleled by the virgin (unfertilized) egg production data, although on average the number of eggs is higher on low food for both types of slow yoyo treatment flies (Fig. 5). Again, as in Exp #1, the variation of weight (and now also the number of eggs) is both dependent on current food, the time flies spent on a specific food, and on whether they began adult life on high or low food. In contrast, the actual effect of food and time on weight differs between Exp #1 and #2 (compare Fig. 3 with Fig. 5).

Egg production

In Exp #2, we also measured the egg production for each female at every transfer. A visual inspection of the data clearly indicates that the relationship between age and number of eggs is not linear (Fig. 6). Therefore, we first tested what the best fit was for the data using a polynomial model with Poisson errors. This was first performed with a GLM (therefore without individual as a random factor). Using AIC as test for improvement of the model, a polynomial model with terms with an exponent of 15 was the best fit, including all (and significant) two-way interactions between age, food, yoyo and start treatments. A GLMM (therefore including

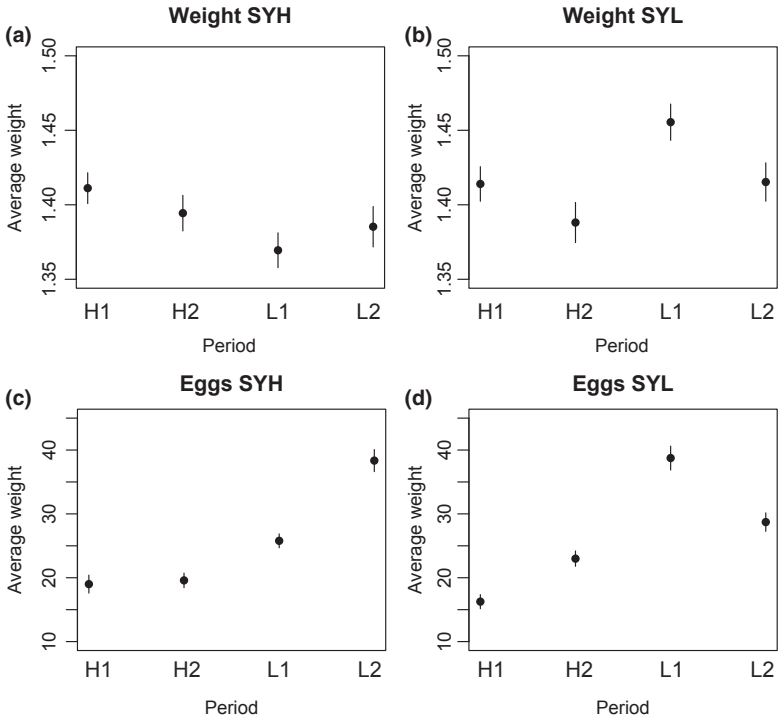

Fig. 5 Average weight (a, b) and number of eggs (c, d) for two food treatments [slow yoyo, high start (SYH), slow yoyo, low start (SYL)]. Error bars indicate 95% confidence interval from a normal distribution with the average trait value as mean. Please note that as these are the slow yoyo lines, the SYH lines first experienced two periods high food (H1 & H2) and then two periods low food (L1 & L2), whereas the SYL first experienced two low food periods (L1 & L2) and thereafter two high food periods (H1 & H2).

individual as random effect) verified that a polynomial model of age with high exponent number was the most significant, whereas the AIC was already lower for a linear model with individuals as random effect compared to the polynomial with exponent 15 without individual as random effect. Further verification of the interaction was performed by fitting a GAM, which uses smoothing functions over a covariate rather than terms for polynomial functions. The best model was one with specific smoothers for every separate food level in every food treatment for the yoyo groups and start treatment for the constant groups, indicating that flies respond differently to food dependent on yoyo treatment and initial vial food level (Table S4). This is the outcome of three separate different statistical models and therefore is perceived to be a robust outcome of the analysis. Therefore, egg number was affected by food level, yoyo treatment, initial adult food level treatment and age. In addition, how flies responded to food was dependent on age, yoyo treatment and initial food treatment (i.e. their interactions). For instance, although on low food the yoyo flies always produced more eggs on average, the difference between egg number on low and high food on consecutive time points is larger in slow yoyo flies compared to fast yoyo flies, and larger for flies that started on low food (for SYL; 27.19, SYH; 25.78, FYL; 16.41, FYH; 13.69 eggs more on low food). Furthermore, as flies get older, they first increase and decrease in plasticity (Fig. 6). Lastly, the improvement of explanatory variation from a GLM to a GLMM indicates that there is substantial variation among individuals. The average number of eggs per individual on both the high and the low food varies between individuals, resulting in more eggs on low food for most, but not all individuals (Fig. S2).

How do the different life-history traits relate?

Weight loss per time step is significantly related to number of eggs ($F_{1,3293} = 243.42$, $P < 0.001$, Fig. S3) they produced in the same time period. This indicates that when flies laid more eggs per time step, they also lost more weight. This effect is much stronger when flies are on high food ($F_{1,3293} = 57.00$, $P < 0.001$; comparable results when tested per food treatment). When a fly gained 0.1 mg per time step, it would on average produce three eggs less, whereas on low food this would be four eggs. This is in addition to the overall negative effect of high food on egg number. In the models, we also took into account age itself as this significantly affected the number of eggs ($F_{9,3293} = 84.64$, $P < 0.001$). This was true for both a linear model with a normal error distribution, as well as for a GLM, with a Poisson error distribution (Fig. S3).

We further investigated the relationship between both the number of eggs, weight, and lifespan by separating the flies into short- and long-lived individuals using median lifespan (Figs S4 and S5). For the two constant food treatment flies, egg production is higher for relatively short-lived individuals early in life, whereas egg

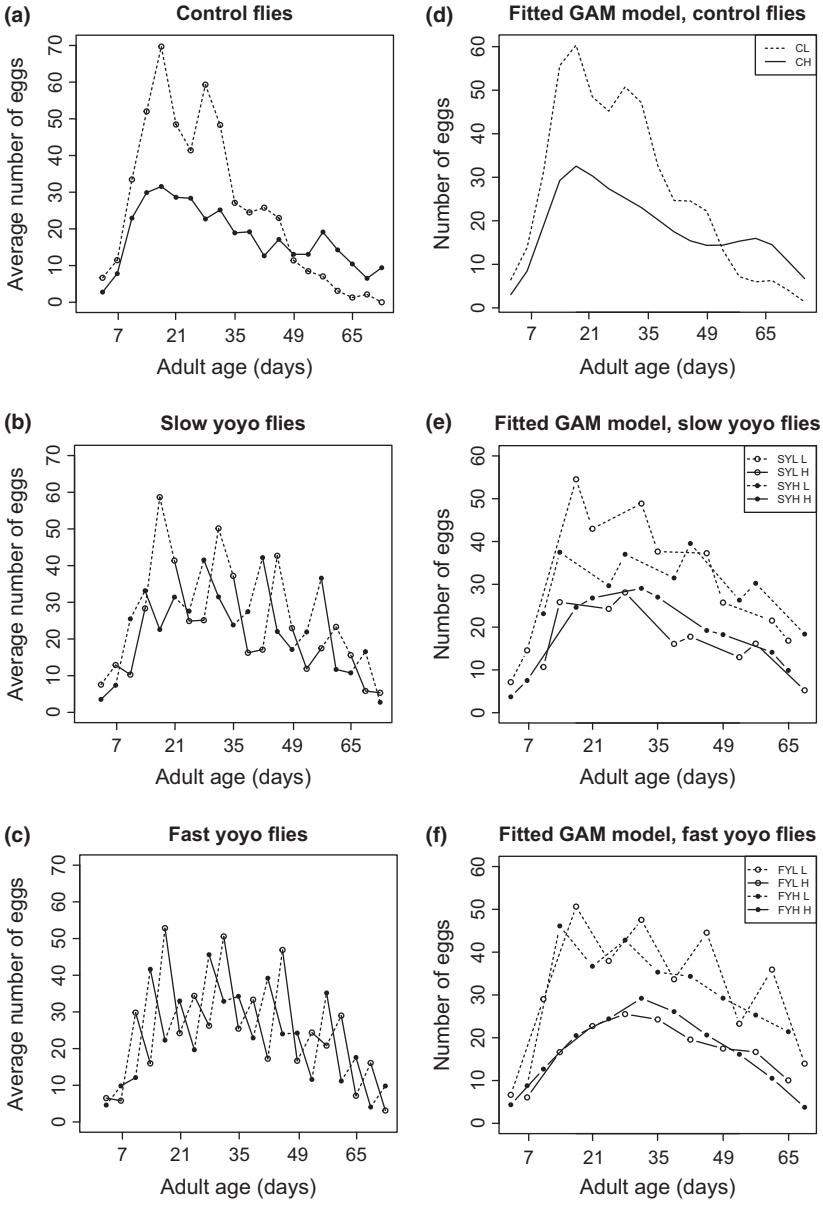

Fig. 6 The average number of eggs per food treatment shown for the two control fly cohorts (a), the slow yoyo flies (b) and the fast yoyo flies (c). In the left column (a–c), filled points indicate the flies that began life on high food, open points the flies that began on low. Dashed lines connect two consecutive data points with low food, solid lines indicate with high food. In the right column, the fitted statistical model is given for the control flies (d), the slow yoyo flies (e) and the fast yoyo flies (f). Here, solid lines indicate fitted smoothers on high food, whereas the dashed lines indicate the fitted smoothers on low food. For the yoyo fly panels (e and f), fitted smoothers are indicated for flies that started on low food by open circles, whereas closed circles indicate flies started on high food.

production is lower later in life. This was significant when tested pairwise at several ages, but also in general the interaction between time and cohort was significant in a full model. The relationship between time-specific egg production and lifespan was less clear for the yoyo treatment flies. Weight was significantly lower for flies that were short-lived, especially in both the slow yoyo and the high fast yoyo flies. In the pairwise tests, few points were significant due to extensive variation in weight. In a full mixed model with age, food treatment and lifespan cohort, correcting for multiple testing within individuals, cohort had a significant effect on weight (fast yoyo: $\chi^2_{\text{d.f.}=1} = 4.18$, $P < 0.05$, slow yoyo: $\chi^2_{\text{d.f.}=1} = 9.09$, $P < 0.005$).

Pooled survival

Finally, we pooled the survival data from the two experiments (Fig. 7). We tested for food treatment effect (six levels) and experiment effect (three levels) where the levels were five individuals unweighed, five individuals weighed and one individual (Exp #2, all weighed). The interaction between these two factors was also examined. The interaction was significant ($\chi^2_{\text{d.f.}=10} = 19.495$, $P < 0.05$), but only marginally so compared to the effect of treatment ($\chi^2_{\text{d.f.}=5} = 88.790$, $P < 0.001$) and experiment ($\chi^2_{\text{d.f.}=2} = 44.100$, $P < 0.001$, see also Fig. 7). The interaction was due to the SYH treatment flies having a higher survival in the experiment with individual flies. The large effect of experiment

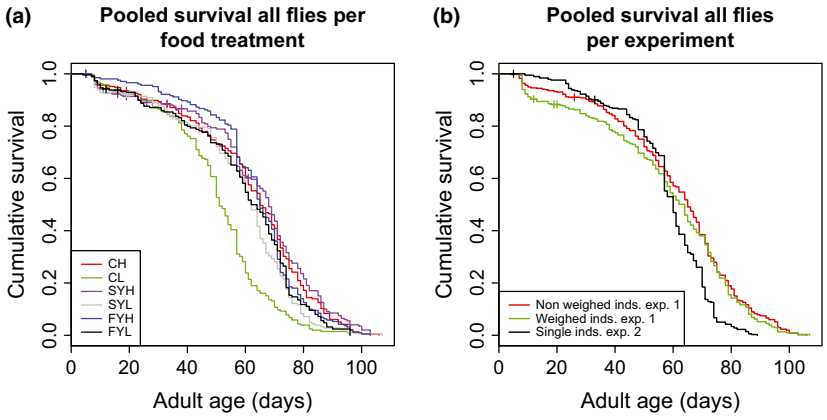

Fig. 7 Survival curves for all pooled flies of Exp #1 and #2 in (a) the effect of food in all the experiments; and in (b) the survival curve per experiment, separated for weighed and unweighed flies in experiment 1. The individuals that died in the first week were omitted.

was caused by a considerably lower survival of the individual flies compared to that of the SYH flies when kept in groups. The effect of treatment was mainly the effect of the CL treatment flies with a much lower survival and that of the SYL treatment flies with a marginally lower survival. In this analysis, the flies that died in the first week were excluded.

Discussion

Integrating of results: some general observations

We examined whether adult flies kept on food that varied over time differed in life-history traits from those maintained on constant food. Figure 8 gives an overview of the effects found of variation in food level on the measured traits. Survival of flies on sustained varying food was not lower than that of controls. The former showed an intermediate survival and the control flies on low food had a decreased survival compared to those on several other food treatments. This suggests that there is little, if any, cost in being variable in weight (Exp #1) or in the number of eggs produced (Exp #2). Strikingly, the lifespan was very similar across experiments when food treatments were compared. Most interestingly, in addition to evidence of adult plasticity, there was also a large effect on life-history traits throughout life of the initial food level experienced by a fly after eclosion. A similar effect of early adult experience was shown by Pearl *et al.* (1927) where flies were kept in bottles with various densities which affected lifespan. For instance, when a fly was transferred from a bottle in which the density was 35 flies to one of 200 at the 16th day of age, they lived longer than flies that lived under a density of 200 throughout life (Pearl *et al.*, 1927). Our study on nutrition and Pearl *et al.*'s study of the effect of density, demonstrate the importance of early adult life experience.

Weight was affected differently by food in the two experiments. Flies on high food had a higher weight in Exp #1. This was true when control flies were compared, but also when the flies on variable food were transferred from low food to high food. This was not, however, repeated in Exp #2. Rather, weight was higher, on average, for CL compared to CH flies. Weight was also higher for all yoyo treatment flies when on low food (except for the SYH). Although the food effect was not significant in Exp #2, the trend was in the opposite direction to Exp #1, indicating that food had a different effect on weight in the two experiments.

Egg production was much higher on low food in Exp #2, whereas flies typically produce more eggs on high food (Lee *et al.*, 2008; Skorupa *et al.*, 2008), although these were mated. Furthermore, gene expression studies of flies kept on high food indicate higher reproductive rates (Pletcher *et al.*, 2002; Doroszuk *et al.*, 2012). Other studies show that weight and reproduction are correlated and higher on high food levels (Morris *et al.*, 2012). In our Exp #2, weight and reproduction are also correlated between food levels, but increased at low food. Furthermore, our FYH and FYL flies tend to be heavier, produce more eggs and have also been shown to up-regulate genes associated with reproduction, for example, gene associated with female gamete production and chorion structure genes (J. van den Heuvel, J. Zandveld, M. Mulder, A. Doroszuk, P. M. Brakefield, T. B. L. Kirkwood, D. P. Shanley & B. J. Zwaan, unpublished data). We therefore suggest that it is likely that the flies on high food in Exp #1 also produced more eggs. This would mean that not only weight, but also reproduction is affected in a different way by food in Exp #1 and #2. In general reproduction can be differentially regulated by the environment, which is matched by the expression of reproduction-related genes.

Methodological reasons for differences between Exp #1 and #2

Our two experiments differed in how flies were treated. In Exp #1, a large proportion of the flies died in the group that was weighed. Therefore, in Exp #2, we did not sedate them during the distribution of flies to vials.

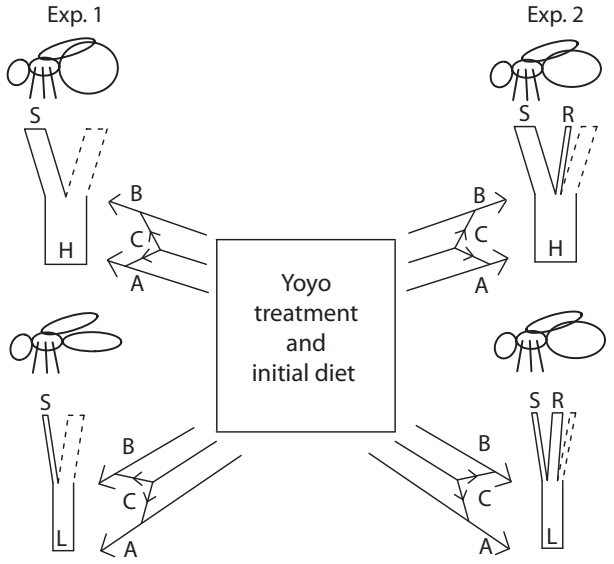

Fig. 8 A schematic overview for the outcome of the two experiments. High and low food treatments are indicated by the H and L at the stem of the 'Y'. We quantified the life-history traits survival (S) and virgin egg production (R) which are indications of how the acquired resources are allocated (by the width of the stem). In experiment 1 and 2, survival is higher at high food represented here by a broader branch towards survival. In Exp #1, no other target for allocation of resource was quantified; we represent any other resource allocation by the dashed branch. The other trait measured, weight, was higher at higher food, indicated by the 'fat' fly at high food and the 'slender' fly at low food. In Exp #2, we also quantified the number of eggs: at high food, allocation to survival was high but to egg production low, whereas the reverse was true for low food. In Exp #2, a smaller amount of the acquired resource has an unknown allocation (dashed branch). Weight was equal between high and low food in Exp #2 shown by the equal flies. In both experiments, the general scenario of differential allocation holds, but the detailed relationships between acquisition and allocation of resource varies with yoyo treatment and especially initial food level experienced in the early adult life of a fruit fly. Yoyo treatment and initial food level could have affected the details of the outcome in three ways, namely (A) by variation in acquisition, (B) by variation in allocation and (C) by a combination of acquisition and allocation. Lastly, there are differences mainly in weight between experiments.

Furthermore, we did not measure egg production in Exp #1 which was increased on low food in Exp #2. Although we repeated Exp #2 and similar differences between food levels were found in egg number, we did not repeat Exp #1, using five flies per vial to count the eggs. Our conclusion that flies are very plastic in response to food and that these responses are in a largely determined by yoyo treatment and initial food level remain, with or without the addition of eggs number in Exp #1, as proof of the involvement of these factors have been found in both experiments.

The effects of living in a group

Although the differences between the experimental outcomes might have been caused by variation in treatments, there could be other, more biological explanations, such as an increased feeding rate of flies when kept at higher densities (Wong *et al.*, 2009). It is known that an increase of sugar and yeast has interactive effects on life-history outcome (Grandison *et al.*, 2009). In other species of fruit flies, it has been shown that the effect of feeding rate on reproduction and lifespan interacts with level of carbohydrate and protein content of food (Fanson *et al.*, 2009, 2012). In our experiment, a difference in feeding rate between Exp #1 and Exp #2 might have led to a change in the relationship between high and low food and the measured life-history traits (see also Fig. 8). In Exp #2, flies on high food produced fewer eggs and tended to have lower weights. If we had only considered these two traits, we might have concluded that more acquisition (i.e. high food) leads to lower resource output (egg and weight), which is opposite of that expected from the difference in nutritional value of the food. According to the Y model, relationships between traits are the composite effect of both variation in acquisition and allocation of resource (Van Noordwijk & De Jong, 1986). Because survival was higher in the flies on high food (when control flies are considered), the Y model is sufficient to explain the variation in life-history traits in Exp #2, where flies on high food might have allocated more resource to maintenance and repair and therefore have the potential to live longer. Hypothetically, they could then have allocated less resource to weight gain and egg production, and therefore, flies on high food are both lighter and lay fewer eggs, while increasing survival. Although the Y model can be extended to contain more loci underlying the variation in traits (De Jong & Van Noordwijk, 1992), it is also important to consider the physiology of more than two traits (Calow & Townsend, 1981; Calow, 1987; Sibly & Calow, 1987; Boggs, 2009).

Furthermore, a particular prediction of the Y model hypothesis is that individuals that have a higher acquisition of resource might show less negative relationships between life-history traits compared to those acquiring fewer resources. A more negative relationship between weight gain and egg production for individuals on high food was found in Exp #2. Similar patterns have been found in *Daphnia*, where on higher food levels, relationships between survival and egg rate have been found to be more negative (Olijnyk & Nelson, 2013). Because it is not clear how much resource any particular trait costs to develop, it is uncertain how relationships between multiple traits play out, even more so when acquisition is varied. In our experiment, the more negative relationship between weight gain and egg production on high food can be explained by the Y model if the increase in egg production were

more costly because of higher allocation to survival on high food. However, it remains unclear how costly these specific functions are and how the costs of these functions relate to each other, and also whether these costs are similar on different food types. These costs must be incorporated into the Y model to completely model the actual relationships between traits on different food types (cf Olijnyk & Nelson, 2013).

Adult plasticity and early adult experience

In this study, we set out to examine the influence of adult acclimation on life-history traits. Survival, weight and egg production were affected by adult plasticity. Interestingly, strong and persistent effects were found of the initial food condition of the adult flies. For instance, the influence of food on fly weight differed between SYH and SYL flies in Exp #1. Furthermore, the FYH and FYL differed widely in how they responded to high food (see Fig. 3, lower panel). Similarly to Exp #1, the effect of initial food experience on weight in the SYH and SYL differed in Exp #2, as well as that on egg production.

We conclude that whereas many studies have considered the influences of developmental plasticity on adult life histories in numerous organisms, the influence of the earliest adult experience, at least in *Drosophila*, is also of great importance. Pearl *et al.* (1927) showed in early work that density in young flies can have a long-lasting effect on their life histories (Pearl *et al.*, 1927). In our study, we have demonstrated long-lasting effects of nutrition in early life on late life history. Because fruit flies cannot perceive changes in environmental nutrition during the pupal stage and rely on information from the larval stage, it might be beneficial for a short-lived organism to be able to alter the life-history decisions immediately dependent on (very) early adult experience. Although these changes are persistent, their adaptive value is likely to be on a short time scale in the field as fruit flies are thought to experience high mortality rates (Dobzhansky & Wright, 1947; Crumpacker & Williams, 1973). Following the main evolutionary theories of ageing (Medawar, 1952; Williams, 1957; Kirkwood, 1977), selection is considered to act primarily on adults early in life which will have affected the life history including the nature and extent of plasticity. Thus, in the ecological context, fixation of life-history traits in very young adult flies is more likely to be adaptive in the early adult life history rather than through any long-lasting effects or predictive abilities of future conditions to be experienced in later life. Nevertheless, we consider that the type of substantial consequences revealed in our experiments of the dietary conditions experienced immediately after eclosion will repay further investigation in other organisms. This may be particularly important in those invertebrates in which some adults in the wild can have extended reproductive lifespans. Such effects could then play a role alongside developmental plasticity in pre-adults in forming predictive responses regarding environments to be experienced later in adult life (Brakefield & Zwaan, 2011; Flatt *et al.*, 2013).

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 (a) Cumulative survival of the flies that were weighed (left), and (b) cumulative survival of the flies that were not weighed (right) of all flies, including the ones that died in the first 4 days.

Figure S2 Individual plasticity in the average number of eggs on low and high food.

Figure S3 Weight loss and number of eggs produced per time step for low food (green) and high food (red).

Figure S4 Egg production for flies that are short- and long-lived within every treatment.

Figure S5 Average weight (in mg) for flies that are short- and long-lived within every treatment.

Table S1 Individuals alive and dead after 4 days per line and weighing treatment.

Table S2 Descriptive statistics and *t* tests for weight of the slow yoyo lines.

Table S3 Descriptive statistics and *t* tests for weight and egg number of the slow yoyo lines.

Table S4 AIC values of the GLM, GLMM and GAM models of egg number.

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