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Aging Differently: Diet- and Sex-Dependent Late-Life Mortality Patterns in *Drosophila melanogaster*

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Diet effects on age-dependent mortality patterns are well documented in a large number of animal species, but studies that look at the effects of nutrient availability on late-life mortality plateaus are lacking. Here, we focus on the effect of dietary protein content (low, intermediate, and high) on mortality trajectories in late life in the fruit fly *Drosophila melanogaster*. According to the two theories that are mainly implicated in explaining the deceleration of mortality rate in late life (the heterogeneity/frailty theory and the Hamiltonian theory), we predict, in general, the occurrence of late-life mortality deceleration under most circumstances, independent of sex and dietary regime. However, the heterogeneity theory of late life is more flexible in allowing no mortality deceleration to occur under certain circumstances compared with the Hamiltonian theory. We applied a novel statistical approach based on Bayesian inference of age-specific mortality rates and found a deceleration of late-life mortality rates on all diets in males but only on the intermediate (standard) diet in females. The difference in mortality rate deceleration between males and females on extreme diets suggests that the existence of mortality plateaus in late life is sex and diet dependent and, therefore, not a universal characteristic of large enough cohorts.

Key Words: Senescence—Dietary restriction—Sexual dimorphism—Mortality plateau—BaSTA.

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MORTALITY rates often increase exponentially from maturity onwards—a pattern that is used as one of the main signatures of aging in biodemography (1). However, mortality rates decrease in late life in a number of different species, including medflies (2), fruit flies (3), seed beetles (4), and humans (5). To explain the decrease of mortality rates after this initial increase, two main theories have been put forward. The demographic heterogeneity theory (6,7) is based on the selective disappearance of individuals with advancing age. Cohorts of individuals are thought to vary in certain traits (eg, stress response; 8,9) from very early on in their lives. For example, nematode worms *Caenorhabditis elegans* with environmentally induced stress resistance have lower penetration of deleterious mutations than their counterparts in which stress resistance was not induced (9). The sum of these characteristics makes up a measure of robustness, which can also be expressed as its complementary variable, frailty (7). Mortality rate trajectories of such within-population cohorts can be described by differently parameterized Gompertz functions, resulting in an overall plateau of mortality rate of the entire population in late life (6,7). Another explanation for late-life mortality plateaus is based on age-independent effects of beneficial genes that are under positive selection earlier in life (also called Hamiltonian theory of late life; 10,11). Because of extrinsic mortality, the force of natural selection declines with age, resulting in the so-called "selection shadow" of late life, which is proposed to be the main cause of aging in general and of age-specific increase in mortality rate in particular. However, when the declining force of selection reaches zero, it cannot decline any further, resulting in a late-life mortality plateau. Note that this theory assumes that strength of selection approaches zero in late life, which may not be a realistic assumption in all taxa. These two theories for the existence of late-life mortality plateaus are not mutually exclusive but have rather different implications for biomedical research aimed at unraveling ways of manipulating late-life mortality to increase healthy life span in humans.

If plateaus are mainly due to demographic heterogeneity and differences in robustness, the therapeutic focus would need to be on very early life stages, probably starting with the quality of sperm and egg cells before fertilization, through to ontogenesis, also taking into account epigenetic differences. Thus, Casanueva and coworkers (9) showed that individuals in isogenic populations of nematode worms differ in their intrinsic robustness because of the differences in the amount of molecular chaperones, which affects the penetration of deleterious mutations. Determining the reason for the difference in robustness between shorter- and longer-lived cohorts would be paramount.

Nutrition has been shown to have strong effects on mortality patterns in a large number of animal species

(12). In particular, diets that prolong life span (measured as mean or median life span), which often have a restricted content of certain dietary components (dietary restriction [DR]), have been the focus of a large number of studies. Although the effects of low and high nutrition on late-life fecundity rate patterns have been investigated before (13), nutritional effects on late-life mortality rates in males and in females have not been experimentally tested to the best of our knowledge. This is surprising, given the great interest in nutritional effects on mortality during earlier life stages, as well as a growing interest in late-life mortality plateaus.

Here, we experimentally tested the effects of three diets, differing in their yeast content (restricted, standard, and excess), on patterns of late-life mortality in male and female fruit flies, *Drosophila melanogaster*. Experimental flies were able to freely interact and mate during the experiment, as we kept them in mixed-sex population cages. We used flies from Dahomey stock, which is a large outbred population with overlapping generations that has been maintained in the laboratory for a very long period of time. Arguably, such populations represent the best research tool for studying mortality patterns in the laboratory for three reasons: (i) they harbor a lot of additive genetic variation for traits (14,15), (ii) they are adapted to their new environment (16,17), and (iii) they are not selected for increased early-life performance as populations kept on discrete generation cycle. We predicted to find decreasing mortality rates in late life in both males and females, irrespective of the diets they were fed on. We further predicted that the onset of mortality rate plateaus would not be diet dependent, as the flies experienced the same conditions for many generations and were randomly sourced from our laboratory population prior to the experimental assay.

We used a novel statistical approach based on Bayesian estimation of age-specific mortality implemented in the software package BaSTA (18,19) to show that although males exhibited mortality deceleration in very late life on all three diets (but realized to a lesser degree on low diet), females did not show late-life plateaus on restricted and excess diets. This nonexistence of mortality plateaus does not support an explanation based solely on the Hamiltonian theory of late life. In general, life span was highest on the intermediate yeast diet, not on the restricted diet, which supports the notion that social interactions in mixed-sex population cages, where flies experience costs of courtship, mating and reproduction, affect the way in which nutrition influences longevity (20).

Methods

Fly Stocks

Experimental flies were all derived from the wild-type and outbred *D. melanogaster* strain Dahomey, which was originally collected from Dahomey (now Benin) in 1970. Since then, fly populations have been kept in population cages containing more than 3,000 individuals of both sexes with overlapping generations, on standard 1.0 SY (sugar/yeast) diet (with Baker's yeast as protein source).

Experimental Design

In the present experiment, flies were raised on standard 1.0 SY diet (with Brewer's yeast) with approximately 180 eggs per vial (plastic, 28.5 × 95 mm) for two generations to remove any potential maternal effects that could arise from unstandardized rearing conditions. Emerging adult flies of the third generation were lightly anesthetized with CO_2 and distributed into experimental population cages, made out of clear plastic (26.5 × 16.5 × 15.5 cm) with one opening, closed by fine nylon mesh. We populated each cage with 300 males and 300 females, that is, 600 flies in total and used two replicate cages per diet. Flies were maintained in a climate chamber at 25°C and 60% humidity on a 12-h:12-h light:dark cycle.

To test for the effects of dietary protein, we provided flies with one of three diets that differed in their yeast concentration (40, 100, or 300 g per 1 L of diet; Table 1). We prepared diets by mixing required quantities of agar, sugar, yeast, and water together. We then boiled the mixture in an autoclave at 121°C for 30 minutes. When the solution had cooled down to 65°C, we added propionic acid and nipagin solution to prevent fungal and bacterial growth and dispensed it into plastic containers (10 × 10 × 4.5 cm; 120 mL in each container).

Mortality was checked three times each week, on Monday, Wednesday, and Friday. Dead flies were removed from the cage with a custom-made electric aspirator, and immediately sexed. Plastic containers with new food were provided twice per week, on Tuesday and Friday, and number and sex of flies that died on the food surface was scored.

Statistics

To model age-dependent mortality trajectories, we fitted logistic models that allow for mortality to level off at later ages:

Table 1. Diet Composition

Diet Concentration (×SY)	0.4 (low)	1.0 (intermediate)	3.0 (high)
Agar (g/L)	15	15	15
Sucrose (g/L)	50	50	50
Brewer's yeast* (g/L)	40	100	300
Nipagin solution† (mL/L)	30	30	30
Propionic acid (mL/L)	3	3	3

Notes: For each diet, distilled water added to 1 L.

Suppliers: Agar (Bageriprodukter AB), sucrose (Nordic Sugar AB), Brewer's yeast (MP Biomedicals), propionic acid (Acros Organics), Nipagin solution (Ph. Eur. from VWR).

*Protein content: 45.94%; carbohydrate content: <0.1%.

†100 g/L methyl 4-hydroxybenzoate (VWR) in 95% ethanol.

$$\mu_x = \alpha e^{\beta x} \Big/ \left[1 + (\alpha\gamma/\beta)(e^{\beta x} - 1)\right] \quad (1)$$

Instantaneous mortality rate (hazard rate) is given by μ_x, parameter α is the intercept and is interpreted as the baseline mortality rate, parameter β is the increase of mortality rate with advancing age, and parameter γ quantifies the extent of the decrease in mortality rate in late life. Parameter γ was originally introduced to model the variance in lifelong frailty of individuals in a population (7). If γ is zero, the logistic model (equation 1) reduces to the Gompertz model, as the denominator in equation (1) becomes 1. We calculated the onset of the decrease in mortality rate as the inflection point of equation (1) in late life, that is, the age at which mortality rate starts to decrease (21).

Modeling was carried out using a modified version of the Bayesian estimation routines in the R package BaSTA (18,19) to account for differences in sampling intervals between populations. This modeling approach uses parametric functions to estimate age-specific mortality trajectories, where x is continuous age and X is defined as a random variable for ages at death. Lifetime mortality is based on the estimation of the hazard rate, or mortality function, which corresponds to:

$$\mathrm{m}(\mathrm{x}|\theta) = \lim_{\Delta \mathrm{x} \to 0} \frac{\Pr(\mathrm{x} \le X < x + \Delta x | X \ge x, \mathrm{q})}{\Delta x} \quad (2)$$

where θ are parameters to be estimated. From the hazard rate, other relevant functions for survival analysis are constructed such as the (i) survival probability:

$$\mathrm{S}(x|\theta) = \Pr(X > x) = \exp\left[-\int_0^x \mu(y|\theta)\mathrm{d}y\right] \quad (3)$$

(ii) the cumulative density function for deaths up to age X:

$$F(x \mid \theta) = \Pr(X < x) = 1 - S(x \mid \theta) \quad (4)$$

and (iii) the probability density function of ages at death, which constitutes the basis for the likelihood function:

$$f(x \mid \theta) = \Pr(x < X < x + \Delta x) = \mu(x \mid \theta)\, S(x \mid \theta) \quad (5)$$

Because time intervals between observations varied among populations, individual deaths were recorded as occurring between the last observation where an individual i was alive, Y_l and the following observation, when the individual was already dead, Y_u. Thus, the likelihood corresponds to the probability of that individual dying within the interval (Y_l and Y_u), also know as interval censored. This likelihood is constructed as:

$$\begin{aligned} L(x \mid \theta) = \Pr(Y_l < X < Y_u) &= \int_{Y_l}^{Y_u} f(x \mid \theta)\, \mathrm{d}x \\ &= F(Y_u \mid \theta) - F(Y_l \mid \theta) \\ &= S(Y_l \mid \theta) - S(Y_u \mid \theta) \end{aligned} \quad (6)$$

The full Bayesian model using equation (1) as the hazard rate is of the form:

$$\begin{aligned} p(\alpha,\beta,\gamma|X) \propto \prod_{i=1}^{n} & L(X_i|\alpha,\beta,\gamma) \\ & \times p(\alpha|a_1,a_2)\, p(\beta|b_1,b_2)\, p(\gamma|r_1,r_2) \end{aligned} \quad (7)$$

where the first term on the left-hand side of equation (7) is the posterior density, whereas the second term corresponds to normal priors truncated at 0 with mean parameters a_1, b_1, and r_1, variances a_2, b_2, and r_2 for parameters α, β, and γ, respectively. All priors were uninformative with variance values set at 10.

Parameter posteriors were obtained by implementing a Markov Chain Monte Carlo procedure that samples parameters using a Metropolis-within-Gibbs algorithm (for additional information, see (19)). To assess convergence, four parallel Markov Chain Monte Carlo's were ran starting at different parameter values and calculated potential scale reduction as proposed by Gelman and coworkers (2004) (22). We ran each Markov Chain Monte Carlo for 200,000 iterations with a burn-in of 50,000 and a thinning interval of 100.

To estimate parameter overlap, we calculated Kullback–Leibler discrepancies (KL; 23) between the posterior densities of the corresponding parameters by sex and treatment (for further details, see (18)). To improve the interpretation of the KL values, we calculated a calibration (KLc) proposed by McCulloch (24). This calibration ranges between 0.5 and 1, where KLc values close to 0.5 indicate full overlap between the posterior distributions, and KLc values close to 1 otherwise.

We tested the effects of sex and diet on mean life span using a mixed-effects model (function lmer in the package lme4; 25) with a random intercept for replicate cages. As a starting model, we used Sex, Diet, and the interactions Sex × Diet and Sex × Diet^2 as fixed effects. To test for importance of fixed effect model terms, we excluded higher order terms sequentially and tested reduced models against full models using likelihood ratio tests with twice the difference in log-likelihoods being chi-square distributed. For all analyses, R version 2.15.0 was used.

Results

All parameters converged appropriately with potential scale reduction values below 1.1. The logistic γ parameter

had a significant effect on hazard rates in all experimental groups except for females on low and high diet, where the values for the γ parameter were extremely low; females on low and high diet showed, therefore, no deceleration of hazard rate late in life (Figure 1; Table 2). The logistic frailty parameter (γ) had an important effect on all experimental groups of males. The start of the late-life mortality deceleration, estimated as the inflection point of the logistic curves, increased gradually from high to low diet (Figures 1 and 3). This later onset of mortality deceleration corresponds to a reversed pattern in the magnitude of the deceleration parameter, γ, with more restricted diet resulting in smaller values (Figures 2 and 3).

The calibrated Kullback–Leibler comparisons showed that only the α parameter for males with low and high treatment overlapped (ie, were not different, mean KLc = 0.518), whereas a moderate overlap was detected in the γ parameter between females in moderate treatment and males in high treatment (mean KLc = 0.714). All other parameters had very significant nonoverlap (KLc > 0.99).

Mean and maximum life span, pooled over two replicate cages, are given in Table 2. The life span model, containing a quadratic effect of diet and interactions between the diet terms and sex as predictors, was better than the reduced models that either had the interaction terms (χ_3 = 19.21, p = .0002) or the quadratic term excluded (χ_2 = 10.84, p = .0044). The effect of diet was nonlinear and dependent on sex (Figure 4). Mean life span did not differ strongly between diets 0.4 and 1.0 but was markedly lower on diet 3.0 in both sexes (Table 3; Figure 4). Contrary to the often reported life-span prolonging effect of restricted diet, flies on diet 0.4 did not live longer than flies on standard 1.0 diet. In fact, life span of females on diet 0.4 showed a trend to decrease compared with life span of females on diet 1.0 (Table 3; Figure 4). A similar result was found by Bass and coworkers (26). In general, the question of which diet constitutes the "restricted" diet does not impinge on our findings about evidence for or against sex-specific late-life mortality leveling off.

Discussion

We observed a deceleration of mortality rates late in life on all diets in males (low-, intermediate-, and high-protein diet) but only on intermediate diet in females. The nonexistent mortality rate deceleration in females on low- and high-protein diets suggest that the existence of mortality plateaus in late life is sex and diet dependent and, therefore, not a universal characteristic of large enough cohorts containing males or females.

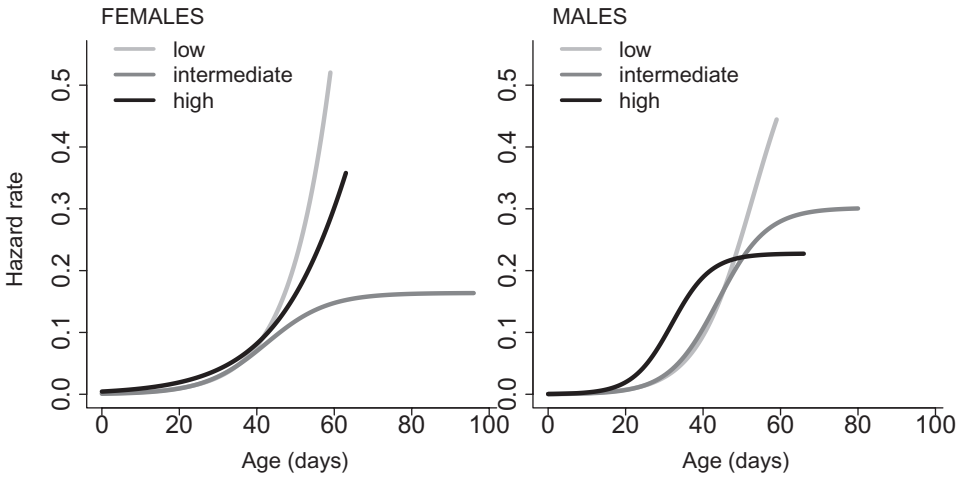

Figure 1. Mortality models. Fitted logistic models for females (left) and males (right). Inflection points are indicated by bars on the x-axes.

Table 2. Parameter Estimates of Fitted Logistic Models (α, β, and γ) and of the IPs

			95% CI (α)			95% CI (β)			95% CI (γ)			95% CI (IP)	
Diet	Sex	α	Lower	Upper	β	Lower	Upper	γ	Lower	Upper	IP	Lower	Upper
Low	Male	0.0005	0.0003	0.0008	0.1351	0.1190	0.1499	0.2107	0.0483	0.3837	54.42	47.47	67.80
Low	Female	0.0012	0.0009	0.0017	0.1047	0.0971	0.1126	0.0201	0.0008	0.0665			
Intermediate	Male	0.0003	0.0002	0.0005	0.1577	0.1418	0.1727	0.5230	0.3944	0.6695	44.41	42.10	47.00
Intermediate	Female	0.0008	0.0005	0.0011	0.1255	0.1126	0.1403	0.7659	0.5834	0.9996	42.99	39.90	46.16
High	Male	0.0004	0.0002	0.0007	0.1986	0.1742	0.2243	0.8725	0.6431	1.1145	32.03	30.07	34.27
High	Female	0.0044	0.0034	0.0054	0.0745	0.0677	0.0834	0.0536	0.0024	0.1680			

Notes: CI = confidence interval; IP = inflection point; α = initial mortality rate; β = increase in mortality rate; γ = decrease of mortality rate at very late ages.

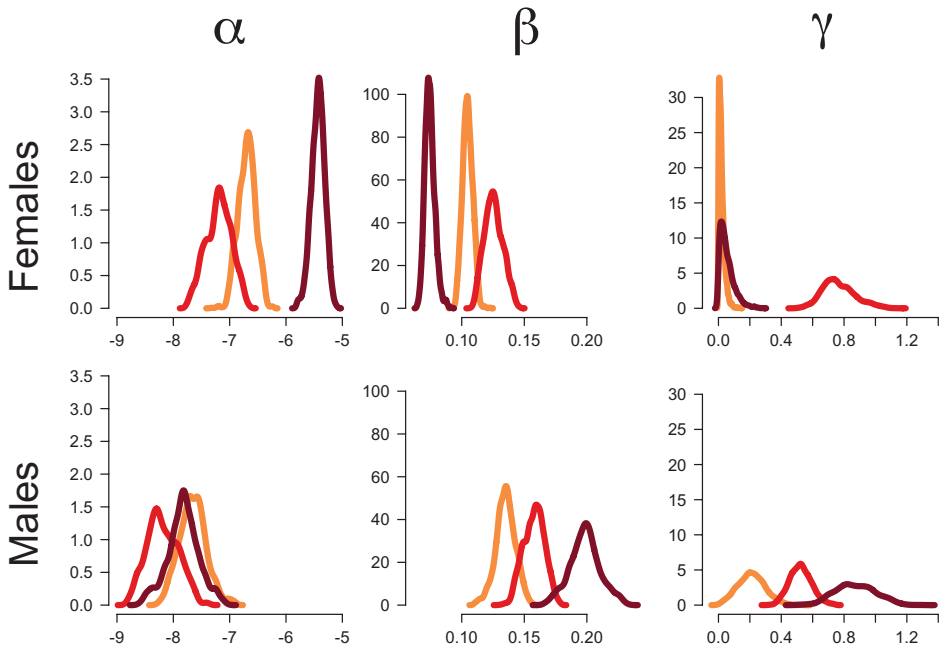

Figure 2. Mortality parameters. Probability density functions of the three parameters from the fitted logistic models for females and males. α: logarithm of the initial mortality rate; β: increase in mortality rate; γ: decrease of mortality rate at very late ages. Orange: low diet; red: intermediate diet; dark red: high diet.

Males subject to more restricted dietary environment considerably postponed the onset of mortality plateaus, and the magnitude of the plateaus gradually decreased with declining dietary protein content. More generally, lower protein diets caused mortality to decelerate at a later age, resulting in a slower leveling off of mortality late in life. In females, the onset of aging-related mortality was distinctly delayed in low- and intermediate-diet groups compared with groups on high diet (see levels of Gompertz α). The aging rate (Gompertz β) was negatively correlated with the initial mortality rate, so that females on high diet showed the highest initial mortality and aged at the lowest rate, whereas females on low and intermediate diet showed lower initial mortality and stronger increase in mortality rate in late life. A similar effect of lower diet decreasing initial mortality and increasing the rate of aging was previously found in female fruit flies (27,28). In male flies, Magwere and coworkers (27) found a similar pattern as in female flies, which contrasts with our findings. But flies in their study were kept in separate sex groups, and the dietary protein to carbohydrate ratio was kept constant, in contrast to the present study where we kept flies in mixed-sex groups and manipulated the dietary protein to carbohydrate ratio—factors that might be responsible for the observed differences.

Age-specific mortality rates have been demonstrated to be influenced to some extent by the population density of flies (29), but the density did not affect existence of deceleration of mortality at older ages and had only minor negligible effects on mortality patterns in very late life (30–32). We did not detect mortality plateaus for females on low and high diets, whereas mortality rates of females on intermediate (standard) diet and males on all tested diets started to decrease in late life. However, in males on low diet, the statistically significant start of mortality deceleration is not very pronounced. If we assume the plateau model of heterogeneity in frailty, we can conclude that extreme diets on either side of the dietary protein content spectrum led to a decreased variance in frailty in females and, to a lesser extent, in males on low-protein diet.

The recent evolutionary history and the immediate environment where development took place were similar for all experimental individuals. This implies that any assumed differences in frailty would have been acquired in adult life, as a direct effect of the nutritional environment. If we assume that age-independent genetic effects are responsible for the deceleration of mortality rates in late life, as under the Hamiltonian theory of late life, we would predict to find a deceleration in all groups, independent of diet and sex. In an experiment using the cactophilic fly *Drosophila mojavensis*, late-life mortality deceleration was found for male and female cohorts on artificial standard laboratory diet only, in contrast to flies with access to fermenting cactus tissue, which is the species' food source in nature (33). The sample size of around 800 individuals per sex and diet treatment should be sufficiently high to detect decelerating mortality rates late in life using standard maximum likelihood fitting techniques, with the minimum sample size ranging between 100 and 500 individuals (34). The authors suggest that the cactus tissue is similar to a dietary restricted environment, as the carbohydrates in this food are not easily accessible to the flies (33). This would fit with our findings for females and could be explained by a potential decrease in the degree

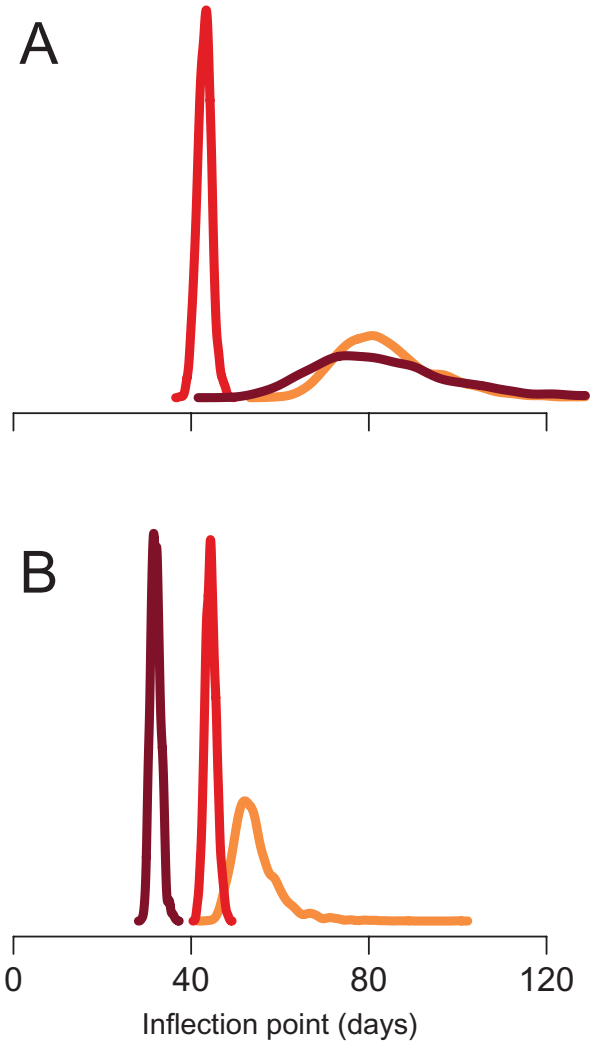

Figure 3. Start of mortality plateaus. Probability density functions of the inflection point of the fitted logistic models for females (**A**) and males (**B**). Orange: low diet; red: intermediate diet; dark red: high diet.

of heterogeneity caused by a restricted dietary environment. However, the *D. mojavensis* flies were already reared as larvae on the experimental diet, which makes a cross-species comparison, based on this study, very difficult.

The experimental diets we used differed not only in their protein to carbohydrate ratios but also in their caloric content due to varying the yeast content, while keeping the sugar content the same. To explain why this might matter, it is useful to know that until recently, the general scientific consensus was that the life-span extending effect of limiting nutrients, without inducing malnutrition, is due to the overall caloric content of the diet. Therefore, this dietary manipulation was coined "caloric restriction." When it was shown in *D. melanogaster* that the effect is rather due to the protein to carbohydrate ratio, the more general term "dietary restriction" was used instead (35). Since then, more nutritionally explicit studies corroborated that caloric restriction is not the major mechanism underlying life-span extension through food restriction, not only in different species of fruit flies (36–38), but also in crickets (39,40) and ants (41), with preliminary evidence in mice (S. J. Simpson, unpublished data). These recent results rather support a low protein to carbohydrate ratio, leading to a lower protein intake compared to diets with higher protein to carbohydrate ratios, as the cause of life-span extension (36–41). Indeed, in *D. melanogaster*, there is evidence that life-span extension, found under DR, is to a large extent due to the imbalance of single amino acids ((42) but see (20)). However, these findings do not tell us about the mechanisms that cause late-life mortality deceleration. Our study cannot analytically differentiate between protein to carbohydrate ratio and caloric content, leaving that specific question open for future work; although previous studies allow us to suggest that calories are unlikely to play a key role in shaping late-life mortality plateaus (see above).

In general, it is expected to find extended life span (measured as the mean, median, or maximum life span) under DR, more specifically, for a diet with a low protein to carbohydrate ratio. In our study, males and females on the intermediate yeast diet (1.0 SY) attained the highest life span. There are three important points to make about this finding. First, there is evidence from other studies on *D. melanogaster* that a diet similar to our intermediate diet falls already in the range of "restricted" diets, where life span peaks. Similar to our study, Bass and coworkers (26) also found a lower median life span of flies on a 0.5 SY diet compared with the flies on a 1.0 SY diet. The absolute difference in median life span between these two treatments was also very similar to the one we found between 0.4 and 1.0 SY in the present study (around 2 days, according to Figure 2 in (26)). But median life span for these two diet treatments were markedly different between the two studies, with flies in our study living about 25 days shorter than flies in the Bass et al. study, that is, the relative change was much stronger in our study. However, in a study from the same lab group, using the same fly stock and the same yeast type and supplier in a different year, flies on a 0.5 SY diet lived 8 days longer compared with flies on the 1.0 SY diet (Figure 3 and Table 1 in (43)). The authors suggested seasonality of food characteristics as a possible reason for this discrepancy. In another study, the same group used the 1.0 SY diet as their DR diet (42).

Second, we used very similar diets and fly stocks compared to the three studies discussed under the previous point, and even this can already lead to some variation on what diet the peak DR life span is expressed. But there also exist differences in how flies were maintained during the experiments (apart from the documented and discussed variation in food quality). In all three studies above, experimental flies were housed in separate sex groups of 10 per vial. In our study, flies were kept in mixed-sex population cages (300 males and 300 females per cage) to have sufficient sample sizes at late life and to improve the ability of the experimental

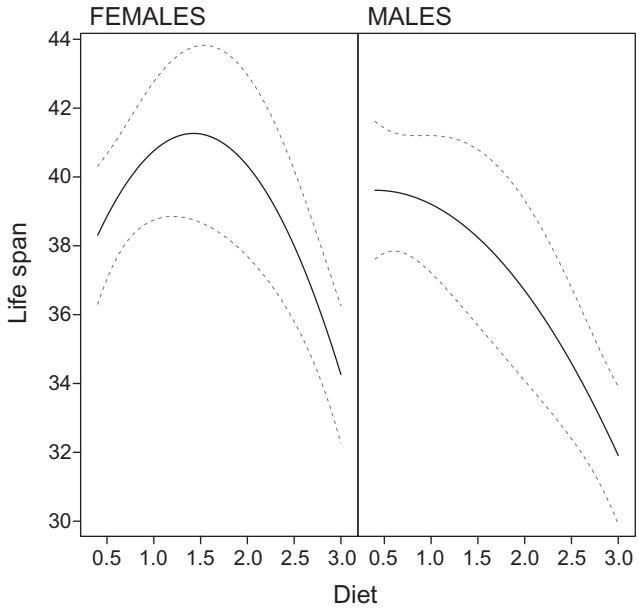

Figure 4. Mean life span. Fitted second-order polynomial models for females (left panel) and males (right panel). Dashed lines represent 95% confidence limits.

Table 3. Summary Statistics for Males and Females on Each Diet

Diet	Sex	Mean Life Span ± *SE* (d)	Maximum Life Span ± *SE* (d)	Sample Size
Low	Male	39.6 ± 0.4 (40)	57.1 ± 0.5	575
Low	Female	38.3 ± 0.5 (40)	55.1 ± 0.3	571
Intermediate	Male	39.2 ± 0.5 (42)	60.1 ± 1.8	591
Intermediate	Female	40.7 ± 0.6 (42)	72.9 ± 2.2	564
High	Male	31.9 ± 0.4 (33)	51.4 ± 0.7	578
High	Female	34.3 ± 0.6 (33)	57.4 ± 0.4	580

Notes: SE: standard error. Median life span is given in parentheses. The statistics were calculated by pooling mortality data from the two replicate cages per diet. Maximum life span is defined as mean life span of the oldest 5% of individuals.

flies to show behaviors in a spatial and social environment more similar to that which the stock of origin, the Dahomey population, has been able to adapt over hundreds of generations. In one of our previous studies, we found a similar pattern in males, with males on 0.4 SY diet living shorter than males on 1.0 SY diet (20). But this pattern was only found for males kept without females (n_{vial} = 40 at age 0). For males in mixed sex groups ($n_{vial\ and\ sex}$ = 20 at age 0), there was no difference in mean life span between these two treatments. In contrast, females showed an increased life span on lower yeast diet, independent of whether in separate or mixed-sex groups. In summary, we find sex differences in the effects of low-yeast diet, and the effects seem to be affected in a sex-specific way by the social or mating environment and the available space. Proximate mechanisms that could lead to such differences are likely related to differences in mating behavior (male–female), sexual competition (male–male), and competition for egg-laying opportunities (female–female). In addition, due to the opportunity to fly in larger population cages (compared with vials in which flies can only crawl), metabolic differences through different energetic demands probably exist.

Third, the fact that we do not seem to find the classic life-span response to DR at first sight does not impinge on our findings about evidence for or against sex-specific late-life mortality leveling off. The existence or lack of mortality leveling off can indeed first be evaluated independent of the question about what diet results in the highest life span. We want to stress here that so far, there has not been a study published that evaluated sex- and diet-specific effects on late-life mortality rates. Our study will, therefore, draw interest to studying late-life mortality patterns in a framework (eg, the *Geometric Framework of Nutrition*) that has the potential to go into more details of which nutritional characteristic causes mortality deceleration in a sex-specific way.

To summarize, we show that the onset and the magnitude of late-life mortality deceleration depend on sex and diet. This result is more compatible with theories that put forward the lifelong heterogeneity of cohorts as a primary explanation for the occurrence of mortality plateaus than with theories that emphasize the role of zero-level selection in late life. Late-life plateaus occur in humans, where there is mounting evidence for postreproductive selection via grandparental care (44,45), suggesting that the heterogeneity theory is

likely to be an important factor shaping mortality rates at old ages (46). This study lends tentative support to the heterogeneity theory in explaining late-life mortality in fruit flies but suggests that more work is needed to understand the full spectrum of this phenomenon across a range of nutritional and social environments in the two sexes. One promising approach is to utilize the Geometric Framework of Nutrition (36,40,47) for the study of sex-dependent late-life mortality plateaus. Major pathways that are responsible for the life-span extending effect of restricted nutrient availability are evolutionary conserved across diverse phylogenetic lineages (12). Therefore, fundamental research on sex differences in late-life mortality, using well-established animal models like *D. melanogaster*, is a very promising avenue to increase our understanding about the effects of dietary manipulations on longevity, which are likely to be sex specific. The potential of fundamental experimental research on aging-related phenotypes in model organisms like fruit flies to inform us on issues related to human health in late life has been noted before (48). There is still much more work to be done to firmly establish invertebrate systems in screening and testing for life span–extending treatments with potential application in humans (49), and the study of late-life mortality should be included in this endeavor. If heterogeneity in frailty is found to play a significant part in mortality decelerations in later life in a wider range of animal models, that is, if heterogeneity is a general cause of late-life mortality deceleration, it would be highly informative to focus on experimental evidence that elucidates the genetic and physiological characteristics of cohorts that exhibit the lowest frailty. In humans, this is already underway by studying centenarians and supercentenarians (50).

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References

1. Carey JR. Insect biodemography. *Annu Rev Entomol*. 2001;46:79–110.
2. Carey JR, Liedo P, Orozco D, Vaupel JW. Slowing of mortality rates at older ages in large medfly cohorts. *Science*. 1992;258:457–461.
3. Drapeau MD, Gass EK, Simison MD, Mueller LD, Rose MR. Testing the heterogeneity theory of late-life mortality plateaus by using cohorts of *Drosophila melanogaster*. *Exp Gerontol*. 2000;35:71–84.
4. Fox CW, Scheibly KL, Wallin WG, Hitchcock LJ, Stillwell RC, Smith BP. The genetic architecture of life span and mortality rates: gender and species differences in inbreeding load of two seed-feeding beetles. *Genetics*. 2006;174:763–773.
5. Vaupel JW, Carey JR, Christensen K, et al. Biodemographic trajectories of longevity. *Science*. 1998;280:855–860.
6. Vaupel JW. Inherited frailty and longevity. *Demography*. 1988;25:277–287.
7. Vaupel JW, Manton KG, Stallard E. The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography*. 1979;16:439–454.
8. Wu D, Rea SL, Yashin AI, Johnson TE. Visualizing hidden heterogeneity in isogenic populations of *C. elegans*. *Exp Gerontol*. 2006;41:261–270.
9. Casanueva MO, Burga A, Lehner B. Fitness trade-offs and environmentally induced mutation buffering in isogenic *C. elegans*. *Science*. 2012;335:82–85.
10. Mueller LD, Rose MR. Evolutionary theory predicts late-life mortality plateaus. *Proc Natl Acad Sci U S A*. 1996;93:15249–15253.
11. Abrams PA, Ludwig D. Optimality theory, Gompertz' law, and the disposable soma theory of senescence. *Evolution*. 1995;49:1055–1066.
12. Fontana L, Partridge L, Longo VD. Extending healthy life span–from yeast to humans. *Science*. 2010;328:321–326.
13. Rauser CL, Hong JS, Cung MB, Pham KM, Mueller LD, Rose MR. Testing whether male age or high nutrition causes the cessation of reproductive aging in female *Drosophila melanogaster* populations. *Rejuvenation Res*. 2005;8:86–95.
14. Gardner M, Fowler K, Partridge L, Barton N. Genetic variation for preadult viability in *Drosophila melanogaster*. *Evolution*. 2001;55:1609–1620.
15. Gardner MP, Fowler K, Barton NH, Partridge L. Genetic variation for total fitness in *Drosophila melanogaster*: complex yet replicable patterns. *Genetics*. 2005;169:1553–1571.
16. Rice WR, Linder JE, Friberg U, Lew TA, Morrow EH, Stewart AD. Inter-locus antagonistic coevolution as an engine of speciation: assessment with hemiclonal analysis. *Proc Natl Acad Sci U S A*. 2005;102(suppl 1):6527–6534.
17. Rice WR, Stewart AD, Morrow EH, Linder JE, Orteiza N, Byrne PG. Assessing sexual conflict in the *Drosophila melanogaster* laboratory model system. *Philos Trans R Soc Lond B Biol Sci*. 2006;361:287–299.
18. Colchero F, Jones OR, Rebke M. BaSTA: an R package for Bayesian estimation of age-specific survival from incomplete mark–recapture/recovery data with covariates. *Method Ecol Evol*. 2012;3:466–470.
19. Colchero F, Clark JS. Bayesian inference on age-specific survival for censored and truncated data. *J Anim Ecol*. 2012;81:139–149.
20. Zajitschek F, Zajitschek SR, Friberg U, Maklakov AA. Interactive effects of sex, social environment, dietary restriction, and methionine on survival and reproduction in fruit flies. *Age (Dordr)*. 2013;35:1193–1204.
21. Witten TM, Eakin T. Multiphasic models of survival: analysis of mortality rate change regions and the issue of finite species lifespan. *Exp Gerontol*. 1997;32:259–285.
22. Gelman A, Carlin JB, Stern HS, Rubin DB. *Bayesian Data Analysis* (Second edition). Raton, FL: Chapman and Hall/CRC; 2004.
23. Kullback S, Leibler RA. On information and sufficiency. *Ann Math Stat*. 1951;22:79–86.
24. McCulloch RE. Local model influence. *J Am Stat Assoc*. 1989;84:473–478.
25. Bates D, Maechler M, Bolker B. *lme4: linear mixed-effects models using S4 classes. R package version 0.999999-0*; 2012.
26. Bass TM, Grandison RC, Wong R, Martinez P, Partridge L, Piper MD. Optimization of dietary restriction protocols in *Drosophila*. *J Gerontol A Biol Sci Med Sci*. 2007;62:1071–1081.
27. Magwere T, Chapman T, Partridge L. Sex differences in the effect of dietary restriction on life span and mortality rates in female and male *Drosophila melanogaster*. *J Gerontol A Biol Sci Med Sci*. 2004;59:3–9.
28. Pletcher SD, Macdonald SJ, Marguerie R, et al. Genome-wide transcript profiles in aging and calorically restricted *Drosophila melanogaster*. *Curr Biol*. 2002;12:712–723.

29. Graves JL Jr, Mueller LD. Population density effects on longevity. *Genetica*. 1993;91:99–109.
30. Curtsinger JW. Density and age-specific mortality. *Genetica*. 1995;96:179–82; discussion 183.
31. Khazaeli AA, Xiu L, Curtsinger JW. Effect of density on age-specific mortality in *Drosophila*: a density supplementation experiment. *Genetica*. 1996;98:21–31.
32. Khazaeli AA, Xiu L, Curtsinger JW. Effect of adult cohort density on age-specific mortality in *Drosophila melanogaster*. *J Gerontol A Biol Sci Med Sci*. 1995;50:B262–B269.
33. Jaureguy LM, Etges WJ. Assessing patterns of senescence in *Drosophila mojavensis* reared on different host cacti. *Evol Ecol Res*. 2007;9:91–107.
34. Pletcher SD. Model fitting and hypothesis testing for age-specific mortality data. *J Evol Biol*. 1999;12:430–439.
35. Mair W, Piper MD, Partridge L. Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLoS Biol*. 2005;3:e223.
36. Lee KP, Simpson SJ, Clissold FJ, et al. Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc Natl Acad Sci U S A*. 2008;105:2498–2503.
37. Skorupa DA, Dervisefendic A, Zwiener J, Pletcher SD. Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. *Aging Cell*. 2008;7:478–490.
38. Fanson BG, Weldon CW, Pérez-Staples D, Simpson SJ, Taylor PW. Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). *Aging Cell*. 2009;8:514–523.
39. Maklakov AA, Hall MD, Simpson SJ, et al. Sex differences in nutrient-dependent reproductive ageing. *Aging Cell*. 2009;8:324–330.
40. Maklakov AA, Simpson SJ, Zajitschek F, et al. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Curr Biol*. 2008;18:1062–1066.
41. Dussutour A, Simpson SJ. Ant workers die young and colonies collapse when fed a high-protein diet. *Proc Biol Sci*. 2012;279:2402–2408.
42. Grandison RC, Piper MD, Partridge L. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature*. 2009;462:1061–1064.
43. Grandison RC, Wong R, Bass TM, Partridge L, Piper MD. Effect of a standardised dietary restriction protocol on multiple laboratory strains of *Drosophila melanogaster*. *PLoS One*. 2009;4:e4067.
44. Lahdenperä M, Lummaa V, Helle S, Tremblay M, Russell AF. Fitness benefits of prolonged post-reproductive lifespan in women. *Nature*. 2004;428:178–181.
45. Hawkes K. Grandmothers and the evolution of human longevity. *Am J Hum Biol*. 2003;15:380–400.
46. Vaupel JW. Biodemography of human ageing. *Nature*. 2010;464:536–542.
47. Simpson SJ, Raubenheimer D. Caloric restriction and aging revisited: the need for a geometric analysis of the nutritional bases of aging. *J Gerontol A Biol Sci Med Sci*. 2007;62:707–713.
48. Kirkland JL, Peterson C. Healthspan, translation, and new outcomes for animal studies of aging. *J Gerontol A Biol Sci Med Sci*. 2009;64:209–212.
49. Conn PM. *Handbook of Models for Human Aging*. Boston, MA: Elsevier Academic Press; 2006.
50. Willcox BJ, Willcox DC, Ferrucci L. Secrets of healthy aging and longevity from exceptional survivors around the globe: lessons from octogenarians to supercentenarians. *J Gerontol A Biol Sci Med Sci*. 2008;63:1181–1185.