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.
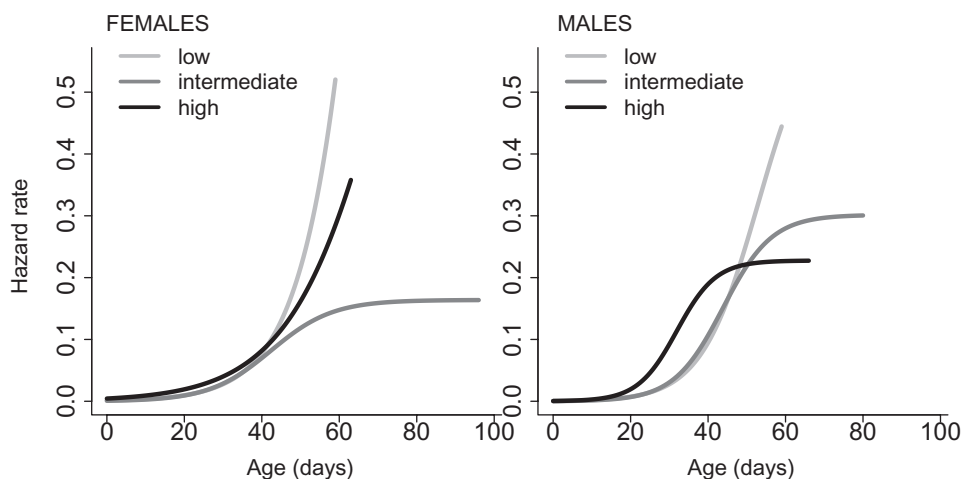

Figure 1. Mortality models. Fitted logistic models for females (left) and males (right). Inflection points are indicated by bars on the x-axes.

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

Diet	Sex	α	95% CI (α) Lower	95% CI (α) Upper	β	95% CI (β) Lower	95% CI (β) Upper	γ	95% CI (γ) Lower	95% CI (γ) Upper	IP	95% CI (IP) Lower	95% CI (IP) 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.
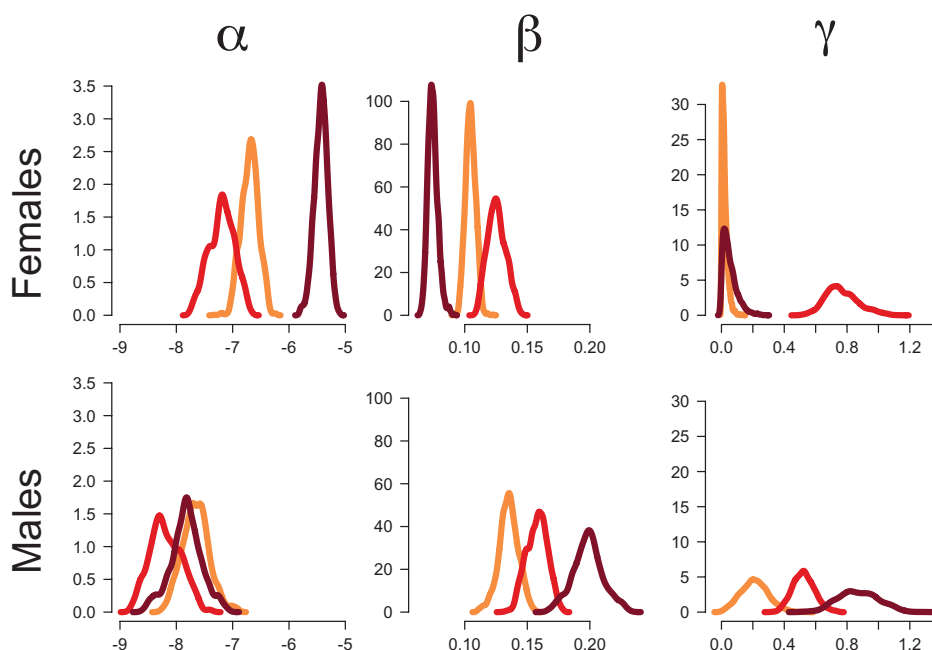
Figure 2. Mortality parameters. Probability density functions of the three parameters from the fitted logistic models for females and males. α: logarithm of the initial mortality rate; β: increase in mortality rate; γ: decrease of mortality rate at very late ages. Orange: low diet; red: intermediate 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
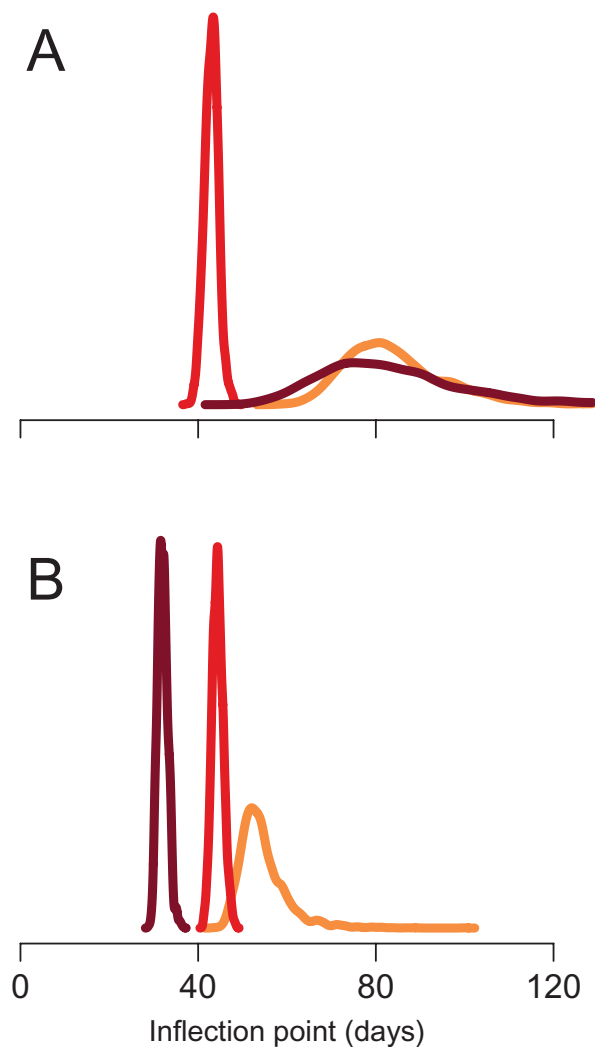
Figure 3. Start of mortality plateaus. Probability density functions of the inflection point of the fitted logistic models for females (**A**) and males (**B**). Orange: low diet; red: intermediate diet; dark red: high diet.

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

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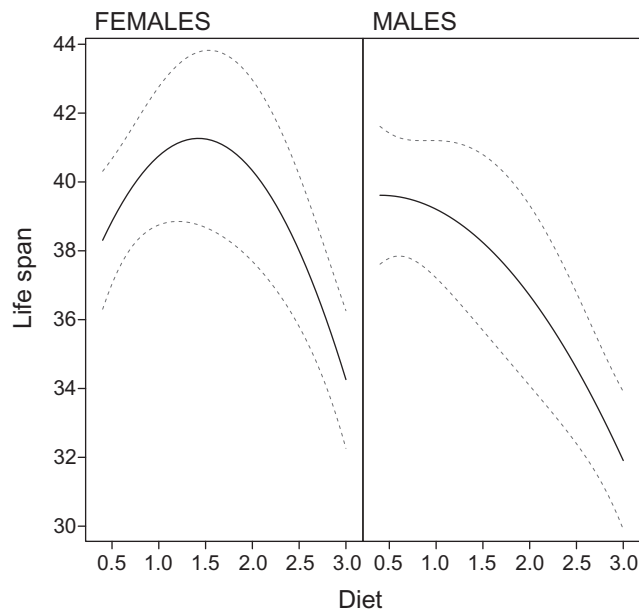
Figure 4. Mean life span. Fitted second-order polynomial models for females (left panel) and males (right panel). Dashed lines represent 95% confidence limits.

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 lifespan 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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