UPPSALA
UNIVERSITET

Dietary effects on late-life mortality rates of male and female *Drosophila melanogaster*

JIN TUO

Degree project in biology, Master of science (2 years), 2012
Examensarbete i biologi 45 hp till masterexamen, 2012
Institutionen för biologisk grundutbildning och Animal Department , Uppsala universitet
Handledare: Alexei Maklakov och Felix Zajitschek
Extern Opponent: Rebecca Dean

Abstract

Age-specific mortality rate has been shown to plateau at very late ages in *Drosophila melanogaster*. But how the mortality rates, especially in late-life, respond to DR (dietary restriction) is rarely investigated. In the present study I mainly focus on detecting if age-specific mortality rate in *Drosophila* levels off, when kept on three different diets (low, medium and high yeast), on how mortality rates differ in detail between the diet treatments, and whether these effects are sex-specific. The results demonstrated that mortality plateaus at advanced ages were affected by diet and this dietary effect differed considerably between the sexes. Males subject to DR considerably postponed the onset of mortality plateaus with decreased magnitude of the plateaus, while females did not show any late-life deceleration on DR and obesity diets. This non-existence of mortality plateaus on extreme diets in females seems to be in support of the lifelong heterogeneity theory. Diets that maximized life span differed between sexes, and DR prolonged life spans mainly by decreasing the initial mortality rate in females and males.

Introduction

Ageing or senescence usually refers to the process of organisms growing older and is always accompanied by functional deterioration, showing decreases in the age-specific survival rates and reproductive rates with chronological age. Natural selection favours adaptation to enhance survival and reproduction, which thereby should eliminate senescence during evolution. Nevertheless, ageing is an inescapable fate for almost all multicellular organisms (Rose 1991). There are two main evolutionary theories that explain the prevalence of ageing: the mutation accumulation hypothesis (Medawar 1952) and the antagonistic pleiotropy hypothesis (Williams 1957). Both theories posit that natural selection on survival and reproduction is weakening with increasing age due to extrinsic (predation, disease and accidents) and intrinsic death risks. Medawar proposed that ageing results from accumulation of late acting deleterious mutations, while Williams extended Medawar's hypothesis to assume a selection for pleiotropic genes with beneficial effects early in life but detrimental effects in relatively late life. There is also the disposable soma theory that physiologically explains the evolution of ageing via accumulation of un-repaired damage in somatic cells (Kirkwood & Holliday 1979). All these theories can be viewed as an optimization of the balance between growth, survival and reproduction throughout life (Partridge & Barton 1993).

The Gompertz model (see below in **Statistics Equation** (1)) has been broadly applied to quantify the ageing process based on an exponential increase of mortality rates with increasing age. According to this model, mortality rates are expected to accelerate continuously in adulthood until all individuals of a population have died off. However, a lot of studies on demography of large cohorts revealed that mortality rates started to level off at very late ages, such as in large medfly *Ceratitis capitata* (Carey, Liedo, & Orozco 1992), fruit fly *Drosophila melanogaster* (Drapeau *et al.* 2000) and humans (Vaupel 1998). These studies seemed to suggest that the Gompertz model failed to correctly predict age-specific mortality rate at very late ages. Moreover, not

only did mortality rates stop accelerating at advanced adult ages, reproductive rates were also shown to stop decelerating in late life (Rauser *et al.* 2006b). Hence, adulthood is demographically divided into two distinct phases: ageing and relaxed ageing at the very late ages, which means the fitness of a cohort deteriorates progressively during ageing, but such deterioration is supposed to attenuate gradually in late-life. The age dividing the two phases in adulthood is sometimes called break-day (Shahrestani, Mueller, & Rose 2009).

There are two main views that have been proposed to explain the existence of the plateaus in late-life: demographic lifelong heterogeneity and an evolutionary theory based on the intensity of natural selection (Shahrestani *et al.* 2009). The former one originally hypothesizes an inherently different robustness among individuals (Beard 1964). An ageing population is therefore assumed to consist of a collection of sub-populations varying in robustness that can be modeled by differently parameterized Gompertz functions (Vaupel & Manton 1979). Those individuals with relatively weak robustness are prone to die off in early life so that they do not have a chance to affect the demographical parameters later on. Only robust enough individuals are able to survive to late-life and it is these individuals that predominantly contribute to the plateaus of mortality rate in late-life (Vaupel 1998; Pletcher & Curtsinger 2000; Carnes & Olshansky 2001). This cohort of late-life survivors is assumed to have lower mortality rate than individuals that die early in life, thereby contributing to the phenomenon of late-life mortality plateaus. This hypothesis is based on heterogeneity of individuals in a cohort from genetic variance or from environmental manipulations (Vaupel & Manton 1979; Vaupel 1990). The largest drawback with this theory is that the robustness of individuals is hard to explicitly measure or even identify. In addition, the theory is also criticized on the grounds that it is not sufficient to explain fecundity plateaus (Rauser *et al.* 2005a).

The second theory, which is sometimes called Hamiltonian theory, explains the plateaus by the fact that genes with age-independent beneficial effects are selected

due to beneficial effect in earlier life and still have beneficial effect in later life (Charlesworth 2001). Hamiltonian theory assumes a function to quantitatively describe the declining strength of natural selection with age (Hamilton 1966). According to this function, the value of the force of natural selection on mortality is equal to 1 in the period before the first reproduction. Then it begins to fall gradually until the end of reproduction. During the post-reproduction stage, it stays 0 until death. Additionally, deleterious mutations accumulate in a population with an approximately constant level throughout life, while intensity of natural selection opposing the accumulation of mutations decreases in adulthood (Shahrestani *et al.* 2009). Hence the pattern of mortality in adulthood is revealed as a result from the trade-off between selection acting on mortality rates and other evolutionary forces, such as mutation rate and genetic drift. With the cessation of force of natural selection in late life, the mortality curve is shaped to gradually plateau in this period (Rauser, Mueller, & Rose 2006a). Mortality rates are therefore expected to eventually reach 100% by mutation accumulation since there is no selection against deleterious effects at late-life. However, genes with age-independent beneficial effects are favoured by selection that have a beneficial effect in late-life to prevent mortality rate to 100% (Charlesworth 2001). Hamiltonian theory also supports the existence of fecundity plateaus in late life, with corroboration from experimental findings (Rauser *et al.* 2005b, 2006b). The plateaus should persist in cohorts regardless of genetic or environmental variation (Khazaeli, Xiu, & Curtsinger; Curtsinger *et al.* 1992).

Environmental variation has been demonstrated to profoundly influence the patterns of ageing in a wide range of species (McCay, Crowell, & Maynard 1935; Norry & Loeschcke 2002). Dietary restriction (DR), which moderately reduces food intake without malnutrition, has been shown to prolong longevity in a range of animal species, including *Caenorhabditis elegans* (Kenyon *et al.* 1993), *D.melanogaster* (Chippindale *et al.* 1993; Magwere & Chapman 2004) and rats (McCay *et al.* 1935). Observations from such diverse species make DR a potential intervention to prolong longevity in mammals though there is still uncertainty about the molecular

mechanisms through which it works (Piper *et al.* 2011). Restriction of calories (CR) in food had been shown to essentially determine lifespan extension by DR in rats (Masoro *et al.* 1989), but not in *Drosophila* (Mair, Piper, & Partridge 2005). Recently, some new findings revealed that extension of lifespan in response to DR in *Drosophila* is primarily attributed to the ratio between specific nutritional components in the diet (Lee *et al.* 2008; Grandison, Piper, & Partridge 2009).

Extension of life span in response to DR in *Drosophila* is achieved through the reduction of the content of yeast and sugar in the diet (Partridge, Piper, & Mair 2005). Between the two components, quality and quantity of yeast tend to mainly contribute to the effect of DR (Chippindale *et al.* 1993; Mair *et al.* 2005), while high density of sugar has little impact on life span (Bass *et al.* 2007). Therefore DR implemented in *Drosophila* is best applied by manipulating the density of yeast in the diet (Partridge, Green, & Fowler 1987; Chapman 1996). For flies maintained on diets that differed in their yeast density, their life span reaches a peak on an intermediate density diet and decline on either side, while their fecundity continuously decrease (Partridge *et al.* 2005). This decrease in life span by increase of nutrition in the diet has been shown to be associated with increased fecundity (Chippindale *et al.* 1993; Chapman 1996), which implicitly corroborates a trade-off between survival and reproduction predicted by the acquisition-allocation model (Jong 1992). Therefore a suitable diet with a lifespan extending effect of DR is supposed to reflect a negative correlation between survival and fecundity (Bass *et al.* 2007).

The effect of DR on lifespan and reproduction has been studied for a long time. Furthermore, the response of age-dependent mortality rate to DR in *Drosophila* has also been empirically shown, but the effect of DR on mortality rates in late-life in both sexes is rarely investigated. More generally, it is still unclear how DR affects plateaus of mortality rates at advanced ages and whether there is any sex difference in late-life mortality rates given varying concentration of diets. In the present study, I mainly focus on detecting if age-specific mortality rate in *Drosophila* levels off on

three different diets (low, medium and high yeast), on how mortality rates differ in detail between the diet treatments, and whether these effects are sex-specific.

Materials and Methods

Fly stocks

Flies for the experiment were all derived from the wild-type and outbred *D. melanogaster* strain Dahomey, which was originally collected from Dahomey (now Benin, in Africa) and then kept in population cages containing >3000 individuals of both sexes with overlapping generations from 1970. They were maintained in a temperature chamber at 25℃ on a 12-h: 12-h light: dark cycle. In the present experiment, flies were raised on standard 1.0 SY diet (see below in **diets**) with around 200 eggs per vial for two generations since two generations of rearing flies in this standardized environment should remove any strong maternal effects.

Experimental design

30 vials (28.5×95 mm) of 1.0 SY, including 20 male and 20 female flies from the original cage respectively were prepared and kept overnight to let flies mate, reproduce and lays eggs. Flies were anesthetized by CO2 during transfer. Then I trimmed (scraped eggs on diet by needle under microscope) each vial to 200 eggs on average. After ten days, the first generation of sexually mature flies was obtained. Experimental flies in this study were from the third generation. I put 300 individuals of each sex in each cage. To test for diet effect, I designed my experiment with two replicates for each of three diet treatments (0.4, 1.0 and 3.0 SY). Cages were made of transparent plastic (26.5×16.5×15.5 cm) with an opening closed by fine nylon mesh.

Diets

Among a number of different protein sources, Brewer's yeast-based diet was shown to be very suitable for DR studies in *Drosophila* (Bass *et al.* 2007). So I selected standard sugar/yeast (SY) diet (1.0) with Brewer's yeast (MP Biomedicals) in my

experiment. According to the variation in the amount of Brewer's yeast per liter diet, experimental diets are classified as restricted diet (0.4 SY), standard diet (1.0 SY) and obese diet (3.0 SY). The three diets are described in Table 1.

Table 1. Recipe of experimental diets with different concentration of yeast

Diet density (SY)	0.4	1	3
Agar (g/L)	15	15	15
Sugar (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

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

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

To prepare diets, I mixed agar, sugar and Brewer's yeast together in a bowl. Then I added water to the desired volume and mixed well. I transferred the mixed solution to a pot and heated it up to boil. During heating, I stirred it constantly, especially for the 3.0 SY, and let it boil for 5 minutes. I waited until the solution cooled down to 65 degrees Celsius and added Nipagen (dissolved in EtOH) and Propionic acid. Meanwhile, I kept stirring the diets well and adjusted the water volume. Lastly, I dispensed the diet into plastic containers (10×10×4.5 cm) and covered them with cheese cloth to leave over night. On the following day, I packed each container in plastic bags and stored it in a cold room (+2℃).

Statistics

To quantify how mortality rates changed with age in the six experimental cages, I used the program WinModest (Pletcher 1999). I compared four mortality models of

the Gompertz family, fitted to the observed mortality rate by maximum likelihood, and determined the best fitted model for each group, with likelihood ratio testing. Fitting the optimal model to each group, the program estimated parameter values with their upper and lower 95% confidence intervals. Whether there was a significant difference in each parameter between models fit to each sex under the same diet or each diet of the same sex was also estimated by hypothesis testing. I assumed as null hypothesis that each parameter is unique between two groups and as the alternative that there is one parameter constrained to be the same for the two groups. The program computed maximum log-likelihood estimates of models and twice the difference between them was taken to be chi-square distributed with one degree of freedom. If the null hypothesis was rejected (P-value < 0.05), the difference was taken to be statistically significant. Additionally, mean life spans with standard error (SE), median life spans and actual sample size of each sex under each diet treatment were also calculated.

The Gompertz model was originally proposed by Benjamin Gompertz in 1825 (Gompertz 1825) and had been used to demonstrate age-specific mortality. The model contains two parameters:

$$\mu_x = ae^{bx} \quad (1)$$

Where μ_x is the mortality rate at age x, *a* is the mortality rate at birth and *b* is the exponential increase of mortality rate with age. However, recently a lot of studies on demography demonstrated that mortality rates can level off or even decrease after an old enough age (Carey *et al.* 1992; Vaupel 1998; Drapeau *et al.* 2000). Parameter *s* is proposed to characterize the deceleration of increase of mortality rate at advanced ages, which can be fit using the Logistic model:

$$\mu_x = ae^{bx}/[1 + (as/b)(e^{bx} - 1)] \quad (2)$$

If *s* is equal to zero in this function, it indicates that there is no deceleration of increase of mortality rate at older ages and that the Gompertz model is adequate to describe changes of mortality rate with age. All the above parameters are age-dependent. However, age-independent factors sometimes also contribute to total mortality rate and a constant parameter *c* is provided to represent this extrinsic and age-independent death. Based on the Gompertz model and the Logistic model, the Gompertz-Makeham (Equation 3) and the Logistic-Makeham (Equation 4) are respectively providing a better fit when mortality rate is also influenced by age-independent factors:

$$\mu_x = c + ae^{bx} \quad (3)$$

$$\mu_x = c + ae^{bx}/[1 + (as/b)(e^{bx} - 1)] \quad (4)$$

I used the program R to plot curves of mortality rates and to calculate the break-day. Based on functions for every treatment estimated from WinModest, I generated graphs on trajectories of mortality rate across ages. I compared the trajectories between sexes in each diet and among diets of each sex to detect sex differences in mortality rates and dietary effects on the curves. For each logistic model, the maximum in the first derivative of the fitted curve, and its corresponding age were also estimated to figure out when mortality rate started to level off, i.e. its break-day.

Data collection

During the experiment, dead flies were removed from each cage three times a week (Monday, Wednesday and Friday), until all flies had died. Normally, I used a mini-sucker (with USB charger bought in Media Market) to collect dead flies from the ground of the cages. The removed flies were immediately put under a microscope to be sexed and counted. Food for each cage was exchanged twice a week (Monday, Friday). Dead flies on the diet were also sexed and counted. Hence, mortality number of flies in each sex at every weekly check included dead flies gathered by mini-sucker

and lying on the diet, except Wednesday's check, where there was no count of dead flies on diet since there was no exchange of food.

Results

Mean life span

The maximum value of mean life span of 40.7 days was found for females on 1.0 SY diet, and the minimum value was 31.9 days, found in males on 3.0 SY diet (Table 2). Males lived significantly shorter than females on 3.0 SY diet, but not on 0.4 SY and 1.0 SY diet, where difference of longevity between the sexes was not significant (ANOVA comparing mean life span between the sexes. For 0.4 SY diet: $P=0.4375$; for 1.0 SY diet: $P=0.2527$; for 3.0 SY diet: $P=0.0109$; Table 2). However, females and males had the same median life span in the three diet treatments. Moreover, males showed a similarly high lifespan on 0.4 SY diet compared to 1.0 SY diet, but lived significantly shorter on 3.0 SY diet (ANOVA test of mean life span between the diets: 0.4 SY vs 1.0 SY: $P=0.9926$; 0.4 SY vs 3.0 SY: $P<0.0001$; 1.0 SY vs 3.0 SY: $P<0.0001$). Life spans of females were significantly longer on 1.0 SY diet than on either 0.4 SY (0.4 SY vs 1.0 SY: $P=0.0083$) diet or 3.0 SY diet (1.0 SY vs 3.0 SY: $P<0.0001$). Consequently, females' lifespan peaked in the 1.0 SY diet treatment and males' life span peaked on 0.4 SY diet, but was not significantly different from life span of males on 1.0 SY diet.

Age-specific mortality rates

Best-fit models of age-specific mortality rates (Table 3) are shown in Figure 1 and 2, on a logarithmic scale, in an inter-sexual and intra-sexual way respectively. Generally, the Logistic-Makeham model provided the best fit for males on all diets and females on 1.0 SY diet, which suggests mortality rate significantly leveled off at advanced ages in these groups (Figure 1, 2). The best model for mortality data of females on 0.4 SY diet was the Gompertz-Makeham model, and for females of 3.0 SY diet it was the Gompertz model. Therefore mortality rates of males leveled off regardless of the

concentration of diet, in contrast to females. Furthermore, fitted mean longevities highly coincided with actual mean longevities (see Table 2, 3).

Table 2. Summary statistics for males and females on each diet. The statistics were calculated by pooling two replicates

Diet	Sex	Mean life span ± SE (days)	Maximum life span ± SE (days)	Sample size
0.4 SY	Male	39.6±0.4(40)	57.1±0.5	575
	Female	38.3±0.5(40)	55.1±0.3	571
1.0 SY	Male	39.2±0.5(42)	60.1±1.8	591
	Female	40.7±0.6(42)	72.9±2.2	564
3.0 SY	Male	31.9±0.4(33)	51.4±0.7	578
	Female	34.3±0.6(33)	57.4±0.4	580

Note: SE means standard error. Median life span of each category is given in corresponding parentheses. Maximum life span of each category is estimated by mean life span of the oldest individuals accounting for 5% of the population

The break-day of each Logistic-Makeham model is listed in Table 3. Flies on 3.0 SY diet had the earliest age at which mortality rate started to plateau (24 days), while the mortality rate of males on 1.0 SY diet started to level off at the latest age (35 day). The break-day was similar for males on diet 0.4 SY and females on diet 1.0 SY, with 32 days and 33 days respectively. Males exhibited a delayed break-day with decreasing concentration of diet from 3.0 SY to 1.0 SY, whereas the break-day slightly increased when concentration of diet decreased from 1.0 SY to 0.4 SY. Interestingly, the degree of deceleration of mortality rates in males always declined with decreased concentration of diet (Table 3). On 1.0 SY diet, there was no sexual difference in the break-day and degree of deceleration of increasing rate of mortality.

Table 3. The best fitted model for each treatment group and estimated parameter values for each model

	Best fitted model	*a*	*b*	*s*	*c*	break day （days)	Predicted mean life span (days)
0.4 males	LM	6.0×10^{-5} (2.0×10^{-5}-1.9×10^{-4}）	0.19 (0.16-0.23）	0.61 (0.36-1.01）	2.03×10^{-3} (1.20×10^{-3}-3.41×10^{-3}）	32	39.6
0.4 females	GM	2.0×10^{-4} (1.1×10^{-4}-3.8×10^{-4})	0.14 (0.13-0.17)	—	4.47×10^{-3} (3.21×10^{-3}-6.25×10^{-3})	—	38.1
1.0 males	LM	2.93×10^{-7} (3.74×10^{-8}-2.30×10^{-6})	0.33 (0.28-0.39)	1.43 (1.08-1.89）	4.17×10^{-3} (3.17×10^{-3}-5.49×10^{-3}）	35	39.1
1.0 females	LM	2.0×10^{-5} (4.71×10^{-6}-8.0×10^{-5}）	0.22 (0.18-0.26）	1.5 (1.13-2.00）	4.01×10^{-3} (2.84×10^{-3}-5.67×10^{-3}）	33	40.8
3.0 males	LM	5.88×10^{-6} (9.38×10^{-7}-4.0×10^{-5}）	0.34 (0.28-0.42）	1.9 (1.41-2.55）	3.24×10^{-3} (2.14×10^{-3}-4.92×10^{-3}）	24	31.9
3.0 females	G	4.14×10^{-3} (3.31×10^{-3}-5.18×10^{-3}）	7.21×10^{-2} (6.63×10^{-2}-7.85×10^{-2}）	—	—	—	34.3

Note: 95% confidence interval for each parameter value is presented in parentheses. G: Gompertz, LM: Logistic-Makeham, GM: Gompertz-Makeham. "—" means there is no value for that parameter. *a*: the mortality rate at birth, *b*: the exponential increase of mortality rate with age, *s*: the deceleration of increase of mortality rate at advanced ages, *c*: the extrinsic and age-independent mortality

In the 0.4 SY diet treatment, all model parameters were significantly different between males and females except parameter *a* (likelihood ratio tests between sexes, in all cases df =1, for *a*, χ^2=1.9531, P=0.1622; for *b*, χ^2=3.9591, P=0.0466; for *c*, χ^2=7.3485, P= 0.0067; for *s*, χ^2=8.8095, P=0.0029). In the 1.0 SY diet treatment, Gompertz parameters (*a* and *b*) significantly differed between sexes (likelihood ratio tests with df=1. In *a*, χ^2=11.0053, P=0.0009; in *b*, χ^2=12.1924, P=0.0004), but of the two other parameters were not found to be sex-specific (in s, χ^2=0.0629, P=0.8019;

in c, χ^2=0.0326, P=0.8565). In the 3.0 SY diet treatment, there was a significant difference between sexes in parameter *a*, *b* and *s* (likelihood ratio tests with *df*=1, difference in *a*, χ^2=49.3753, P<0.0001; difference in *b*, χ^2=51.6324, P<0.0001; difference in *s*, χ^2=32.8112, P<0.0001), but parameter *c* was not found to be sex-specific (likelihood ratio test with *df*=1, χ^2=0.7056, P=0.4009). In conclusion, the exponential increase in rate of mortality with age in females was dramatically lower compared with that of males on 3.0 SY diet, which predominantly contributed to the elevation of life spans in females.

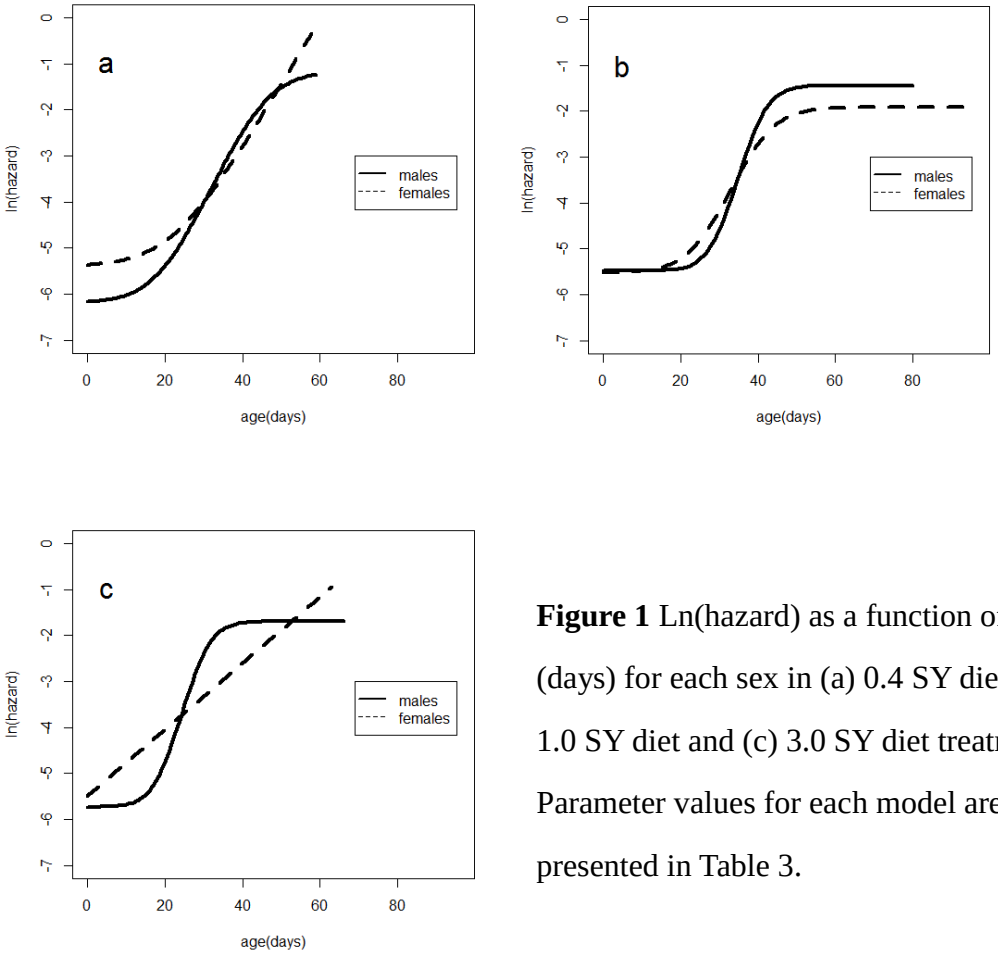

Figure 1 Ln(hazard) as a function of age (days) for each sex in (a) 0.4 SY diet, (b) 1.0 SY diet and (c) 3.0 SY diet treatment. Parameter values for each model are presented in Table 3.

In addition to the illustration of inter-sexual differences in mortality patterns (Figure 1), I also wanted to compare intra-sexual differences between the three diets. Hence, mortality curves for the three levels of diet in each sex are presented in Figure 2. For

models of males kept on 0.4 and 1.0 SY diets, the difference of each parameter was significant (likelihood ratio tests with *df*=1, for *a*, χ^2=21.6938, P<0.0001; for *b*, χ^2=20.7450, P<0.0001; for *s*, χ^2=10.0661, P=0.0015; for *c*, χ^2=7.2609, P=0.0070). Similarly, comparing models of males kept on 0.4 and 3.0 SY diets, estimated parameters significantly differed, except for the age-independent factor *c* (likelihood ratio tests with *df*=1, in *a*, χ^2=5.0221, P=0.0250; in *b*, χ^2=20.1593, P=<0.0001; in *s*, χ^2=17.5442, P<0.0001; in *c*, χ^2=2.0680, P=0.1504). Parameters estimated in models for males maintained on 1.0 and 3.0 SY diets did not significantly differ between these two categories, except for the baseline mortality rate *a* (likelihood ratio tests, *df*=1 for each difference, difference of *a*, χ^2=4.4591, P=0.0347; difference of *b*, χ^2=0.0594, P=0.8074; difference of *s*, χ^2=1.9188, P=0.1659; difference of *c*, χ^2=1.0462, P=0.3063). In summary, prolongation of life span in males that were exposed to diets 3.0 SY and 1.0 SY mainly relied on decreased initial mortality rates, while those on diets 1.0 SY and 0.4 SY primarily accounted for a decline of increase in rate of mortality with age.

Intra-sex difference of parameters for female flies showed the same pattern of significance between each comparison of diets. Specifically, parameter *a, b* and *s* between any two of three diets exhibited a significant difference (likelihood ratio tests between diets with *df*=1. In *a*, 0.4 SY: 1.0 SY, χ^2=11.1844, P=0.0008; 0.4 SY: 3.0 SY, χ^2=45.7506, P<0.0001; 1.0 SY: 3.0 SY, χ^2=122.2587, P<0.0001. In *b*, 0.4 SY: 1.0 SY, χ^2=18.1711, P<0.0001; 0.4 SY: 3.0 SY, χ^2=49.9983, P<0.0001; 1.0 SY: 3.0 SY, χ^2=83.1015, P<0.0001. In *s*, 0.4 SY: 1.0 SY, χ^2=224.1696, P<0.0001; 1.0 SY: 3.0 SY, χ^2=122.2587, P<0.0001), except for comparison of *s* between 0.4 SY and 3.0 SY since there was actually no value of the parameter on both diets. But the age-independent mortality factor *c* was not found to be different significantly among the comparisons (likelihood ratio tests with *df*=1. 0.4 SY: 1.0 SY, χ^2=0.2057, P=0.6501; 0.4 SY: 3.0 SY, χ^2=2.3086, P=0.1286; 1.0 SY: 3.0 SY, χ^2=0.9235, P=0.3365). DR increased life spans in female entirely by a decline of the baseline mortality rates, while the slope of mortality curve was increased by DR.

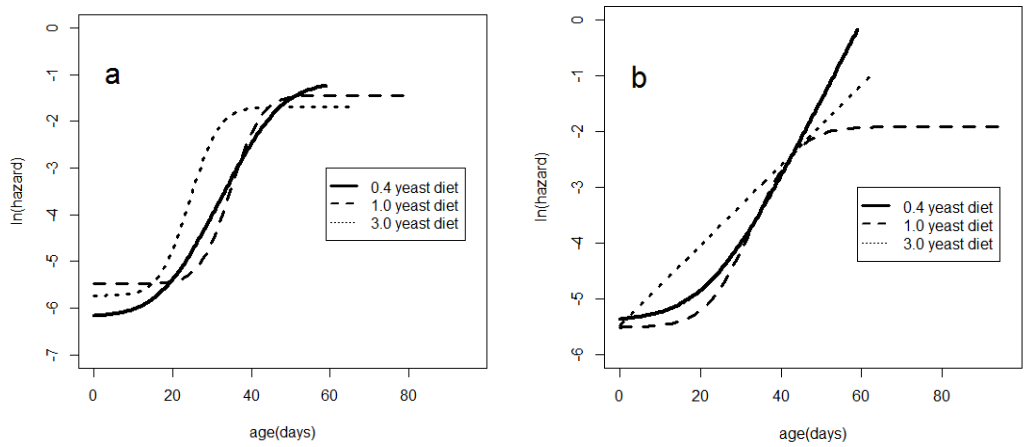

Figure 2 Fitted curves to describe how ln(hazard) changes with age(days) in (a) males and (b) females on the three experimental diets.

Discussion

The principal findings of this study are that plateaus of mortality rate at advanced ages were affected by diet and this dietary effect differed considerably between the sexes. Mortality plateaus were observed in males on all three experimental diets, while females did not show any late-life deceleration on DR (0.4 SY) and obesity (3.0 SY) diets. There was no sexual dimorphism in mortality plateaus on the standard 1.0 SY diet. Intra-sexual mortality plateaus in response to DR could only be compared in males. Males subject to DR considerably postponed the onset of mortality plateaus and the magnitude of the plateaus in response to DR gradually decreased with declining protein quantity. More generally, DR caused mortality to start to decelerate at a later age, resulting in a slower leveling off of mortality at plateau ages. Although DR did not affect the existence of the deceleration of mortality rates at advanced ages in males, the on-set of ageing-related mortalities was distinctly delayed in groups 0.4 SY and 1.0 SY, compared to group 3.0 SY.

The results also demonstrated an inter-sexual difference in life span and a switch of intra-sexual life spans in response to different concentration of diets. Inter-sexual

difference in mean life span was only present on 3.0 SY diet where females lived significantly longer than males, mainly due to the decreased rate of accelerating mortality rates with age. Diets that maximized life span differed between sexes, with females peaking on 1.0 SY diet and males peaking on 0.4 SY diet, although in males, life span on 0.4 SY was only slightly higher than on 1.0 SY. One potential explanation for this sex difference is that the concentration of diet decreasing from 3.0 SY to 1.0 SY was a DR for both sexes, further reduction of concentration in diet from 1.0 SY to 0.4 SY might have been a starvation for females, but not for males. Furthermore, in the demographic analysis, DR prolonged life spans mainly by decreasing the initial mortality rate in females. Interestingly, the slope of mortality curve was mildly increased under the DR treatment, which is similar to findings from a previous study (Magwere & Chapman 2004). Similarly, prolongation of longevity under DR on 1.0 SY, compared to 3.0 SY in males can also predominantly be attributed to a reduction of the initial mortality rate. Therefore the differential response of males and females to density change of diets suggests that DR effects on longevity can be sex-specific, and that diet that maximizes lifespan is not likely to occur in both sexes simultaneously.

Contrary to what I predicted, I did not detect any plateaus of mortality rates for females on the two extreme diets (0.4 SY and 3.0 SY). Age-specific mortality rate had been demonstrated to be influenced by the density of flies (Graves & Mueller 1993; Curtsinger 1995),but the density did not affect existence of deceleration of mortality at older ages (Khazaeli & Xiu 1996). Moreover, three hundred flies of each sex in a cage is supposed to be a sufficient cohort size to correctly describe the function of late-life mortality pattern using maximum likelihood estimation of the Logistic model in WinModest and to decrease the variance of estimated model parameters (Pletcher 1999), suggesting that the absence of the deceleration is not an artifact of small sample size. A possible explanation for undetected mortality plateaus is that different concentrations and components of the diet affect mortality trajectories differently in both sexes, and to a greater extent in females (Müller *et al.* 1997; Carey *et al.* 1998).

These studies however utilized *Ceratitis capitata* as experimental subjects and the diets differed substantially from the ones used here, making a direct comparison difficult. A lack of the plateaus of mortality rates on DR was also documented previously in rats (Pletcher, Khazaeli, & Curtsinger 2000). Another potential explanation I can not exclude is that deceleration of mortality in female flies resulted from demographic heterogeneity. The heterogeneity among individual flies may diminish on extreme diets, which could lead to a reduction or complete disappearance of late-life plateaus. Potential mechanisms that would lead to this pattern are not evident and it would require further research to shed light on them.

The break-day can be defined as the age when late-life started. Previous studies on mortality rates in late-life preferred to characterize the break-day as the age at which age-specific mortality rate stopped increasing exponentially, by fitting a two-stage Gompertz model to mortality rates (Drapeau *et al.* 2000; Rose *et al.* 2002; Shahrestani *et al.* 2012). For ages before the break-day, age-specific mortality rate was modeled by the Gompertz equation. For ages exceeding the break-day, mortality rates were expected to be equal to a constant that was independent of age. However, in the present study I selected one mortality model (out of four candidate models) that fit mortality rates best. I then defined the break-day as the age when the first derivative of the function, i.e. its slope, reached the maximum value. In this case, age-specific mortality rate does not have to be a constant in the late-life period and may still increase, but at a lower rate than before.

A large number of studies have been done to investigate age-specific mortality rate in response to DR in fruit flies (Mair *et al.* 2003; Magwere & Chapman 2004; Bross, Rogina, & Helfand 2005), but many of them did not specifically study changes of mortality rates in late life, and conversely did not allow mortality curve to plateau at late ages, by only fitting the Gompertz model. Moreover, some of my results seem to be inconsistent with them as they generally found DR to result in life span extension in females, and if both sexes were included, an even larger effect of DR on

life span in females, compared to males. Rauser et al. used *D.melanogaster* to look at the mortality response in late life to DR in both sexes, but they exclusively focused on plateaus in late life fecundity and used only two dietary treatments (2005b). Interestingly, there is a strong difference in the effect of diet on plateaus in fecundity and mortality rates. Nutritional level does not affect the existence of plateaus of fecundity at advanced ages in either male or female fruit flies (Rauser *et al.* 2005b). However, the mortality plateaus in response to nutrition level were found to be sex-specific, with the plateaus in female flies eliminated on the two extreme diets. Since heterogeneity among individuals may come from environmental variation (Vaupel & Manton 1979; Vaupel 1990), these observed mortality patterns in female flies in my experiment seem to be in support of the lifelong demographic heterogeneity theory for the evolution of late-life in fruit flies, rather than supporting the theory proposed by Rose et al. (1991) that does not seem to accommodate the non-existence of mortality plateaus as easily.

DR had a strong effect on late-life mortality plateaus but mediated mortality trajectories in fundamentally different ways in male and female *Drosophila*. DR delayed the onset of mortality plateaus and lowered the magnitude of the plateaus in males, but completely eliminated them in females. Interestingly, obese diet also resulted in the elimination of mortality plateaus in females. The lack of lifespan extension in my study underlines the potential for small differences in experimental design to exert a strong effect on lifespan and suggests that DR effects may not be universally robust. Future work should focus on the role of sex-specific selection in generating sexual dimorphism in age-specific mortality rates across all life-history stages, including late-life.

Acknowledgements

I would like to thank Associate Professor Alexei A. Maklakov, Post Doctor Felix Zajitschek for all of their great ideas, invaluable advices and much needed criticism.

Nothing in this thesis would be accomplished without their considerate help. Playing the role as my supervisor, Felix treated me as a student by teaching me how to start a scientific research step by step and also as a friend by caring me so much. My gratefulness to them goes beyond my words. I must thank all my group mates from Ageing Research Group for their help, friendship and support in the lab and in the journal club. Thanks must be given to all my families and friends for all of their supporting hands and listening ears. It's not easy for me to live and to study aboard for the first time, especially in the last academic year full of stress. It wouldn't be possible for me to finish this thesis and my master program on time without them as my lifeblood. Thank you all sincerely.

References

Bass, T.M., Grandison, R.C., Wong, R., Martinez, P., Partridge, L. & Piper, M.D.W. (2007) Optimization of dietary restriction protocols in *Drosophila*. *The Journals of Gerontology. Series A, Biological sciences and medical sciences*, 62, 1071-1081.

Beard, R. (1964) Some observations on stochastic processes with particular reference to mortality studies. *International Congress Actuaries*, 3, 463-477.

Bross, T.G., Rogina, B. & Helfand, S.L. (2005) Behavioral, physical, and demographic changes in *Drosophila* populations through dietary restriction. *Aging Cell*, 4, 309-317.

Carey, J.R., Liedo, P., Müller, H.G., Wang, J.L. & Vaupel, J.W. (1998) Dual modes of aging in Mediterranean fruit fly females. *Science*, 281, 996-998.

Carey, J., Liedo, P. & Orozco, D. (1992) Slowing of mortality rates at older ages in large medfly cohorts. *Science*, 258, 457-461.

Carnes, B.A. & Olshansky, S.J. (2001) Heterogeneity and its biodemographic implications for longevity and mortality. *Experimental Gerontology*, 36, 419-430.

Chapman, T. (1996) Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. *Proceedings of the Royal Society B: Biological Sciences*, 263, 755-759.

Charlesworth, B. (2001) Patterns of age-specific means and genetic variances of mortality rates predicted by the mutation-accumulation theory of ageing. *Journal of Theoretical Biology*, 210, 47-65.

Chippindale, A.K., Leroi, A.M., Kim, S.B. & Rose, M.R. (1993) Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. *Journal of Evolutionary Biology*, 6, 171-193.

Curtsinger, J.W. (1995) Density and age-specific mortality. *Genetica*, 96, 179-182.

Curtsinger, J.W., Fukui, H.H., Townsend, D.R. & Vaupel, J.W. (1992) Demography of genotypes: failure of the limited life-span paradigm in *Drosophila melanogaster*. *Science*, 258, 461-463.

Drapeau, M.D., Gass, E.K., Simison, M.D., Mueller, L.D. & Rose, M.R. (2000) Testing the heterogeneity theory of late-life mortality plateaus by using cohorts of *Drosophila melanogaster*. *Experimental Gerontology*, 35, 71-84.

Gompertz, B. (1825) On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philosophical Transactions of The Royal Society of London*, 54, 513-583.

Grandison, R.C., Piper, M.D.W. & Partridge, L. (2009) Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature*, 462, 1061-1064.

Graves, J.L. & Mueller, L.D. (1993) Population density effects on longevity. *Genetica*, 91, 99-109.

Hamilton, W.D. (1966) The moulding of senescence by natural selection. *Journal of Theoretical Biology*, 12, 12-45.

Jong, G.D. (1992) Acquisition and allocation of resources: genetic (co) variances, selection, and life histories. *American Naturalist*, 139, 749-770.

Kenyon, C., Chang, J., Gensch, E., Rudner, A. & Tabtiang, R. (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature*, 366, 461-464.

Khazaeli, A. & Xiu, L. (1996) Effect of density on age-specific mortality in *Drosophila*: a density supplementation experiment. *Genetica*, 98, 21-31.

Khazaeli, A.A., Xiu, L. & Curtsinger, J.W. Stress experiments as a means of investigating age-specific mortality in *Drosophila melanogaster*. *Experimental Gerontology*, 30, 177-184.

Kirkwood, T.B.L. & Holliday, R. (1979) The Evolution of Ageing and Longevity. *Proceedings of the Royal Society B: Biological Sciences*, 205, 531-546.

Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soran, N. & Raubenheimer, D. (2008) Lifespan and reproduction in *Drosophila*: New insights from nutritional geometry. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 2498-2503.

Magwere, T. & Chapman, T. (2004) Sex differences in the effect of dietary restriction on life span and mortality rates in female and male *Drosophila melanogaster*. *The Journals of Gerontology: Biological Sciences*, 59(1), 3-9.

Mair, W., Goymer, P., Pletcher, S.D. & Partridge, L. (2003) Demography of dietary restriction and death in *Drosophila*. *Science*, 301, 1731-3.

Mair, W., Piper, M.D.W. & Partridge, L. (2005) Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLoS Biology*, 3, e223.

Masoro, E.J., Iwasaki, K., Gleiser, C.A., McMahan, C.A., Seo, E.J. & Yu, B.P. (1989) Dietary modulation of the progression of nephropathy in aging rats: an evaluation of the importance of protein. *The American Journal of Clinical Nutrition*, 49, 1217-1227.

McCay, C.M., Crowell, M.F. & Maynard, L. (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. *Journal of Nutrition*, 10, 63–79.

Medawar, P.B. (1952) *An Unsolved Problem of Biology*. H. K. Lewis, London.

Müller, H.G., Wang, J.L., Capra, W.B., Liedo, P. & Carey, J.R. (1997) Early mortality surge in protein-deprived females causes reversal of sex differential of life expectancy in Mediterranean fruit flies. *Proceedings of the National Academy of Sciences of the United States of America*, 94, 2762-2765.

Norry, F.M. & Loeschcke, V.R. (2002) Longevity and resistance to cold stress in cold-stress selected lines and their controls in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 15, 775-783.

Partridge, L. & Barton, N.H. (1993) Optimality, mutation and the evolution of ageing. *Nature*, 362, 305-311.

Partridge, L., Green, A. & Fowler, K. (1987) Effects of egg-production and of exposure to males on female survival in *Drosophila melanogaster*. *Journal of Insect Physiology*, 33, 745–749.

Partridge, L., Piper, M.D.W. & Mair, W. (2005) Dietary restriction in *Drosophila*. *Mechanisms of Ageing and Development*, 126, 938-950.

Piper, M.D.W., Partridge, L., Raubenheimer, D. & Simpson, S.J. (2011) Dietary restriction and aging: a unifying perspective. *Cell Metabolism*, 14, 154-160.

Pletcher, S. (1999) Model fitting and hypothesis testing for age-specific mortality data. *Journal of Evolutionary Biology*, 12, 430-439.

Pletcher, S.D. & Curtsinger, J.W. (2000) The influence of environmentally induced heterogeneity on age-specific genetic variance for mortality rates. *Genetical Research*, 75, 321-329.

Pletcher, S.D., Khazaeli, a a & Curtsinger, J.W. (2000) Why do life spans differ? Partitioning mean longevity differences in terms of age-specific mortality parameters. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 55, B381-389.

Rauser, C.L., Abdel-Aal, Y., Shieh, J.A., Suen, C.W., Mueller, L.D. & Rose, M.R. (2005a) Lifelong heterogeneity in fecundity is insufficient to explain late-life fecundity plateaus in *Drosophila melanogaster*. *Experimental Gerontology*, 40, 660-670.

Rauser, C.L., Hong, J.S., Cung, M.B., Pham, K.M., Mueller, L.D. & Rose, M.R. (2005b) Testing Whether Male Age or High Nutrition Causes the Cessation of Reproductive Aging in Female. *Rejuvenation Research*, 8, 86-95.

Rauser, C.L., Mueller, L.D. & Rose, M.R. (2006a) The evolution of late life. *Ageing Research Reviews*, 5, 14-32.

Rauser, C.L., Tierney, J.J., Gunion, S.M., Covarrubias, G.M., Mueller, L.D. & Rose, M.R. (2006b) Evolution of late-life fecundity in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, 19, 289-301.

Rose, M.R. (1991) *Evolutionary Biology of Aging*. Oxford University Press, New York.

Rose, M.R., Drapeau, M.D., Yazdi, P.G., Shah, K.H., Moise, D.B., Thakar, R.R., Rauser, C.L. & Mueller, L.D. (2002) Evolution of late-life mortality in *Drosophila melanogaster*. *Evolution*, 56, 1982-91.

Shahrestani, P., Mueller, L. & Rose, M. (2009) Does aging stop? *Current Aging Science*, 2, 03-11.

Shahrestani, P., Quach, J., Mueller, L.D. & Rose, M.R. (2012) Paradoxical physiological transitions from aging to late life in *Drosophila*. *Rejuvenation research*, 15, 49-58.

Vaupel, J.W. (1990) Relatives risks: frailty models of life history data. *Theoretical Population Biology*, 37, 220-234.

Vaupel, J.W. (1998) Biodemographic Trajectories of Longevity. *Science*, 280, 855-860.

Vaupel, J. & Manton, K. (1979) The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography*, 16, 439-454.

Williams, G.C.. (1957) Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, 11, 398-411.