

# A Comparison of Two Different Diets and Their Restricted Groups in Relation to Developmental Time and Viability in *Drosophila melanogaster*

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## Abstract

Effects of dietary conditions on many life history traits have been studied for a long time with *Drosophila*. However, many laboratories use different diets and techniques, which make it difficult to compare the results obtained from various studies. For this reason an optimization protocol for dietary restriction (DR) studies seems to be necessary and in fact recently an optimization protocol published by Bass et al. [1]. In our study we compared the standard diet (S) that we use in our laboratory and the proposed diet (P) with the restricted groups of both diets. In our work, differences between two different dietary protocols with respect to egg-to-adult viability and developmental time were investigated. The P diet did not show any significant differences between the groups with respect to developmental time. On the contrary, the S diet showed significant differences in the yeast and yeast + sugar restricted groups when compared with the respective control and sugar restricted groups.

## INTRODUCTION

Effects of dietary restriction (DR) in ageing studies have been assessed for 70 years since the positive correlation between DR and median life span was first shown by McCay et al. in 1930's [2]. There are strong evidences coming from several studies that show the prolongation of adult life span when they are fed on the diluted food medium [3-5]. Otherwise, it may be assessed to be a species-specific effect rather than a universal rule for all organisms [6]. However, there are lots of evidences coming from

*Drosophila* studies, which already inspire scientists to focus on the effects of DR on ageing [7]. Dietary restriction is applied to *Drosophila* mostly at the adult stage and there are clear evidences that indicate lifespan extension with respect to restricted food medium when compared with standard medium [3,4,7]. However, different laboratories use different sources of yeast and different concentrations of sugar, yeast, and agar for DR [3,5,8-10]. From that point of view, Bass et al. [1] investigated for an optimization protocol, which can be applied to life history trait studies in *Drosophila melanogaster*. They mainly compared the life span and fecundity responses of flies with respect to different diets containing different quantities of yeast and sugar.

The developmental theory is best known as non-

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evolutionary theory of ageing between the other two evolutionary theories, which are mutation accumulation and antagonistic pleiotropy. According to this theory, development is a continuous process and ageing is the part of development [11]. From that point of view, some studies have been focused on larval environmental conditions including diet, which may effect longevity [5,12]. In most of these studies, the relationship between developmental time and ageing has been pointed out as the most important one. Some of the studies showed positive correlation between development time and adult longevity when developmental time increases with respect to larval density [12]. There are also evidences that temperature plays an important role on developmental time which is prolonged at lower temperatures (16°C and 25°C) when the individuals reared at lower and intermediate temperatures for five years (16°C and 25°C) [13], but adult longevity is not inversely related to developmental temperature and developmental time had not a causal determinant of adult longevity [14]. The relationship between increased developmental time and adult longevity has been investigated by several researchers, because of the fact that dietary restriction extends developmental time. In general, dilution of food medium retards developmental time, but the increase in developmental time has no effect on adult lifespan [15,16]. However, it must be stressed that this problem needs to be investigated in a broader sense.

In this study, we tried to find out an optimum food medium for developmental time for *Drosophila melanogaster*. Differences and optimization levels in developmental time and egg-to-adult viability were assessed by comparison of two different food mediums; one of them is our standard diet (S) we use for about 40 years and the other is the proposed diet (P) from the study of Bass et al. [1].

## MATERIALS AND METHODS

### Strain and Culture Conditions

The samples of *Drosophila melanogaster* used in the study were collected from *Girne* in Cyprus 2007 (Table 1). Laboratory stocks of these populations were constructed immediately after collection and they have been maintained since then in half-pint bottles with overlapping generations on a 12-12 h light-dark cycle at 21°C and 55% R.H. (relative humidity).

Table 1. Geographical locations (as latitudes) of the population and some relevant climatic parameters for the sampling site.

Population	Latitude	T <sub>year</sub> (C°)	R <sub>year</sub> (mm)	H <sub>year</sub> (%)
Girne	35°07'	19.00	402.8	68.4

T<sub>year</sub>: Total yearly temperature; R<sub>year</sub>: Total yearly rainfall; H<sub>year</sub>: Total yearly humidity.

### Media

Experiments were based on two different sugar-yeast food mediums with having different quantities to assess the effects of different food mediums and their restricted groups on developmental time. The experiment was conducted with eight different food regimes as shown in Table 2. Two of the food regimes were held as the controls, one is the standard which we use to maintain our laboratory stocks (*S Diet*) [17] and the other is the proposed (*P Diet*) as used in the study of Bass et al. [1]. The other six regimes were modified with respect to pertinent controls.

### Egg collection

About 500 flies were taken from each population to be the parents of the experimental flies and were transferred to 15 laying pots in approximately equal numbers containing yeasted corn meal medium (*S Diet*). After an acclimation period of 24 h at 21°C, flies were transferred to fresh medium for a 2 h pre-lay period and then transferred again to fresh medium for 4 h at 21°C for egg collection. Eggs were collected 4 h after the midpoint of the laying

Table 2. Nutritional composition of experimental food types.

Food Type		Nutritional Composition (Grams of Components per Liter Water)		
		Sugar (g)	Yeast (g)	
<i>S Diet</i>	1	Control 1 (C 1)	94	19
	2	DR sugar / Control yeast 1 (DR-S 1)	47	19
	3	Control sugar / DR yeast 1(DR-Y 1)	94	9.5
	4	DR sugar and yeast 1 (DR-SY 1)	47	9.5
<i>P Diet</i>	5	Control 2 (C 2)	50	100
	6	DR sugar / Control yeast 2 (DR-S 2)	25	100
	7	Control sugar / DR yeast 2(DR-Y 2)	50	50
	8	DR sugar and yeast 2 (DR-SY 2)	25	50

period. Eggs were placed in vials containing 7 mL of two base mediums and their restricted groups for at least ten replicates consisting of ten eggs per vial.

### Developmental Time

Developmental times were measured as egg-to-adult mean developmental times and numbers of adults were scored every 12 h a day until the adults did not emerge from the vial for 72 h period.

### Viability

Viability (egg-to-adult survival) was measured as the ratio of the total number of emerged flies to initial egg density of vials. Controls and collecting of emerged adults were made every 12 h after pupal darkening. Flies were distinguished by sex at the time of collection. Collecting the flies was terminated when no adults were observed in the vial for 72 h period.

### Statistical Analyses

We calculated average development times according to sex for each individual vial during the experiments. Variation between the food regimes and between sex specific developmental time were tested using a one way ANOVA test (SPSS 15.0).

## RESULTS

### Developmental Time

Dietary restriction is applied in *Drosophila* by the simultaneous dilution of nutrient in the standard corn meal medium (*S diet*) in which the yeast is the only source of protein and sugar as a main source of carbohydrate. We tested the separate effects of sugar and yeast on developmental time and its difference of the proposed food medium (*P diet*) for DR studies by Bass et al. [1] from our standard food medium and calculated average developmental times for each experimental food regime by sex.

Mean values of developmental time for both females and males are given in Table 3. Average developmental times for different food regimes varied between 271.06 and 345.27 h. In *P diet*, mean developmental times of the flies were shorter when compared with the flies that developed in the *S diet*. Analysis of variance (ANOVA) in developmental time comparisons between control and restricted groups are given in Table 4. A significant interaction was found in the *S diet* and their restricted food media when developmental time is selected as a dependent variable ( $P < 0.0001$ ). No

Table 3. Analyses of variance performed to test the differences between male and female egg-to-adult mean developmental time.

Food type	Female			Male			P values*
	n	Mean (hours)	SE	n	Mean (hours)	SE	
C1	56	290.93	3.290	50	289.24	3.636	1.00
DR-S 1	53	293.53	3.530	47	298.70	3.942	1.00
DR-Y 1	45	345.27	3.094	34	339.82	4.617	1.00
DR-SY 1	33	327.55	4.037	50	324.52	3.234	1.00
C2	34	271.06	1.352	26	278.31	1.334	<0.05
DR-S 2	29	273.93	1.793	22	282.55	1.717	<0.05
DR-Y 2	31	276.00	1.113	24	286.00	1.560	<0.001
DR-SY 2	20	272.40	2.320	31	280.65	1.067	0.056

\*Statistical significance of the differences was tested with Games Howell test.

significant differences in developmental time were observed in the *P diet* groups (C2, DR-S2, DR-Y2 and DR-SY2). However, developmental time differences between sexes were observed in the *P diet* (Figure 1). The females have a significantly (Table 3) shorter means of developmental time than the males. There were no significant developmental differences between sexes in *S diet* (Figure 1). In *S diet* yeast restriction and sugar together with yeast restriction prolonged developmental time significantly against their control (Figure 1).

Table 4. Results of one-way ANOVA of mean developmental time of *D. melanogaster* reared on S and P media with the restricted groups.

Food type	S Diet			P Diet		
	d.f.	MS	F	d.f.	MS	F
Food type	3	57459.412	90.790***	3	72.170	1.528
Error	370	632.882	-	221	47.244	-

\*\*\*p < 0.001. d.f.: degree of freedom, MS: mean square.

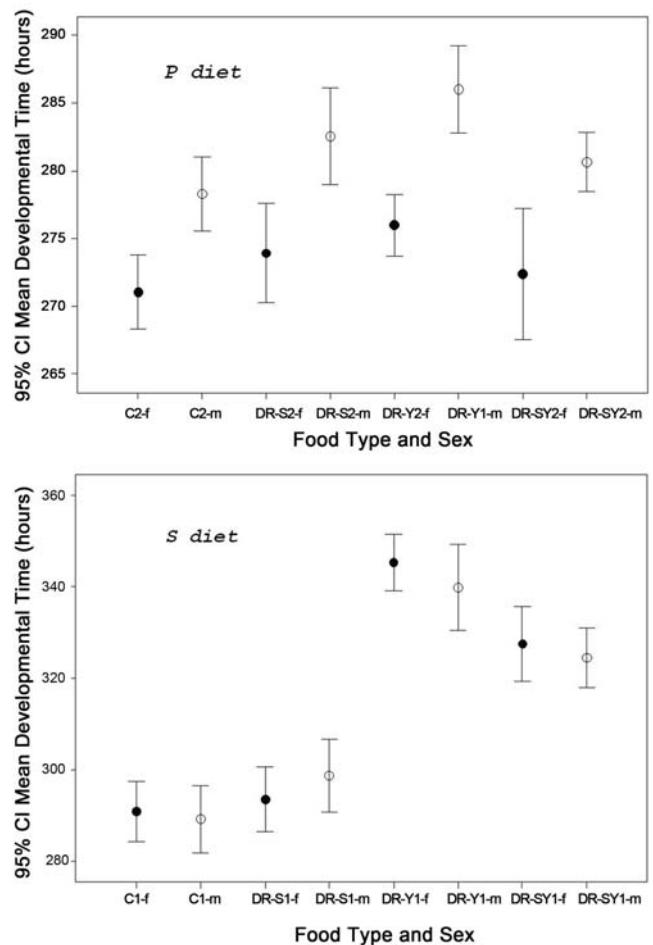


Figure 1. Mean developmental times and 95% confidence intervals of the females and males from S and P diets. f: female, m: male.

## Viability

More flies survived at the *S diet*. However, the viability in the *P diet* is significantly decreased (Table 5,  $P < 0.0001$ ). The decreased viability has no interaction with restriction of sugar or yeast, the unsuccess is a general effect of the *P diet*. Figure 2 shows a plot of mean egg-to-adult viability, and the decrease of viability is clearly seen in the *P diet*.

Table 5. Analyses of variance on egg-to-adult viability in both diet groups and their restrictions.

Group	<i>n</i>	Viability †	SD
C1	150	0.707***	0.158
DR-S 1	120	0.833	0.078
DR-Y 1	100	0.790**	0.129
DR-SY 1	100	0.820*	0.155
C2	100	0.610***	0.137
DR-S 2	100	0.550***	0.143
DR-Y 2	100	0.550***	0.135
DR-SY 2	100	0.510***	0.173

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

†The ratio of the total number of emerged flies to initial egg density of vials.

*n*: Sample size, SD: Standard deviation.

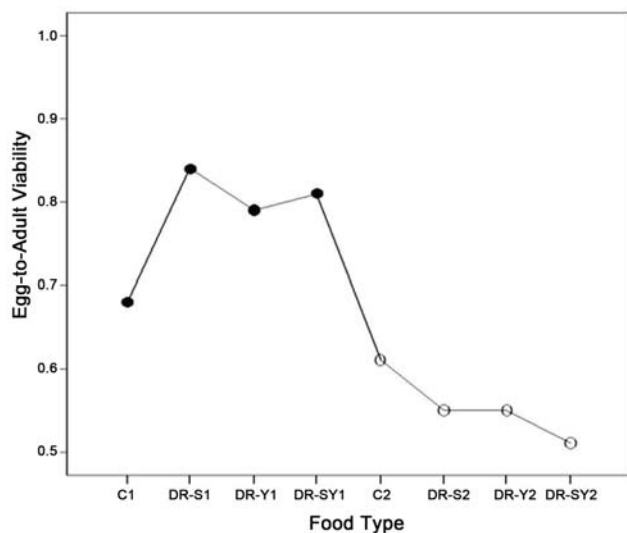


Figure 2. Plot of egg-to-adult viability of *S diet* (black circles) and *P diet* against the diet restriction of the food medium with their controls.

## DISCUSSION

As we mentioned in the introduction, there are some differences between the diets and techniques of different *Drosophila* laboratories, which make it

difficult to compare the outcomes of DR studies. Therefore, it seems necessary to optimize dietary protocols to get more reliable results and to make trustworthy comparisons between the results of different studies. In this respect, Bass et al. [1] proposed an optimization procedure to show which diets are suitable for DR studies in *Drosophila*. In that study, they primarily focused on the differences of life span and fecundity with respect to diets with different quantities of sugar and yeast. Only one of the experimental diets showed optimum effects on survival and fecundity and this diet was proposed as an optimal diet for DR studies in *Drosophila* [1]. They did not only study with different quantities of yeast in the diet but also with five different types of yeasts. Indeed, effects of yeast on life span and fecundity seem to be depending on yeast type and quantity. Bass et al. [1] showed that life span is longer at 100 g/L yeast concentration in all yeast types but female fecundity maximized at the levels of 100 g/L and 200 g/L yeast depending on the type of the yeast (In two types of yeast 200 g/L yeast decreased fecundity when compared with 100 g/L yeast). They also showed the detrimental effects of high sucrose levels on life span ( $> 50$  g/L) and female fecundity ( $> 100$  g/L). 50 g/L sucrose seems to be the optimum level both for life span and fecundity.

According to developmental theory of ageing, ageing is the part of development. Also, there are clear evidences that *Drosophila* life span is in relation with the environmental conditions (temperature, larval density, food abundance, habitat, etc.) where they grown up. For this reason, we think that it is also crucial to make an optimization for the developmental stage in the concept of food medium. Additionally we tested the effect of diet, which we use in our laboratory (*S diet*) on developmental time.

*Drosophila* can be maintained in the laboratory on a



combination of sugar, yeast and water [18]. Bass et al. [1] found that addition of sugar above 50 g/L in the culture media was destructive for egg laying and has little effects on life span. Similarly in our study, there are no significant effects of decreased levels of sugar on developmental time in both *S* and *P diet*. Actually, these findings support that *Drosophila* has a very low necessity for free sugar for maximal life span, fecundity [1] and developmental time. However, some other experiments showed that sugar levels above 50 g/L could affect the feeding behavior in *Drosophila* [6,19,20].

Yeast has been shown as the most important compound of the food medium in *Drosophila* studies by several researchers [1,5,6,15]. Life history traits like ageing, fecundity, viability and development are directly affected by the levels of yeast used in the food medium. Previous studies link to a possibility of dose-dependent toxicity in yeast level [1,21]. Bass et al. [1] compared five different yeast types and found in context to the yeast type variable effects of fecundity and life span. Yeast extract at high concentration is detrimental to fecundity in addition to negatively affecting life span [1].

We found some significant developmental time differences in *S diet* with yeast restriction in contrast to *P diet*; there were no developmental time differences in *P diet* with or without yeast restriction. There was also a 50% decrease in egg-to-adult viability in *P diet* which gives us the idea that the yeast levels in *P diet* is very high. It is possible that the high levels of yeast have a toxic effect at the early stages of development. Furthermore, developmental time differences between sexes were observed only in *P diet*, females were developing faster than males at higher levels of yeast.

The yeast level (100 g/L) in the *P diet* seems to be high when compared with *S diet*. High levels of

yeast may be optimum for life span and fecundity but it is not for the developmental rate. High levels of yeast retards development and decreases egg-to-adult survival as can be seen in our results. Even we used the same diet given by Bass et al. [1], the commercial baker yeasts are different which may also cause some differences. In conclusion, we want to emphasize that our findings do not support the reference study of Bass et al. [1] completely, but tries to give some detailed approach of food composition effect on the developmental time and viability. Whereas the previous authors did not study these traits. Ageing and development are closely related events as mentioned by several studies. Thus we cannot think development and ageing to be independent from each other. For this reason we conclude that, the diet in *Drosophila* must be optimized for all life history traits. Therefore, further studies will probably may help to understand the interaction between diet and development as well as ageing.

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