The effect of dietary restriction on developmental time in *Drosophila melanogaster* and its sibling *D. simulans.*

Önder, B.Ş.*, and M. Yilmaz. Hacettepe University, Faculty of Science, Department of Biology, 06800, Ankara, TURKEY. *Corresponding author: bdalgic@hacettepe.edu.tr

Abstract

We investigated developmental time difference in response to dietary restriction (DR) in two sibling species, *Drosophila melanogaster* and *Drosophila simulans*, which were collected at the same time from two different localities in Turkey. Different diets used in this experiment were: standard (C), sugar reduced (DR-S), yeast reduced (DR-Y), and sugar with yeast reduced (DR-SY) diets. When the species developmental times in response to different DR were analyzed, both of the species showed the same pattern. We did not observe significant difference in relation to developmental time between different populations of *D. melanogaster* whereas two *D. simulans* populations showed significant developmental time differences. As a major result, egg-to-pupa developmental time was observed to be prolonged-mostly due to yeast restriction.

Introduction

D. melanogaster and *D. simulans* are sibling species of the *melanogaster* species complex. Previous studies with these two sibling species have shown many slight but significant differences due to the divergence of the ecological niches (*e.g*., David *et al.,* 2004). Although limited in scope and number, studies of life history traits in *Drosophila* have exposed considerable interspecific variability (Markow and O'Grady, 2006). The intra- and interspecific variability of life history traits can be explained not only by the genetic constitution of species or populations but also by environmental effects (food abundance, heat, etc.), and genotype by environment interaction (James *et al.*, 1997; Gibert *et al.*, 2004; Lazzaro *et al.*, 2008). Developmental time, a very important life history trait, is largely affected by environmental conditions (James and Partridge, 1995). Nutritional manipulation is one of the mostly used ways to expose the effects of food as an environmental variable on aging and development of the organisms. *Drosophila* is being increasingly used as a laboratory model for life history evolution (Powell, 1997). *Drosophila* is an organism that breeds and feeds in ephemeral substrates; therefore, the larval developmental time is a very important trait (Chippindale *et al.*, 1997; Soto *et al.,* 2006; Folguera *et al.,* 2008). Important levels of genetic variation in developmental time occur in natural populations (Cortese *et al.,* 2002; Fanara *et al.*, 2006).

The effects of DR have been investigated for more than 70 years in various organisms. Although DR is known to extend the life span of a wide range of organisms, species-specific effects of DR restriction have also been recorded (*e.g*., Mockett *et al.,* 2006). Restriction of yeast levels prolonged the life span in *D. melanogaster* (Chippindale *et al.*, 1993; Mair *et al.,* 2005; Min and Tatar, 2006; Piper and Partridge, 2007). There are various DR studies that were focused on the adult stage of *Drosophila*, but only a few studies were conducted to investigate the effects of DR on juvenile stages (Tu and Tatar, 2003).

Species that have the ability to move away from unsuitable conditions are unable to increase

their longevity with DR, because leaving an area is the best and easiest strategy (Bourg and Minois, 2005). The quality of the larval medium is very important with respect to developmental time (Chippindale *et al.*, 1997; Soto *et al.*, 2006; Folguera *et al.,* 2008), as larvae with limited dispersal ability should complete their development in the poor medium conditions.

In the scope of this study we addressed these questions: 1) What is the response of the two closely related species, *D. melanogaster* and *D. simulans*, to different DR regimes, which are applied at early developmental stages? Are there any differences between and within species? 2) Which component of the food medium affects the developmental time?

Materials and Methods

Culture and Diet

The stocks (*Drosophila melanogaster* and *Drosophila simulans*) used in this experiment were constructed from the samples taken in Edirne in September 2006 and in Antalya in May 2006 in Turkey (Table 1). The stocks have been maintained in half pint bottles with overlapping generations with a 12-12 h light-dark cycle at 25°C and 60% R.H.

Table 1. Geographical locations (as latitudes) of the populations of the two species and some relevant climatic parameters for the sampling sites.

Population	Latitude	T_{year} (C°)	R_{year} (mm)	H_{year} (%)
Edirne	41° 39’	13.56	585.9 mm	70
Antalya	36° 54’	18.17	1068 mm	64

T_{year}: Total yearly temperature; R_{year}: Total yearly rainfall; H_{year}: Total yearly humidity

Four different food types were used to measure developmental time differences due to DR. One of the food types was standard cornmeal medium, which we use to maintain our laboratory stocks. The other three food types were the restrictions with respect to the standard. The components of these food types are given in Table 2.

Table 2. Nutritional composition of experimental food types.

Food Type	Nutritional Composition (Grams of Components per Liter Water)	
	Sugar	*Yeast*
Control (C)	94 g	19 g
DR sugar / Control yeast (DR-S)	47 g	19 g
Control sugar / DR yeast (DR-Y)	94 g	9.5 g
DR sugar / DR yeast (DR-S/DR-Y)	47 g	9.5 g

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 cornmeal medium. After an acclimation period of 24 h at 25°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 25°C for egg collection. Eggs were collected 4 h after the midpoint of the laying period. Eggs were placed in vials containing 7 mL food media, as five replicates consisted of fifty eggs per each experimental group.

Developmental time

Developmental time was measured as the mean egg-to-pupa developmental time, and numbers of pupae were scored every four hours a day until the number of the pupae in each vial did not change for 72 hours.

Statistical Analyses

First, we tested for differences in the mean egg-to-pupa developmental times among food types by the analysis of variance. Two way ANOVA between food type and populations within species was carried out, and also three-way ANOVA between species, population, and food type was applied to investigate the effect on developmental time.

In all cases the datasets were checked if the assumptions of normality and homogeneity of variances required for ANOVA were satisfied. All tests were done by using SPSS 15.0.

Results

DR applied in *Drosophila* by the simultaneous dilution of nutrient in the standard cornmeal medium in which the yeast was the only source of protein and sugar as the main source of carbohydrate. We tested the separate effects of sugar and yeast on egg-to-pupa developmental time and calculated average developmental time for each species and population. In this experiment, average egg-to-pupa developmental times for different populations and different food types varied between 126.08 and 214.89 hours (Table 3 and Table 4). A two-way ANOVA was carried out to evaluate the differences in the egg-to-pupa developmental time with respect to population and food type (Table 3 and Table 4). For both species a two-way ANOVA showed significant interactions between developmental time with yeast and sugar/yeast restriction. Table 3 shows the mean egg-to-pupa developmental time and significant comparisons of *D. melanogaster* populations. Developmental time of both populations that fed on DR-SY were observed to be prolonged significantly (Table 3). However, when we compared the developmental time of yeast restricted and sugar-yeast restricted populations, we found that yeast restrictions developed more slowly. In addition, developmental time of sugar restricted *D. melanogaster* Antalya population was significantly different from those of DR-Y and DR-SY groups ($p < 0.001$).

Table 3. Results of multiple comparisons of ANOVA and descriptive statistics for developmental time of *D. melanogaser*'s population.

Species / Population	Food Type	n	Mean ± S.E. (in hours)	Significant comparisons†
D. melanogaster ANTALYA	1. Control	221	142.70 ± 0.80	1-3***
	2. DR-S	219	138.32 ± 0.64	1-4*
	3. DR-Y	216	182.39 ± 1.28	2-3***
	4. DR-SY	226	163.84 ± 0.91	2-4***
D. melanogaster EDİRNE	1. Control	218	138.17 ± 0.72	
	2. DR-S	204	152.61 ± 1.13	1-3***
	3. DR-Y	211	169.00 ± 1.14	1-4*
	4. DR-SY	232	157.00 ± 0.84	

n = sample size; *S.E.*= Standard Error
*p<0.05, ***p<0.001
†significative values after Bonferroni correction for multiple comparisons.

D. simulans egg-to-pupa mean developmental times are given in Table 4. When compared to the control group, we found statistically significant differences in developmental times of all DR groups (DR-S, DR-Y, DR-SY). The Control and the DR-S groups developed faster than the DR-Y and DR-SY groups. Also the longest developmental time from egg-to-pupa was observed in the yeast-restricted group.

Table 4. Results of multiple comparisons of ANOVA and descriptive statistics for developmental time of *D. simulans*'s population.

Species / Population	Food Type	n	Mean ± S.E. (in hours)	Significant comparisons†
D. simulans ANTALYA	1. Control	209	158.01 ± 1.10	1-2**
	2. DR-S	172	135.88 ± 1.00	1-3***
	3. DR-Y	191	214.89 ± 1.73	1-4***
	4. DR-SY	194	184.52 ± 1.18	2-3***
				2-4***
				3-4***
D. simulans EDİRNE	1. Control	242	126.74 ± 0.76	1-3***
	2. DR-S	224	126.08 ± 0.82	1-4***
	3. DR-Y	185	172.84 ± 1.13	2-3***
	4. DR-SY	192	153.58 ± 1.10	2-4***
				3-4*

n = sample size; *S.E.*= Standard Error
*p<0.05, **p<0.01, ***p<0.001
† significative values after Bonferroni correction for multiple comparisons.

Table 5. Results of the analysis of variance for developmental time testing for differences between species, populations and food types.

Source of variation	*df*	*Mean Square*	*F*	*P-value*
Food	3	9363.356	152.2993	0.000
Population	1	4507.203	73.3117	0.000
Species	1	239.778	3.9001	0.053
Species*Population	1	3186.045	51.8225	0.000
Species*Food	3	957.387	15.5723	0.000
Population*Food	3	748.278	12.1711	0.000
Species*Population*Food	3	4.187	0.0680	0.978
Error	64	61.480		
Corrected Total	79			

df: degrees of freedom

Both populations of these sibling species were observed to have an extended and significant ($p < 0.001$) mean developmental time with respect to yeast restriction.

The effects of the food type, species, and population on egg-to-pupa developmental time were analyzed using a three-way analysis of variance (ANOVA) in which food type (C, DR-S, DR-Y, DR-SY), species (*D. melanogaster, D. simulans*), and population (Antalya, Edirne) constituted the three factors in the analysis (Table 5). Significant effects were established of food and population ($p < 0.001$) on developmental time. We found that the species did not affect developmental time significantly, but in margin ($p = 0.053$). There is a significant two-way interaction between species and food type ($p < 0.001$), species and population ($p < 0.001$), food type and population ($p < 0.001$), whereas we did not find any significant interactions between species, food type, and population ($p = 0.978$).

We found that yeast restriction had highly pronounced effect on developmental time and that yeast restriction put a high delay on larval development in all populations of the two species. Figure 1 shows the means of egg-to-pupa developmental times with respect to DR variation. Additionally,

the feeding in the DR-SY medium prolonged developmental time, too. The sugar-restricted groups have small, insignificant effects on developmental time.

The developmental time patterns within and between species are mostly similar (Figure 1). However, while there was a significant difference between two populations of *D. simulans* ($p < 0.001$), we did not find any significant difference between two populations of *D. melanogaster* ($p = 0.770$).

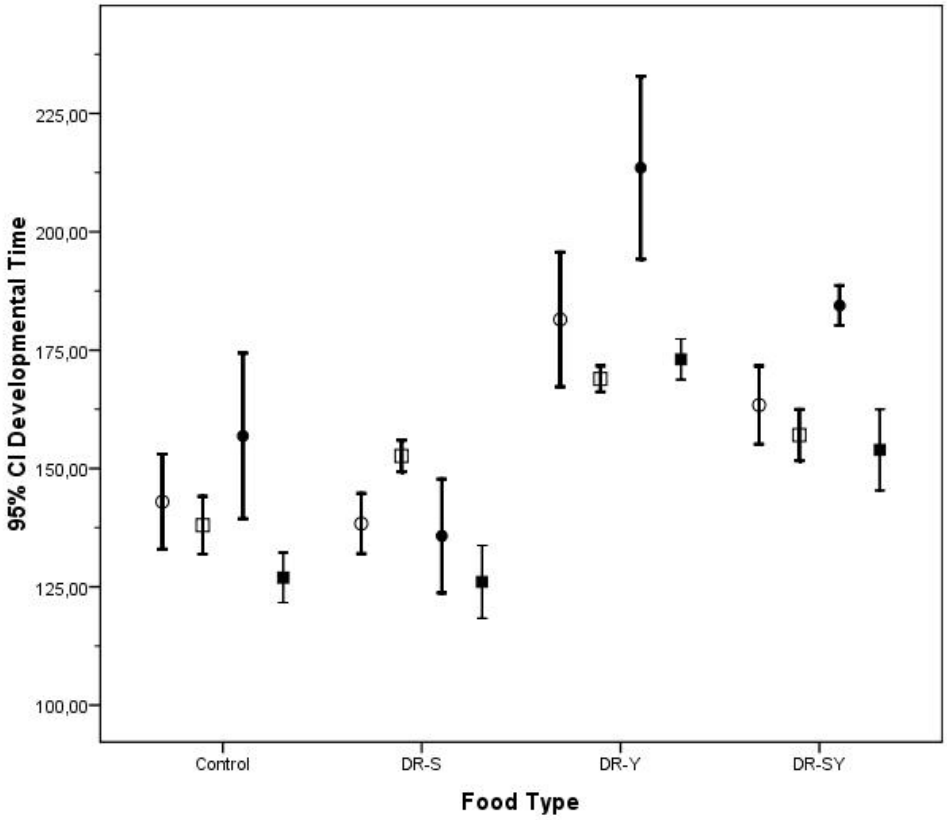

Figure 1. A 95% confidence intervals of egg-to-pupa developmental times in Antalya and Edirne populations of *D. melanogaster* and *D. simulans* feeding on different food types. (*D. melanogaster*, open labels; *D. simulans*, black labels; Antalya populations, round labels; and Edirne populations, square labels).

Discussion

As a major result of our study, developmental time is affected at-most with yeast restriction, and this observed pattern seems not to be depending on species or their populations. As seen in Tables 3 and 4, two populations of *D. melanogaster* have nearly the same overall average developmental time (slightly shorter in Edirne; not significant), but populations of *D. simulans* differ significantly from each other. The comparisons between the DR groups within population show us that restriction of yeast lengthened the developmental time at most, regardless of species or population. This may be due to the larval tendency to appropriate more protein from the yeast restricted food medium, which postpones pupation time (*e.g.*, Gebhardt and Stearns, 1993).

Nearly all of the DR studies are conducted at the adult stage. Only a few studies focused on juvenile DR and its effects (Tu and Tatar, 2003). In the study of Tu and Tatar, they found that although many adult traits were affected by larval nutrient conditions, nutrition seems to be affecting adult aging only when applied at the adult stage. They emphasize that *D. melanogaster* DR applied at the larval stage does not have an impact on adult aging, and larval yeast restriction did not cause an increasing rate of mortality. However, in our previous study we found that age-specific mortality and mean longevity is affected by larval dietary restriction depending on species, population, and sex (Onder *et al.*, 2009). There are also other evidences which indicates developmental time's correlation with some adult fitness components (Cortese *et al.*, 2002; Folguera *et al.*, 2008).

It is interesting to note that the developmental time patterns in response to DR in these siblings are quite similar. As mentioned before, the major component of food medium, which affects egg-to-pupa developmental time, is yeast. Grandison *et al.* (2009) find that dietary essential amino acids affected lifespan and fecundity in *D. melanogaster*. Especially adding methionine alone increases fecundity without shortening lifespan by female flies. The results of Grandison *et al.* imply that further investigations should focus on the effects of variable amino acid concentrations on developmental time. We conclude that further dietary restriction studies should focus on the relationship between developmental time and some other life history traits.

Acknowledgments**:** The authors would like to thank Nihal Ata, Ozge Erisoz Kasap, and Ergi Deniz Ozsoy for help and comments on the manuscript.

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