

The effects of larval diet restriction on developmental time, preadult survival, and wing length in *Drosophila melanogaster*

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Abstract: Environmental stress effects on life history traits have been shown by many studies up to the present. Organisms are frequently affected by nutritional stress in nature. Nutritional restriction has been used as an artificial environmental stress by scientists since 1935. As a result of such studies, it is known that nutritional intake during developmental stages can affect many life history traits as a response to environmental stress in *Drosophila melanogaster*. In this study, we used 15 different diet regimes with variable yeast and sugar concentrations to test the effects of dietary changes on viability, developmental time, and wing size. Our data showed no specific relationship between yeast-sugar concentrations and larva-to-pupa or larva-to-adult viability. However, over 90% of all larvae that achieved the pupal stage could develop to the adult stage. As expected, the developmental time was moderately affected with respect to yeast-sugar concentrations and their interactions. In addition, the developmental time was extended with decreases in yeast and sugar concentrations. When calculating the pupation and larval times, we came up with the result that longer larval developmental time was buffered under restricted conditions by a shorter pupation period. In other words, dietary stress extended the larval development period and shortened the pupation period to make up for the developmental time delay. Our study indicated that sugar-free larval nutrition reduced the common positive effects between nutrition and body size. Measurement of wing lengths presented sex-specific fluctuations with increased sugar concentration, which showed variable interaction with yeast concentration.

Key words: Larval nutrition, wing size, developmental time, viability, *Drosophila melanogaster*

1. Introduction

Studies on different life history trait strategies under stressful environments are crucial in understanding how evolution shapes an organism under extreme conditions (Roff, 1992; Stearns, 1992; Flatt, 2011). Effects of dietary restriction (DR) as an environmental stress on life history traits have been assessed over the past 80 years, since the first positive correlation shown by McCay et al. (1935) between DR and median life span. Nutritional manipulation is one of the most frequently used ways to expose the effects of food as an environmental variable on the life history traits of organisms (Onder and Yilmaz, 2009). Subsequent studies with model organisms have indicated that life history traits change with respect to decreasing food medium (Mair, 2005; Speakman and Sharon, 2011).

The amount and quality of nutrients consumed by organisms have a particular impact on life history traits,

including developmental time, body size, and survival. Exposure to stress during developmental periods is significant for all organisms, especially for holometabol insects; moreover, instability in developmental periods can affect many adult characters. A nutrition intake decrease in the developmental stage extends developmental time and reduces adult body and organ size (Shingleton et al., 2008). Roff modeled the interaction between growth, developmental time, and body size in 2000 (Roff, 2000). Depending on the model, all variables have close relations with each other and are also affected by environmental stress. Variations in the quantity or quality of an acceptable diet can have profound effects on insect development (Chapman, 1998).

Developmental stage stability is highly related to an organism's fitness, which critically depends on growth and development, as in the model of Roff (2000). However,

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shorter developmental time, decreased sexual maturity age, and generation time increase larval survival by shortening the developmental period (Flatt, 2011). Survival is one of the most important traits that Darwin put forward in 1859 (Darwin, 1859). In particular, the preadult developmental period is under high predatory risk; for this reason, longer developmental time is critical for larval viability. On the other hand, long developmental time causes a longer growth period and bigger body size (Stearns, 1992; Stearns et al., 2000). Adult body size in insects is utterly relevant to growth during larval stages because of the growth-restricted exoskeleton in adults (Shingleton, 2011). The total body size and the size of external organs are fixed at the point of metamorphosis. From this point of view, restriction in larval nutrition during development reduces the body size in a wide range of insects.

For dipterans, larval nutrition affects all fitness components by mediating various aspects of adult body size; this trait is directly related to fecundity (Bergland et al., 2011). Additionally, environmental signals play an effective role during larval body development. For example, the imaginal discs of *Drosophila* larvae will metamorphose to the adult body with a mass increase; they will also gain responsively against both internal and external environmental signals.

The time of metamorphosis is determined by the size of the final larval instar in *D. melanogaster*, which is called critical size or critical weight. Reaching the critical size is incorporated with initiation of the hormonal cascade that ends in metamorphosis (Shingleton, 2010). After reaching the critical size, larval feeding and body growth persist for a period of time. This delay provides a final period of growth for the larvae, which is called the terminal growth period (TGP). During the TGP, *Drosophila* larvae can more than triple their mass (Shingleton et al., 2008). In *Drosophila*, body size is regulated by critical size and the growth reached during the TGP (Davidowitz et al., 2003; Nijhout et al., 2006; Shingleton et al., 2008). In general, body size in holometabolous insects is regulated by 3 major factors: the critical size, TGP, and growth rate, which are all affected by environmental conditions.

The aim of this study was to test the effects of larval nutrition on viability, developmental time, and wing size

with their interactions. In nature, the most important and often limiting macronutrients for *Drosophila* larvae are proteins and carbohydrates (Mattson, 1980; Markow et al., 2001); therefore, we chose to investigate the effects of different concentrations of sugar and yeast in larval nutrition on viability, developmental time, and adult wing length.

2. Materials and methods

2.1. Flies and husbandry

The *Canton-S* inbred laboratory strain of *D. melanogaster* was used in the present experiments. Flies were maintained in half-pint bottles of nonoverlapping generations on a 12:12 h light:dark cycle at 21 °C and 55 ± 5% relative humidity on a standard cornmeal (CSY) medium (50 g of cornmeal, 50 g of sugar, 50 g of yeast, 10 g of agar, and 6 mL of acid in 1 L of water).

2.2. Breeding design, developmental time, and viability

Experiments were performed using 15 different CSY food media, which were modified from the standard medium proposed by Bass et al. (2007) (Table 1).

Flies of the parental generation were raised on a standard diet. Subsequently, 30 female and 30 males were transferred into each of the 15 laying pots containing agar plates with yeast paste. After an acclimation period of 24 h, flies were transferred to fresh agar plates with yeast paste for a 2-h prelaying period and then transferred again to fresh plates for 4 h at 21 °C for egg-laying. The eggs were allowed to hatch, and developed at 21 °C until they reached the first instar larval stage. First instar larvae were collected and placed in vials containing 7 mL of each of the 15 media for 5 replicates, which consisted of 20 larvae per vial, for a sum of 100 larvae per medium. Developmental times were measured as larva-to-pupa, larva-to-adult, and pupa-to-adult. Developmental times and numbers of pupae and adults were scored every 12 h each day until no more adults emerged from the vial for a 72-h period. Additionally, viability (larva-to-pupa, larva-to-adult, and pupa-to-adult) was measured as the ratio of the total numbers of pupae and emerged flies to the initial larval density of vials.

Table 1. Nutritional composition of experimental food mediums. (Water 1000 mL; Agar 10 g).

S/Y	sugar	yeast	S/Y	sugar	yeast	S/Y	sugar	yeast
0/100	0 g	100 g	50/100	50 g	100 g	100/100	100 g	100 g
0/75	0 g	75 g	50/75	50 g	75 g	100/75	100 g	75 g
0/50	0 g	50 g	50/50	50 g	50 g	100/50	100 g	50 g
0/25	0 g	25 g	50/25	50 g	25 g	100/25	100 g	25 g
0/10	0 g	10 g	50/10	50 g	10 g	100/10	100 g	10 g

2.3. Wing size measurements

To obtain flies for the size measurements, all adults were distinguished by sex shortly after eclosion, and the wings were fixed in Entellan under coverslips on microscope slides. Images were captured using a Leica DFC295 live camera attached to a Leica dissecting microscope. Images were processed using TPS-Dig2 software (developed by Rohlf FJ, 2006; <http://life.bio.sunysb.edu/morph/>), in which wing lengths were measured along the third longitudinal wing vein from its intersection with the anterior cross vein to the tip of the wing vein (Joubert and Bijlsma, 2010) (Figure 1).

2.4. Statistical analyses

Nested analyses of variances (ANOVAs) were used to test the effects of sugar concentration and yeast concentration and their interaction on viability, developmental time, and wing length. Larva-to-pupa and larva-to-adult viability was expressed as the proportion of larvae that resulted respectively in pupae and eclosed adult flies, calculated separately for each vial. Pupa-to-adult viability was calculated as the proportion of pupae resulting in eclosed adult flies. An average development time was calculated for each individual vial during the experiments. Wing-size analyses were performed for each sex separately. All statistical analyses were performed by using SPSS 17.

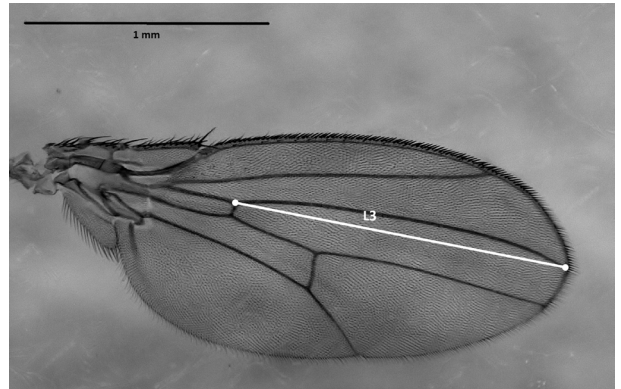


Figure 1. Line for wing length measure extended from the anterior crossvein to the end of the second longitudinal vein.

3. Results

Dietary restriction was applied to the *Drosophila* by the simultaneous dilution of the standard CSY medium. Table 2 summarizes mean values and standard error for developmental times. We tested the separate effects of sugar and yeast on developmental time and their difference from each other with calculated average developmental times for each experimental food regime and for both developmental stages, i.e., larva-to-pupa and larva-to-adult (Table 3). The

Table 2. Mean values ($\bar{x}$) and standard errors (SE) (in hours) of developmental times and the pupation time as percentage (pupation time / Σ developmental time * 100). N, number of analyzed individuals.

Food type S/Y	Larva-to-Pupa			Larva-to-Adult			Pupation Time %
	N	$\bar{x}$	SE	N	$\bar{x}$	SE	
0/100	70	142.74	2.35	62	267.48	2.31	47
0/75	57	145.54	2.09	48	272.00	2.61	46
0/50	71	142.37	2.21	64	269.54	2.51	47
0/25	86	154.33	1.91	74	280.31	2.03	45
0/10	54	167.11	2.74	49	295.67	3.60	43
50/100	85	130.12	1.52	76	258.08	2.10	50
50/75	59	131.19	1.81	56	252.00	2.00	48
50/50	79	139.49	1.56	77	254.86	1.91	45
50/25	37	134.59	2.84	33	251.39	3.39	46
50/10	52	143.85	3.02	44	255.82	3.24	44
100/100	52	130.62	3.23	43	239.26	4.36	45
100/75	68	125.76	2.31	64	231.87	2.27	46
100/50	73	135.73	1.60	64	230.13	1.87	41
100/25	84	142.19	1.59	79	245.97	1.84	42
100/10	57	144.35	2.44	55	240.44	2.70	40

Table 3. Summary of the results from 2-way ANOVA testing effects of yeast and sugar and interaction between sugar and yeast concentration on larva-to-pupa and larva-to-adult developmental time (DT).

Trait	Source of variation	d.f.	Mean Square	F-ratio	P-value
larva-to-pupa DT	sugar	2	22864.258	75.583	<0.0001
	yeast	4	9333.170	30.853	<0.0001
	sugar × yeast	8	1315.605	4.349	<0.0001
	error	969	302.504		
larva-to-adult DT	sugar	2	114471.851	315.664	<0.0001
	yeast	4	4506.193	12.426	<0.0001
	sugar × yeast	8	2641.910	7.285	<0.0001
	error	874	362.638		

d.f.: degrees of freedom

separate effects of sugar and yeast concentrations and their interaction on developmental time were highly significant (Table 3; $P < 0.0001$). Larva-to-pupa developmental time was delayed most in the 0 g/L sugar and 10 g/L yeast groups, and was significantly different from the results of the other yeast concentrations (Table 2, Figure 2). A similar pattern was observed in the larva-to-adult period, but the differences were less than in the larva-to-pupa period. In addition, the pupation period was estimated as the difference between larva-to-adult developmental time and time to pupation. The pupation stage shows a notable result. The longer the larval period, the shorter the pupation

period of the overall developmental time (Table 2; Figure 2). In each sugar group (0 g/L, 50 g/L, 100 g/L), the 10 g/L yeast concentration diets have the shortest pupation time and the longest larva-to-pupa developmental time (Table 2; Figure 2).

Viability of larva-to-pupa was calculated with the ratio of the total number of scored pupae to initial larval density of vials. Table 4 shows the proportions of viability in different stages. Viability of larva-to-adult was calculated with the ratio of the total number of emerged flies to the initial larval density of the vials. Viability of pupa-to-adult is the ratio of the total number of emerged flies to the

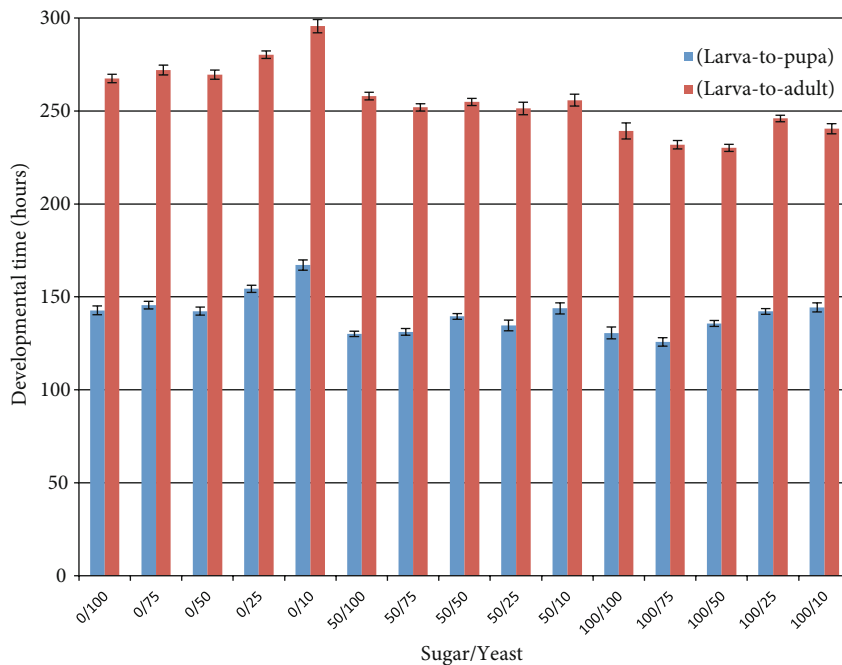
**Figure 2.** Developmental times in hours for larva-to-adult and larva-to-pupa on different diets. The error bars represent standard errors of means.

Table 4. Analyses of variance on larva-to-pupa, larva-to-adult, and pupa-to-adult viability in all diet groups.

Food type (S/Y)	Viability (Larva-to-pupa)	SE	Viability (Larva-to-adult)	SE	Viability (Pupa-to-adult)	SE
0/100	0.70*	0.10	0.62	0.10	0.88	0.03
0/75	0.57***	0.07	0.48*	0.08	0.84	0.06
0/50	0.71*	0.07	0.64	0.07	0.90	0.04
0/25	0.86	0.03	0.77	0.06	0.89	0.05
0/10	0.68*	0.07	0.61*	0.04	0.92	0.06
50/100	0.85	0.04	0.76	0.06	0.89	0.03
50/75	0.71	0.04	0.70*	0.04	0.98	0.02
50/50	0.79	0.04	0.77*	0.03	0.98	0.01
50/25	0.46***	0.04	0.41**	0.05	0.89	0.06
50/10	0.46***	0.07	0.44**	0.06	0.97	0.03
100/100	0.59***	0.03	0.54**	0.03	0.92	0.03
100/75	0.88	0.03	0.80	0.04	0.92	0.04
100/50	0.73	0.05	0.64*	0.05	0.87*	0.02
100/25	0.84	0.06	0.79	0.07	0.94	0.03
100/10	0.71	0.02	0.69**	0.02	0.97	0.02

*P < 0.05, ** P < 0.01, ***P < 0.001. SE: Standard error.

scored pupae. Larva-to-pupa and larva-to-adult viability was significantly affected in all diet groups (Table 4). However, pupa-to-adult viability was not affected by the larval diet (Figure 3). In other words, all larvae that became pupae could develop into adult flies. The viability in 50/25 and 50/10 S/Y concentrations was significantly decreased,

but in general the viability has no pattern with the variable sugar or yeast concentration, although their interaction on viability is highly significant (Table 5; $P < 0.0001$).

Wing length was used as an estimate of body size (Sokoloff, 1966). The mean wing lengths for females and males raised on different food types are given in Table 6.

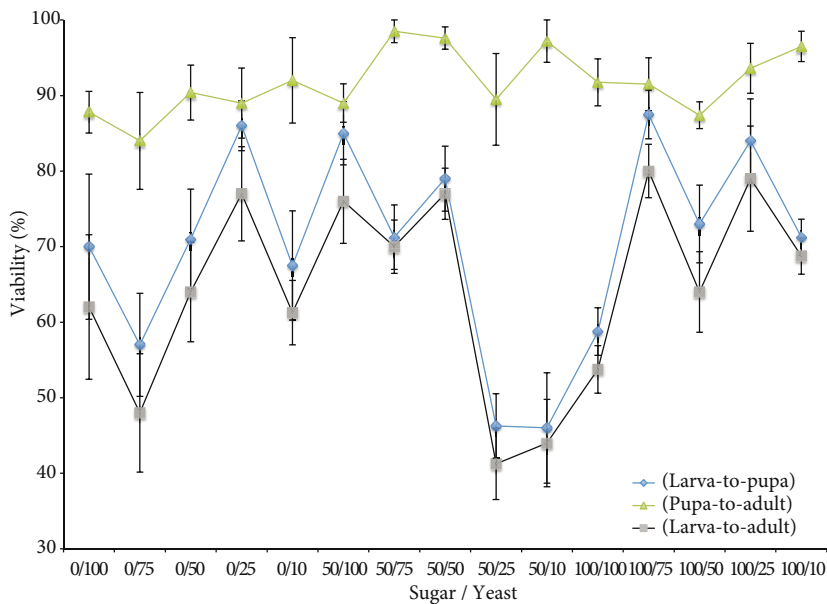


Figure 3. Larva-to-pupa, larva-to-adult, and pupa-to-adult viability (number of adults as a proportion of the number of larvae transferred) as a percentage of different diets. The error bars represent standard errors of means.

Table 5. Summary of the results from 2-way ANOVA testing effects of yeast and sugar and interaction between sugar and yeast concentration on larva-to-pupa, larva-to-adult, and pupa-to-adult viability.

Trait	Source of variation	d.f.	Mean Square	F-ratio	P-value
larva-to-pupa viability*	sugar	2	0.049	3.241	0.047
	yeast	4	0.033	2.161	0.086
	sugar $\times$ yeast	8	0.112	7.403	<0.0001
	error	54	0.015		
larva-to-adult viability*	sugar	2	0.037	2.291	0.111
	yeast	4	0.020	1.264	0.295
	sugar $\times$ yeast	8	0.110	6.804	<0.0001
	error	54	0.016		
pupa-to-adult viability*	sugar	2	0.020	3.078	0.054
	yeast	4	0.006	0.989	0.422
	sugar $\times$ yeast	8	0.007	1.046	0.414
	error	54	0.006		

d.f.: degrees of freedom, *percent

Table 6. Mean values ($\bar{x}$) and standard errors (SE) (in mm) of wing lengths. *N*, number of analyzed individuals.

Food type S/Y	Female wing length			Male wing length		
	N	$\bar{x}$	SE	N	$\bar{x}$	SE
0/100	34	1.5891	0.0121	16	1.4181	0.0122
0/75	24	1.5838	0.0124	21	1.4271	0.0127
0/50	26	1.5885	0.0166	28	1.4186	0.0088
0/25	36	1.5881	0.0113	33	1.4209	0.0092
0/10	20	1.6015	0.0132	24	1.4150	0.0094
50/100	35	1.5986	0.0091	37	1.4211	0.0088
50/75	30	1.6017	0.0113	19	1.4421	0.0121
50/50	34	1.6224	0.0107	37	1.4643	0.0072
50/25	15	1.5533	0.0175	13	1.4154	0.0094
50/10	22	1.6073	0.0179	19	1.4379	0.0105
100/100	18	1.5839	0.0168	21	1.4495	0.0076
100/75	31	1.6232	0.0086	30	1.4353	0.0098
100/50	29	1.6414	0.0098	30	1.4413	0.0117
100/25	34	1.6026	0.0112	36	1.4444	0.0083
100/10	29	1.5431	0.0152	25	1.4028	0.0115

Results of the 2-way ANOVA test revealed that there were significant differences in the effects of diet on male and female wing lengths (Table 7). Results show that yeast has a significant effect on female wing length, whereas sugar has an impact on male wing length. Interestingly, if the diet is

void of sugar, body size shows no differences with variable yeast concentrations (Figure 4). We repeated the analysis for 0 g/L sugar separately, and we found no correlation between yeast concentration and wing length ($P = 0.941$ in females and $P = 0.950$ in males, data not shown).

Table 7. Summary of the results from 2-way ANOVA testing effects of yeast and sugar, and interaction between sugar and yeast concentration on wing lengths.

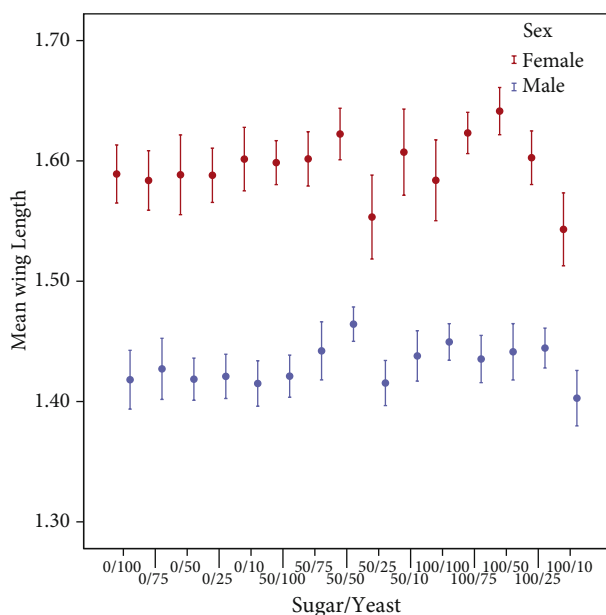
Trait	Source of variation	d.f.	Mean Square	F-ratio	P-value
female wing length	sugar	2	0.003	0.616	0.540
	yeast	4	0.018	4.114	0.003
	sugar $\times$ yeast	8	0.018	4.117	<0.0001
	error	402	0.004		
male wing length	sugar	2	0.009	3.593	0.028
	yeast	4	0.006	2.202	0.068
	sugar $\times$ yeast	8	0.006	2.480	0.012
	error	374	0.003		

d.f.: degrees of freedom.

On the other hand, if the diet contains sugar, the yeast concentrations affect body size dependent on the sugar concentration in different ways (Figure 4). The interaction of sugar and yeast shows a significant effect on body size (Table 7; $P < 0.0001$ in females and $P < 0.012$ in males).

4. Discussion

Results for all investigated traits showed variable responses to diet. Larval protein (yeast) to carbohydrate (sugar) ratio was found to have significant effects on larva-to-pupa and larva-to-adult developmental times. Developmental time increased with a decrease of yeast and sugar concentration.

**Figure 4.** Mean wing length (mm) of females and males developed on different diets. The error bars represent standard error of the mean.

However, the time of the total developmental period spent in pupation clearly declined with decreased yeast concentration; restricted sugar concentration had a slight effect on pupation time. Negative correlations were observed between pupation time and larval developmental time when the complete development from larva to adult was considered. These results supported the finding that the developmental delay occurred in the larval stage, possibly in the TGP (Layalle et al., 2008). The results of the present study could suggest that under unsuitable nutritional conditions an individual's life-history strategy for pupation time may be determined by the larval developmental time. Notably, on a medium lacking sugar, larvae develop substantially more slowly, providing evidence that larvae have fitness disadvantages in the absence of dietary sugar.

Viability was not affected by the concentrations of yeast and sugar independently, but the interaction of yeast and sugar concentrations was found to decrease viability significantly (Table 5). Our results showed that pupal viability was high whereas larval viability was low. However, 90% of the larvae that managed to survive to the pupal stage were able to develop to the adult stage. The outcome of diet selection was clearly dependent on the larval stage. This suggests that variation of diet composition leads to selection pressure on larval survival. Joshi and Mueller (1996) indicated that food requirements for successful pupation may well be an accurate reflection of food requirements for the completion of development to the adult stage. Sisodia and Singh (2012) found that flies developed under protein-rich conditions had reduced egg-to-adult viability. We did not find any correlation between yeast concentration and viability in general. However, our viability results obtained with different yeast concentrations changed with the amount of sugar in the diets. There is also evidence for the interaction between sugar and yeast. Hence, it is possible that the different sugar

and yeast concentrations may result in different qualities of nutrients, which may influence larval survival. Indeed, if we look at the sugar-free groups, our results are parallel with those of Sisodia and Singh (2012), wherein larval survival decreased slightly in protein-rich groups.

Feeding is stimulated at a low diet concentration. For example, Min et al. (2007) showed that flies that were fed on high yeast/low sugar diet concentrations fed more, produced more eggs, and gained more mass than flies that were fed on high yeast/high sugar. Our data also suggest that larvae developed on a sugar-free diet fed more and were able to reach an adult size comparable to that of the larvae developed on a sugary diet. It is probable that high sugar concentration inhibited feeding behavior in the larvae on the diet that included high sugar (100 g/L) in our data. We did not obtain data that showed a negative effect with decreased yeast concentration on body size in our experimental “sugar-free” groups. When the diet contained sugar, the wing size increased with a rise of yeast concentration, showing a peak at the 50 g/L yeast concentration; after this point, wing size decreased with increased yeast level. Prolonged developmental time causes a longer growth period and bigger body size (Stearns, 1992; Stearns et al., 2000). In general, our results support these correlations between developmental time and body size, except for some diet types such as the 0-g sugar and 10-g yeast group and sugar-enriched diet (100 g sugar). However, a sex-specific correlation was found in different diet regimes for wing length. Females showed a

higher correlation between developmental time and body size. Likewise, the effects of yeast and sugar concentrations on wing length were found to be more important in females than in males (Table 7). It is known that egg production in females developed on a protein-enriched diet is higher than that of females developed on a carbohydrate-enriched medium (Sisodia and Singh, 2012). From this point of view, yeast is an important nutrient for female reproductive success. This may suggest that a limiting resource is divided between the 2 sexes, and larval competition occurs against males.

Our data demonstrated many interesting differences in life history traits between flies developed on 15 different diet regimes. These results raised the question of whether the protein and carbohydrate ratio causes variation for these traits. It is clear that no single diet can optimize all fitness components (Cotter et al., 2011).

In summary, our results imply that larval feeding determined not only the developmental time and preadult survival. Feeding at the postembryonic level can influence many adult traits, such as morphological phenotypes. The causal mechanisms underlying the effects of sugar-free nutrition need more investigation. It is clear that preadult diet composition affects larval survival, developmental time, and adult body size in different ways, although further research is needed. It would be of interest to investigate the effects of preadult and adult nutrition on other aspects of life-history traits.

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