The roles of food odorants and diet in larval development of *Drosophila melanogaster*

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Abstract:

Resource assessment via sensory information has been shown to have internal effects on physiology as well as more noticeable behavioral effects. Specifically, the regulation of longevity and fecundity in *Drosophila melanogaster* adults is connected to diet restriction and olfactory sensing of nutrient availability. The effects of this food odorant/diet restriction response on the development of *D. melanogaster* larvae have never been tested, despite the possibility that nutrient availability, and, therefore, nutrient sensing, may be more important to this developmental stage than to an emerged adult fly. This study was performed to determine the effects of diet restriction and food odorants on the time and success of pupation and emergence in *D. melanogaster*. Equal numbers of eggs from two lines of flies, a mutant for the olfactory receptor gene *Or83b* and a control with the same genetic background, were exposed to four treatments of normal diet or restricted diet with or without yeast odorants. It was found that genotype and diet, but not odorant exposure, had a great impact on larval development.

Introduction:

For an organism to live and reproduce successfully, it must be able to sense and interpret information about its environment, and then act on it. The obvious uses of these senses are for hunting, predator evasion, and the location of food and potential mates. A less obvious effect of sensory information is the regulation of internal processes—such as fecundity, longevity, development, and metabolism—in response to an assessment of resource availability in the environment (Mair *et al.,* 2004; Walker *et al.,* 2005; Carey *et al.,* 2002). As stated in Libert and Pletcher (2007), the evolution of advanced sensory systems meant that "assessing the current and future state of the environment became more reliable, and increased fitness presumably came to those that could properly use the information to their reproductive advantage." Although no direct mechanisms have been detailed in the fruit fly, the effects have been observed.

Genetic analysis of the response to dietary restriction has revealed that the regulation of genes coding for odorant-binding proteins is significantly altered (Libert *et al.,* 2007). Olfactory cues are also important in the nematode *Caenorhabditis elegans* and in *Drosophila melanogaster* for avoiding pathogens (Pradel *et al.,* 2006; Beale *et al.,* 2006), finding food, and regulating longevity and fecundity in response to nutrient availability (Tu and Tatar, 2003). Low food availability during adulthood has been found to increase longevity in *D. melanogaster* (Libert *et al.,* 2007; Libert and Pletcher, 2007), and this effect has been documented across many classes and kingdoms of organisms (Masoro, 2006). This induced longevity in response to diet restriction is reversed in *D. melanogaster* by the presence of yeast odorants, which signal high nutrient availability even without ingestion or direct access to the source of the odorants (Libert *et al.,* 2007).

Both the longevity and stress resistance of adult flies in that study increased when introduced to restricted diets without odorants. Meanwhile, dietary restriction of larvae has been shown to produce adults with small body size, reduced fecundity, but unchanged longevity (Tu and Tatar, 2003). Larvae with disrupted nutrient monitoring pathways (dependent on fat body reserves) also exhibit growth restriction, delay in larval development, increased mortality in the larval stage, and the production of adults of reduced body size and weight (Colombani *et al.,* 2003). The combined effects of diet restriction and altered odorant signaling, while demonstrated in adult flies, have not been tested in the larval stage. Given the larva's main function of eating and growing, it may have greater sensitivity than the adult to sensory information regarding nutrient availability.

This study was performed to assess the effects of odorants, diet restriction, and altered odorant detection on pupation and emergence of *D. melanogaster* exposed to these conditions from egg to adult. *Or83b* is a necessary gene for the function of many olfactory receptors across olfactory sensory neurons. The inactivation of *Or83b* has a generalized disruptive effect on normal olfactory receptor localization on the neurons and, therefore, on chemotaxis and other behaviors associated with olfaction (Larsson *et al.,* 2004; Benton *et al.,* 2006). Consequently, an *Or83b* mutant line (w^{*}; w^{+*} $Or83b^{2}$) was used in the study performed by Libert *et al. (*2007) to specifically determine the role of olfaction in the regulation of longevity in response to dietary restriction and odorant treatment on adults. This line and a genetically matched control (w^{1118}; P{$ry^{+t7.2}$=70FLP}10) were used in a similar manner for this experiment. Larvae were subjected to restricted or normal diets with and without yeast odorants. Developmental

time and success were recorded for each diet/odorant treatment. Odorant treatments were found to not affect development of larvae on full diet, and the *Or83b* mutant benefitted more from being on full diet than the wild type did.

Materials and Methods:

Drosophila lines:

The two lines, *w**; w^{+*}, $Or83b^{2}$ and w^{1118}; P{$ry^{+t7.2}$=70FLP}10, were acquired from the Bloomington Drosophila Stock Center. The flies were maintained in vials of standard yeast media prior to their use in the experiment.

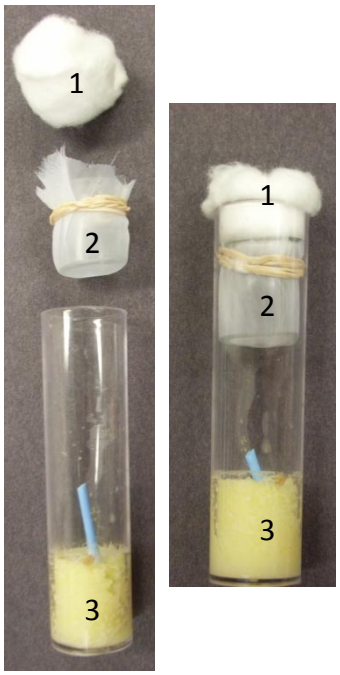

Figure 1. The vial and odorant device consisting of 1. cotton ball with dab of yeast paste to act as odorant 2. mesh attached around plastic tubing with rubber band 3. vial of media (eggs were stuck to the moistened cotton swab, which was then inserted into the medium)

Vials:

The vials were designed to prevent access of larvae and flies to the yeast paste used as food odorant. The vials were filled with 10ml of media, and fitted with 2.5cm segments of 3/16 x 3/16 Nalgene clear plastic vacuum tubing. Nylon mesh was secured around the tubing with rubber bands, which also formed a seal between the vial and the tube. The tubing was then plugged with cotton dabbed with yeast paste (Figure 1).

Media:

The experimental diets were based on the developmental diet used in Libert *et al.*, (2007), and were formulated as follows: 1 L of distilled water, 10 g agar, 55 g dextrose, 30 g sucrose, 60 g cornmeal, 3 ml propionic acid, and 25 g yeast or no yeast for the full and restricted diets, respectively. Each larval treatment vial and each adult treatment vial received 10 ml of diet medium. The odorant dabbed on the cotton ball was made with 8 g active dry yeast, 4 g sucrose, and 10 ml of distilled water.

Grape juice medium was made with 271.5 ml distilled water, 227.5 ml of grape juice, 11 g agar, 29 g dextrose, 14.5 g sucrose, 5.6 ml of acid mix, and 9.0 g active dry yeast. Acid mix was made of 1:1 dilute phosphoric acid to dilute propionic acid. Dilute phosphoric acid was made with 41.5 ml of concentrated phosphoric acid and 458.5 ml distilled water; dilute propionic acid was made with 418 ml propionic acid and 82 ml of distilled water.

Egg collection and standardization:

Flies from both lines were maintained in bottles of yeast media prior to their transfer to the containers of grape juice media. Flies were left in these containers overnight. Parent flies were then collected under CO_2 and discarded. The egg laying containers were then flooded with warm water and the eggs dislodged with a paintbrush. The water containing the eggs was strained through black cloth. Cotton swabs were used to pick up and sort eggs into the appropriate media vials. Vials from the first replicate had a count of 10 eggs per vial, while the second replicate had 12 eggs per vial. Vials were then capped with the appropriate odor treatment. Cotton and yeast paste were

replaced when dry. The developmental data recorded were time to pupation, time to emergence, and number of successful emergences.

Experimental design:

Fly eggs were collected for the first replicate to have 320 eggs per genotype distributed equally among the four diet and odorant treatment combinations: Restricted diet with odorant (RO), restricted diet without odorant (RØ), normal diet with odorant (NO), and normal diet without odorant (NØ) (Figure 2). Larval vials in the first replicate were sorted 10 eggs to a vial, while second replicate were given 12 eggs each to lessen the effect of unviable eggs. Both replicates had 8 larval vials per treatment. The number of days until pupation was measured in the restricted larval treatments of the first replicate, and all treatments of the second. The number of days until emergence was measured for all vials. Adults were transferred to adult containment vials upon emergence. Data collection for the first replicate ended 25 days after egg collection. The

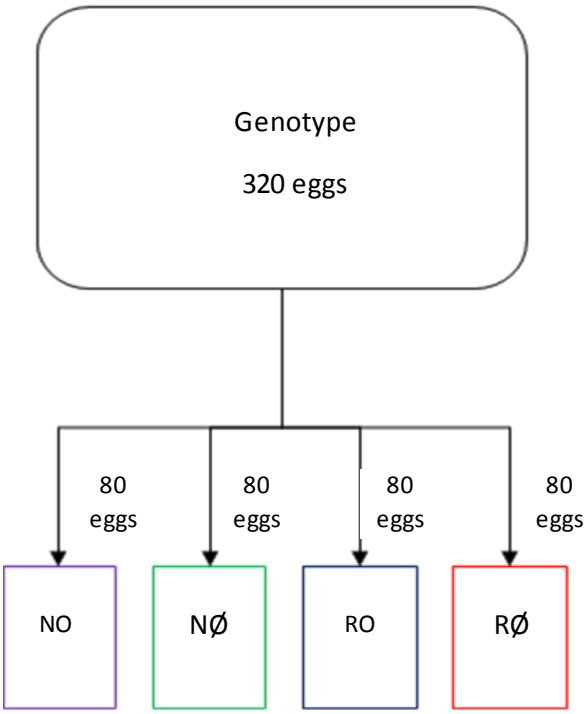

Figure 2. Diagram represents the experimental design as applied to one genotype in the first replicate; both genotypes were treated in this manner. Eggs were distributed evenly into vials across the four treatments (N=full diet, R= restricted diet, O= odorants present, Ø= no odorants). Second replicate treatments received 12 eggs per vial, and therefore had 96 eggs per treatment.

second replicate was ended 23 days after egg collection, and by this point, pupations and emergences had stopped.

Statistical analysis:

The effects of genotype, diet, odorant presence, and experimental replicate on number of pupae formed, adult flies emerged, time to pupation, and time to adult fly emergence were evaluated using analysis of variance implemented in SAS 9.1 (Cary, N.C.). Statistical interactions among genotype, diet and odorant were also tested to determine whether the two genotypes responded differently to the various diet and odorant combinations, and whether the effects of odorant varied across the two diet treatments. Least squares means and standard errors were estimated from the linear models over the three-way interaction genotype*diet*odorant.

Results:

The data analyzed were: time to pupation for the larvae on restricted diets in the first replicate and all larvae in the second replicate; time to emergence for all larvae; the dates of all emergences; and the counts of all emerged flies.

An analysis of variance was applied to test the effects of genotype, diet, and odorant treatment on larval development and survival. The effect of odorant is only significant for time to pupation in restricted diets (p=.0208), but the sample size for successful pupation in the restricted diet with odorant is too small to make a definite conclusion (n=4). The effect of diet was significant across all treatments and developmental data (p<.0001) (Tables 1-4). As expected, the number of larvae surviving to

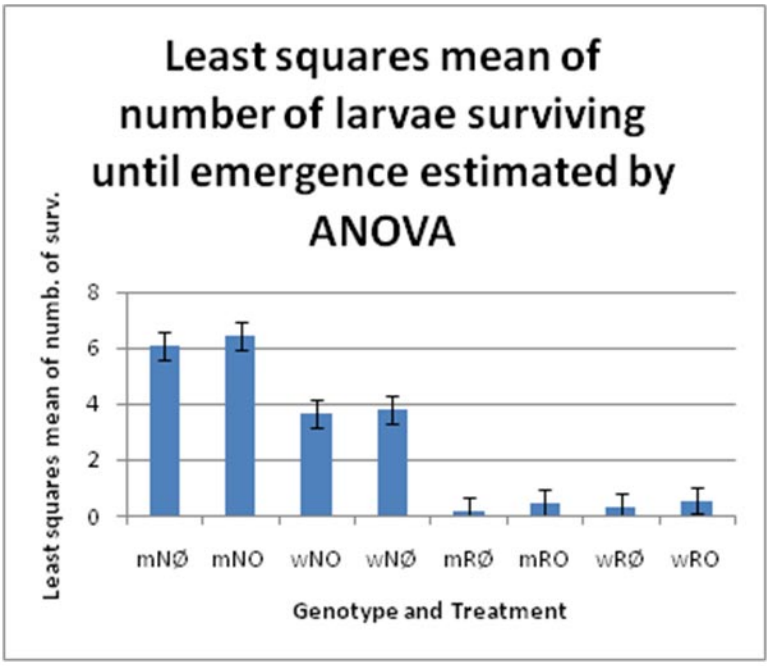

Figure 3. Bars show the least squares mean of emergences for each genotype/treatment combination (w=wild type, m= *or83b* knockout, N=full diet, R= restricted diet, O= odorants present, Ø= no odorants). Error bars show mean +/- 1 standard deviation.

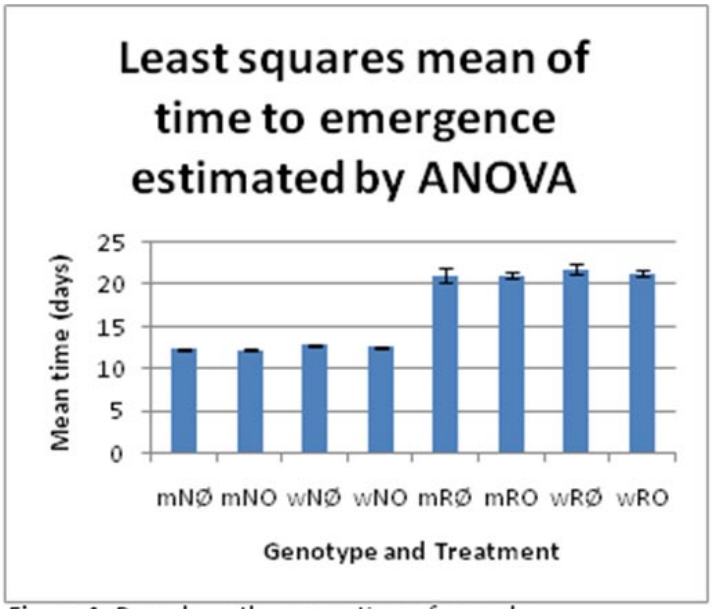

Figure 4. Bars show the mean times for each genotype/treatment combination (w=wild type, m= *or83b* knockout, N=full diet, R= restricted diet, O= odorants present, Ø= no odorants). Error bars show mean +/- 1 standard deviation.

pupation and emergence was lower for those on restricted diet, and they took more time to pupate and emerge than larvae on normal diet (Tables 1-4; Figures 3-4). *Or83b* mutant flies on normal diet had a higher number of larvae surviving until emergence and pupation than wild type flies on normal diet (p=.0002; p=.0049) (Figure 3) (Tables 1 and 3), but the times to pupation and emergence were not affected (Tables 2 and 4).

Larvae Survival to Emergence

Variables	Mean square	F Value	P value
Date	29.528375	7.53	0.007
Genotype	45.660888	11.65	0.0009
Odorant	1.1997024	0.31	0.5812
Diet	685.08981	174.73	<.0001
Odorant*Diet	0.1621909	0.04	0.8392
Genotype*Diet	56.269691	14.35	0.0002
Genotype*Odorant*Diet	0.4440006	0.11	0.893

Table 1. Data from analysis of variance is presented, displaying significance and distribution data for larvae survival to emergence. For genotype data m= *Or83b* mutants, w= wild type; for odorant data No=no odorant, Yes= odorant present; for diet data N= full diet, R= restricted diet.

geno	odorant	diet	mean (emergences)	stdev
m	No	N	6.1	0.5
m	No	R	0.2	0.5
m	Yes	N	6.4	0.5
m	Yes	R	0.4	0.5
w	No	N	3.8	0.5
w	No	R	0.3	0.5
w	Yes	N	3.7	0.5
w	Yes	R	0.6	0.5

Time to Emergence

Variables	Mean square	F Value	P value
Date	5.390152	4.74	0.0302
Genotype	2.882187	2.53	0.1124
Odorant	0.518983	0.46	0.4999
Diet	1168.314	1026.86	<.0001
Odorant*Diet	0.098691	0.09	0.7685
Genotype*Diet	0.006479	0.01	0.9399
Genotype*Odorant*Diet	0.247312	0.22	0.8047

Table 2. Data from analysis of variance is presented, displaying significance and distribution data for time to emergence. For genotype data m= *Or83b* mutants, w= wild type; for odorant data No=no odorant, Yes= odorant present; for diet data N= full diet, R= restricted diet.

geno	odorant	diet	mean (days)	stdev
m	No	N	12.3	0.1
m	No	R	21.1	0.8
m	Yes	N	12.2	0.1
m	Yes	R	21.1	0.4
w	No	N	12.8	0.1
w	No	R	21.8	0.5
w	Yes	N	12.6	0.1
w	Yes	R	21.3	0.4

Larvae Survival to Pupation

Variables	Mean square	F Value	P value
Date	0.5790205	0.17	0.6834
Genotype	21.57313	6.24	0.0144
Odorant	6.1220435	1.77	0.1868
Diet	147.01563	42.53	<.0001
Odorant*Diet	2.6095819	0.76	0.3874
Genotype*Diet	28.893874	8.36	0.0049
Genotype*Odorant*Diet	1.3530983	0.39	0.6773

Table 3. Data from analysis of variance is presented, displaying significance and distribution data for larvae survival to pupation. For genotype data m= *Or83b* mutants, w= wild type; for odorant data No=no odorant, Yes= odorant present; for diet data N= full diet, R= restricted diet.

geno	odorant	diet	mean (pupae)	stdev
m	No	N	5.2	0.7
m	No	R	0.3	0.5
m	Yes	N	4.8	0.7
m	Yes	R	1.3	0.5
w	No	N	2.4	0.7
w	No	R	0.5	0.5
w	Yes	N	3.2	0.7
w	Yes	R	1.3	0.5

	Variables	Mean square	F Value	P value
Time to Pupation	Date	188.2251	61.02	<.0001
	Genotype	9.406435	3.05	0.0826
	Odorant	16.785123	5.44	0.0208
	Diet	1256.3796	407.32	<.0001
	Odorant*Diet	22.463945	7.28	0.0077
	Genotype*Diet	2.137124	0.69	0.4064
	Genotype*Odorant*Diet	20.821658	6.75	0.0015

Table 4. Data from analysis of variance is presented, displaying significance and distribution data for time to pupation. For genotype data m= *Or83b* mutants, w= wild type; for odorant data No=no odorant, Yes= odorant present; for diet data N= full diet, R= restricted diet.

geno	odorant	diet	mean (days)	stdev
m	No	N	10.9	0.4
m	No	R	17.6	0.9
m	Yes	N	10.8	0.4
m	Yes	R	21.7	0.4
w	No	N	11.2	0.5
w	No	R	20.8	0.6
w	Yes	N	11.1	0.4
w	Yes	R	20.3	0.4

Discussion:

This study found no effect of odorants on larval development. Full diet greatly shortened time to pupation and time to emergence, and increased survivability to pupation and emergence for both lines. The degree of responsiveness to the full diet was higher in *Or83b* mutant larvae than in wild type larvae. Additionally, *Or83b* mutants were generally more likely to survive to pupation and emergence than the wild type, irrespective of treatment.

The low survivability/delayed pupation observed in all larvae exposed to the restricted diet prevented adequate analysis of odorant effects. The limitations of the restricted media resulted in only 4 successful pupations in restricted diet treatments with odorants, and low survivability across those without odorants. As opposed to the

0%/25% yeast diets used in this study, Libert *et al.* (2007) used 3% as the most restrictive diet for their adult flies and 15% as full diet. Libert *et al.* (2007) also saw odorant effects in adult flies in restricted diet treatments only. In Libert *et al.*, "longevity was not affected by yeast odorants when flies were fully fed." In this present study, odorants would therefore only have had an effect in the restricted diet, and having such low survivability in the restricted diet resulted in not having enough data to adequately test odorant effects.

The perceived higher responsiveness of *Or83b* mutants may be an artifact of low survivability; *Or83b* may always survive better than wild type, even in restricted diet, but this was not detectable with such low survival. A less restrictive diet with higher larval survivability may show that the survival of both lines of flies increases equally with full nutrient availability, although the mutant line may always have higher base survivability.

In the case that the higher responsiveness of *Or83b* mutants is not an artifact of small sample size, it may be due to compensatory feeding, which has been occurred in *D. melanogaster* under dietary restriction (Carvalho *et al.,* 2005). This behavior has not been a problem in previous studies using *Or83b* mutants and was not monitored in this study due to the intensive measures necessary to do so. Other flies under diet restriction (of both sucrose and yeast) are stimulated to increase their intake to counter the lower nutrient availability (Carvalho *et al.*, 2005). *Or83b* mutants, due to their interrupted olfactory reception, may be under a constant perceived dietary restriction due to their limited ability to sense nutrient availability. This could produce a permanent compensatory feeding behavior in *Or83b* mutants, causing them to always have a higher intake and, therefore, a faster growth in relation to wild type flies. A similar, but

opposite, effect has been shown in flies unable to assess nutrient availability due to a disruption of a fat body monitoring pathway (Colombani *et al.,* 2003). Internal nutrient monitoring pathways such as this may be the reason that odorant had no effect on flies or larvae on full nutrient media in this study and in Libert *et al.* (2007), but the control of longevity by nutrient sensing must at least be shared by odorant-dependent pathways, otherwise there would not have been an effect of odorant in Libert *et al.,* (2007).

Another potential explanation for the superiority of the *Or83b* mutant line to the wild type is hormesis. The definition used in Masoro (2006) for hormesis is "the beneficial effects resulting from cellular responses to repeated mild stress." Hormesis has often been suggested as a mechanism by which stressors, such as dietary restriction, could increase longevity and stress resistance (Masoro, 2006). By this theory, the perception of constant diet restriction in the *Or83b* mutant line could induce the organism's repair mechanisms, resulting in larvae that are healthier and more robust than the wild type.

The lower survivability of the wild type flies may be due to genetic elements introduced in the production of the line. w^{1118} is a white eye marker which, besides altering the physical appearance of the eyes, has also been linked to vision defects, altered locomotor behavior, and homosexual courtship of male flies with overexpression of w^{1118} (Liu *et al.,* 2007; Lloyd *et al.,* 2002) . This altering of locomotor behavior has also been observed in $Or83b^{2}$ (Liu *et al.,* 2007). This white eye marker may have had unexpected pleiotropic sublethal effects that contributed to the lower survivability of the recue line as compared to the *Or83b* mutant line.

This topic of larval development and metabolism deserves more study before any definite conclusions can be made. In particular, the dietary needs of *D. melanogaster* must be investigated, particularly since at the time of writing there is no consensus as to what counts as dietary restriction versus full diet versus malnutrition (Min *et al.*, 2007), especially not for larval development. Until these parameters have been set, attempts at defining dietary restriction and its effects on physiology will be confounded by the different methods of limiting caloric and nutrient intake.

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