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Original Research Article

# Local adaption in life history traits of *Drosophila melanogaster* in extreme conditions of humidity

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***Abstract***

*Our study was aimed at studying the local adaptation in life history traits of wild type Drosophila melanogaster in extreme conditions of humidity. The control line was designated as SC (starvation control) and the line which was starvation resistant was designated as ST (starvation tolerant). For obtaining ST lines about 100 flies were placed in empty bottle. The cotton plug for bottle was saturated with water and thus flies were held in a high humidity environment in constant proximity to moisture. The average dry weight of four independent SC male line was 0.267mg while SC female lines weighed 0.530mg, ST males weighed 0.283mg and ST female weighed 0.573mg.The statistical differences among SC& ST males lines was: F value=16.484,P value=0.007 . The statistical differences in SC& ST females was: F value=153.447 P value=0.000.*

**Key words:** *Drosophila melanogaster ,local adaptation, humidity, life history traits*

## 1. Introduction

Life history evolution has been extensively studied in *D. melanogaster* evidence indicating a major role of lipid and carbohydrate reserves in dating adult life history tradeoffs in *D. melanogaster* came from the observations that (a) selection for increased lifespan results in correlated increases in starvation and desiccation resistance and lipid content (Service *et al*., 1985: Service *et al*.,1987) (b) selection for increased starvation or desiccation resistance leads to a correlated increase in lifespan (Rose*etal*.,1992) and dry weight at ecolosion(Chippindale etal.,1996; Djawdan *et al*.,1997),(d) selection for increased desiccation resistance lead to increased carbohydrate but not lipid content at exclusion (Djawdan *etal*.,1997; Chippindale*etal*.,1998). Selection for increased starvation or desiccation resistance in *D. melanogaster* for wild caught flies yield a somewhat different patterns of correlated responses to selection (Hoffmann *et al*., 1989a, b, 1993a, b; Harshman *et al*.,1999). In these studies, the evolution of increased desiccation resistance was accompanied by increase in starvation resistance (Hoffman *et al*., 1989a) and lifespan (Hoffmann *et al*., 1993b). Selection for increased starvation resistance led to significant correlated increase in weight, absolute lipid content, desiccation resistance and a marginally significant increase in carbohydrate content of starvation selected populations (Harshman *et al*., 1999).They not only play an important role in adult life history, starvation and desiccation resistance are also important because of their likely involvement in determining climatic adaptation and geographical distribution in drosophila species (David et al.,1983: van Herrewege *et al*., 1997: Hoffmann *et al*.,1999; Hoffmann *et al*., 2001a).The ability to survive starvation in an important fitness trait. Survival strategies may include genetic, physiological or behavioral changes (Hoffmann *et al*., 1991; Hoffmann *et al*., 1999; Zou*et al*., 2000; Lee *et al*., 2004; Harbison *et al*., 2005). Starvation resistance is correlated to lifespan and reproduction. There is a positive genetic correlation between starvation and longevity, and a negative correlation between starvation and early fecundity(Leroi *et al*., 1994a. b; Prasad *etal*.,2003**,** Marta L Wayne *et al*., 2006).The abundance of lipids, the most important energy storage molecules is commonly correlated with starvation resistance as well(Chippindale *et al*., 1996; Wang *et al*., 2005). Starvation resistant flies can survive starvation by increasing their lipids reserves or by reducing the rate at which the reserves are used under starvation conditions (Rion *et al*., 2007).

## 2.Materials and Methods

Our study was aimed at studying the local adaptation in life history traits of wild type *D. melanogaster* in extreme

conditions of humidity. We took two experimental lines for our studies from Israna (Panipat, Hr). The control line was designated as SC (starvation control) and the line which was starvation resistant was designated as ST (starvation tolerant). For obtaining ST lines about 100 flies were placed in empty bottle the cotton plug for bottle was saturated with water and thus flies were held in a high humidity environment in constant proximity to moisture. The plugs were inspected every 12 hours to ensure that they remained wet. After approximately 50% mortality, the survivors, including males and females were transferred to the standard medium to produce the next generation. After one round of selection the flies obtained were further used for experimental work.

*2.1 Collection and culturing of Drosophila melanogaster*

*Drosophila melanogaster* individuals were collected by bait trap method from different location of Israna Panipat fruit flies were attracted to different fermenting or decaying organic matters such as banana. For this purpose we took an empty bottle and cut into c-shape then some rotten bananas were crushed and introduced into the bottle. This bottle was then placed in a nursery for some time so that flies were attracted towards the rotten bananas. In order to catch the fruit flies the open mouth of bottles were covered with a black cloth piece. Flies were captured by inserting on empty glass vial into covered mouth of bottles. The flies entered the vials partly due to phototropic nature and partly due to the disturbance caused by vigorous shaking of the bottle. The vials were then quickly plugged with cotton. The flies were collected twice a day from all sides.

The Drosophila was reared on standard Drosophila food medium or axenic medium consisting of fixed proportions of the following ingredients.

| | |
|---|---|
| Agar-agar | 20 gm |
| Dried yeast | 24 gm |
| Brown Sugar | 64 gm |
| Corn meal powder | 72 gm |

After cooking the standard food medium, 1gm Sodium para methyl benzoate (fungicide) and 3ml propionic acid (bactericide).while in anexic food medium 7.5gm of nipagine in 18ml of ethanol was added. Anexic food medium provided sufficient food and energy to larvae and due to its high protein content the resulting flies were very healthy for performing various experiments.

*2.2 Dry weight and Protein Assays*

To conduct this assay we took 20 flies for each line and sex. All the flies taken were 6-7 days old and not virgins. Dry weight would be the most accurate in estimating the exact energy stored in bodies of the flies. Body weight was determined after previously frozen flies were lyophilized for at least 24 hours. The masses of the flies were determined using a Sartorius M2P Microbalance (1µg to 1 gram). Samples were weighed within 2 hours after removal from vacuum drier.

Soluble protein was measured using BCA Protein Assay kit (Pierce Company, Rockford illinos). Five male or female lines from each were homogenized in 250µl of tris HCL pH 8.0. The homogenate was duplicated in 1:3 in homogenizing buffer and 10 µl was added in a 96- well plate for the Micro plate Assay procedure. Following standard concentrations of proteins were employed: 2mg/ml, 1mg/ml, 0.5mg/ml,0.25mg/ml and 0.125mg/ml, the BCA reagents were added and after 30 minute incubation at $37^0$ C, the plates were read in a spectrophotometer at 562nm.

*2.3 Fecundity*

Virgin males and females were collected within 8 hours after ecolosion and one male and female from each line were put into a vial, containing standard fly food, for mating. Flies were transferred to new vials every day and eggs were counted daily. For each control or selection line, there were ten replicates if a male or female died during the experiment a male or female of the same age replaced it.

*2.4 Total Lipid Assay*

Total lipid was measured using a method described by Van Handel (1985) adopted by Zhao and Zera (2002) and slightly modified for the present study. We placed all the flies in $-20^0$C freezer in preparation for the lipid assay .Three female flies were homogenized in 1.5ml append off tube containing 100µl of a chloroform /methanol (2:1) solution using plastic pestles and then centrifuged for 3 minutes. Triolein (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 and3.0 mg/ml) solutions in 2:1 chloroform: methanol were used for standards. The sample supernatant and standards were transferred into glass tubes and put into an 85-$90^0$C water bath for 5 minutes. This step was conducted to evaporate the solvent before addition of sulphuric acid which was followed by an additional incubation in an 85-$90^0$ C water bath for 10 minutes. The tubes were removed and allowed to cool at room temperature before addition of 1 ml of vanillin-phosphoric acid reagent, which was followed by a gentle vortex. 250µl of each sample or standard was transferred to a well in a 96 well plate and read in spectrophotometer at 525nm.

*2.5Triglyceride Assay*

Three flies for each lines were homogenized in 1ml chloroform/methanol(2:1) solution. Then 250µl of a 0.88% KCL solution was added to extract the lipids from the solvents and then mixtures were centrifuged for 5minutes at 7000rpm. Trillion (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 and3.0 mg/ml) solutions in 2:1 choloroform: methanol were used for standards. The sample supernatants and standards were transferred into glass tubes and put into an 85-90°c water bath for 5 minutes. This step was conducted to evaporate the solvent before addition of sulphuric acid which was followed by an additional incubation in an 85-90°c water bath for 10 minutes. The

tubes were removed and allowed to cool at room temperature before addition of 1 ml of vanillin-phosphoric acid reagent, which was followed by a gentle vortex. 250µl of each sample or standard was transferred to a well in a 96 well plate and read in spectrophotometer at 525nm

## 3. Results

The data was analyzed with one way ANOVA by using various online statistical calculators from the following websiteslikehttp://www.danielsoper.com/statcalc/calc43.aspx, http:// easy calculation.com/statistics/standard-deviation.php.

### *3.1 Dry Weight and Protein content*

The average dry weight of four independent SC male line was 0.267mg while SC female lines weighed 0.530mg, ST males weighed 0.283mg and ST female weighed 0.573mg.the statistical differences among SC& ST males lines was (F value=16.484,P value=0.007 ). The statistical differences in SC&ST females was (F value=153.447 P value=0.000).
The statistical difference of protein content between ST&SC male was (F value=38.927 P value=0.001).The statistical difference of protein content between ST&SC females was (F value=109.202 P value =0.000).

Table1. Representing the dry weight data for 4 independent individuals from each line and each sex

| SC MALE | ST MALE | SC FEMALE | ST FEMALE |
|---|---|---|---|
| 0.2767 | 0.2814 | 0.5229 | 0.5709 |
| 0.2592 | 0.2798 | 0.5235 | 0.5724 |
| 0.2681 | 0.2857 | 0.5332 | 0.5821 |
| 0.2648 | 0.2863 | 0.5231 | 0.5687 |

Table2. Representing the soluble protein data of 4 independent individuals from each line and sex

| SC MALE | SC FEMALE | ST MALE | ST FEMALE |
|---|---|---|---|
| 0.74 | 1.62 | 0.68 | 1.82 |
| 0.75 | 1.66 | 0.65 | 1.84 |
| 0.73 | 1.68 | 0.69 | 1.8 |
| 0.77 | 1.64 | 0.67 | 1.85 |

### *3.2Fecundity*

Fecundity was measured by the sexual production of single pairs of flies upon 4 hrs of enclosure and isolation. Figure 2 shows the daily pattern of accumulated fecundity or egg production for ST&SC females. ST females had lower fecundity than SC lines (Fvalue=0.701, P value=0.410). This indicated increased starvation resistances decreases fecundity. Thus we found out that there exist a life history tradeoff between longevity /starvation resistance and reproduction cost.

Table 3.The accumulated fecundity of SC&ST females. Each point represents the mean accumulated egg number of single female of replicate vials.

| DAY | SC FEMALES | ST FEMALES |
|---|---|---|
| 1 | 5 | 4 |
| 2 | 11 | 9 |
| 3 | 25 | 16 |
| 4 | 36 | 24 |
| 5 | 49 | 34 |
| 6 | 64 | 46 |
| 7 | 77 | 58 |
| 8 | 91 | 67 |
| 9 | 102 | 82 |
| 10 | 120 | 94 |
| 11 | 133 | 110 |
| 12 | 145 | 125 |
| 13 | 162 | 139 |
| 14 | 184 | 157 |

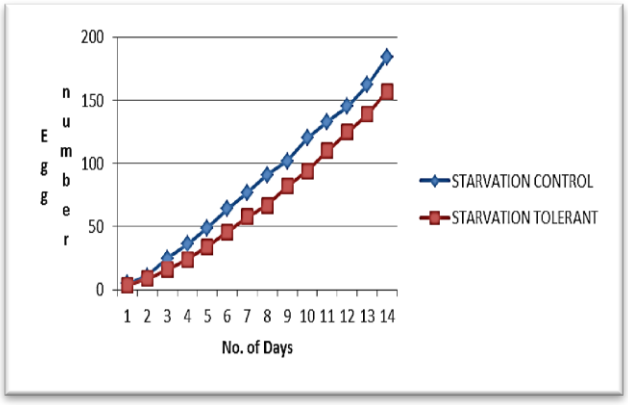


Figure 1. Representing the fecundity data

### *3.3 Lipid and triglyceride assay*

Total Lipid content of female flies was measured at three different stages: - 1st after 1 hr of exclusion. After 1 hr of eclosion ST females did not have significantly higher content SC females (F value=26.014 P value=0.007). This indicated that selected flies did not store much lipid during the larval stage but relied on food consumption at the adult stage to increase their lipid level .our results is contrary to chippindale *et al*., (1996) found. They found out that larval lipid acquisition played a major role in adult starvation resistance but this was not the case in our study we observed increase in lipid content of both SC&ST females after 7 days of eclosion (F value=76.196 , P value=0.001).After 7 days of ecolosion the flies were starved for 36 hrs at this stage we found out that lipid content in ST females were more than SC females( F value=163.925, P value=0.000).

Table 4.Representing the total lipid contents at three different stages. Flies that have emerged for 1 hr.seven day old flies, seven day old flies with another 36 hr of starvation.

| TIME | ST Females | SC Females |
|---|---|---|
| After 1hr | 0.52 | 0.47 |
| | 0.55 | 0.49 |
| | 0.54 | 0.46 |
| 7 days old | 0.76 | 0.62 |
| | 0.73 | 0.6 |
| | 0.75 | 0.64 |
| after 36 hr starvation | 0.65 | 0.44 |
| | 0.64 | 0.42 |
| | 0.61 | 0.43 |

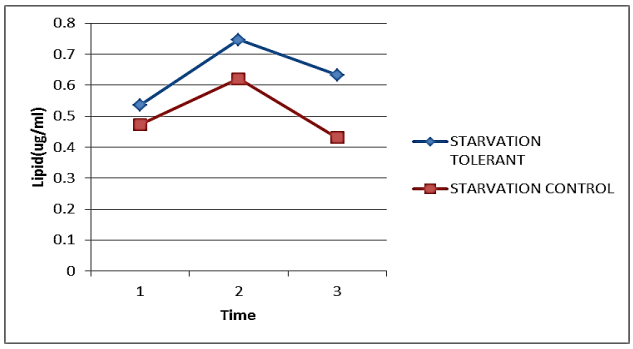


Figure 2.Representing the total lipid contents at three different stages. Flies that have emerged for 1 hr. Seven day old flies ,seven day old flies with another 36 hr of starvation. The figure is modified to show lipid content of single flies

Table 5. Triglyceride levels at different starvation time points. Both ST&SC females were starved for 0 hr, 8 hr,16hr, 24 hr,32 hr, 36hr.each point is the mean of triglycerides of single female

| TIME(in hours) | ST Females | SC Female |
|---|---|---|
| 0 | 0.59 | 0.55 |
| 8 | 0.54 | 0.49 |
| 16 | 0.58 | 0.54 |
| 24 | 0.55 | 0.5 |
| 32 | 0.59 | 0.48 |
| 36 | 0.57 | 0.46 |

Triglycerides level was measured at different starvation time points to see how triglycerides were used in response to starvation.ST females had stable triglyceride level after being starved for 0 hr,8 hr,16 hr, 24hr,32 hr,36 hr .while SC females showed decreased triglyceride level after 36 hr of starvation (F value=16.063 P value=0.002).

## 4. Discussion

### *4.1 Starvation resistance and life history traits*

Selection for starvation resistance over 20 generations showed a significant response in the present study. Chippindale *et al*., (1996) found that increased starvation resistance can also have an indirect response on larval

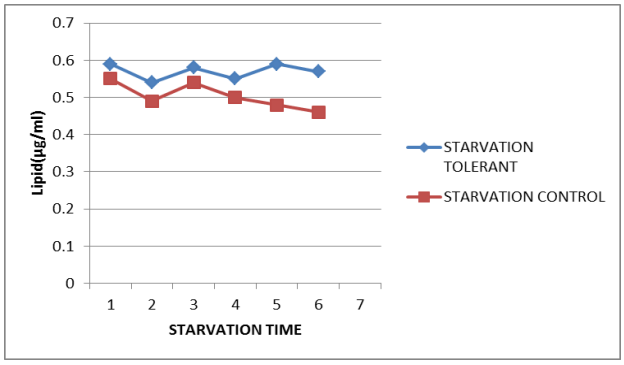


Figure 3.Representing triglyceride data

development time, and can cause higher adult lipid content and larger body size. In our selection lines, we also found that resistant female flies had a higher lipid content (7 days post eclosion), higher early fecundity, and higher dry weight, all of which are consistent with the findings of the aforementioned study. However, the selected male flies did not show significant differences in body size, protein content or even triglyceride level, but they did have higher starvation resistance. It will be an interesting topic for further research to explore why these males can survive under famine without extra energy stores. In our selection regime, we selected both males and females. Resistant females had more lipids and lowered their reproduction cost to extend their lifespan and survive during starvation, but none of these observations were made in males. What kind of physiological mechanism did resistant males use to survive starvation? It could be that ST males might reduce their mating frequency or courtship costs to conserve energy and resist starvation. They may have behavioral adaptations or other modified physiological mechanisms that enable starvation resistance. Increased body weight has been associated with starvation resistance in some studies (Chippindale*et al*.,1996; Hoffman *et al*.,1999), and body weight may reflect the total reserves of energy storage compounds such as lipids, carried by organisms (Djawdan*et al*.,1998). Chippindale*et al*., (1996) showed that the progeny of starvation resistant flies had higher lipid levels 3 hours after eclosion than controls but we did not observe the same thing when measurements were taken within one hour after eclosion. The different outcomes of our experiments might have arisen from discrepancies in lipid content measurements or selection regimes.

### *4.2 Total lipid declination under starvation*

SC and ST females didn't show much divergence in their lipid content soon after eclosion. Not until 7 days after eclosion on regular fly food did ST females have higher lipid content than SC females. This result revealed that resistant females gained more weight after eclosion. One possible explanation is that ST females had more efficient feeding strategies during their adult stage but this notion needs to be experimentally confirmed. Higher feeding rates would also be a correlated response to starvation

resistance, but this hypothesis has not been tested (Rion *et al.*,2007). In relation to energy distribution between reproduction and survival, it is also possible that SC females allocate more lipids to reproduction. Overall, the resistant females in this study did have higher levels of lipids, a finding which is corroborated by other studies. Although, these previous findings do not contain any lipid level data at eclosion. We think that resistant females don't have more lipids stored during larval stage (there was no lipid difference when SC and ST flies were eclosed) or they may use other forms of energy for storage, such as carbohydrates. ST females also had an altered biosynthesis or metabolism that increased body weight as well as lipid content after eclosion. When we starved 7 day old ST or SC females for another 36 hours, we found that the lipid content of both lines declined. The rate of this decrease seemed similar between the ST and SC females, but ST females still had more lipids in their bodies. These results can only tell us that starvation resistance is highly associated with lipid content, which is not surprising. Meanwhile, the utilization rate of lipids under starvation in both populations also reflected that ST females didn't have a slower metabolism rate (Harshman *et al.*,1998) because SC and SC females had a similar rate of decrease in lipid content.

*4.3 Triglycerides don't decline during starvation*

Although there was no difference in the total lipid decline, we used the same idea to test if there was a change in triglycerides at different starvation time points. We showed that SC females reduced their lipid contents but that ST females could maintain stable levels of triglycerides in their bodies after 36 hours of starvation. This outcome was contradictory to the total lipid result. How did resistant flies decrease their lipid but not their triglycerides levels? Theoretically, since triglycerides are important storage lipids in *Drosophila*, resistant flies would have to burn triglycerides as an energy source under starvation. Did resistant flies gain triglycerides or convert other lipids or resources into triglycerides from someplace else in the body? Or is it possible that ST females had stable triglycerides not because they didn't use any of them but because they were able to replenish them? In insects, the lipid trade-off between somatic tissues and reproductive tissues is also the trade-off between triglycerides and phospholipids. For example, long-wing crickets have more triglycerides in their bodies and lower phospholipids in their ovaries while short-wing crickets have less triglyceride in the body and more phospholipids in their ovaries (Zhao *et al.*, 2002). We speculated that ST females might retrieve lipids from ovaries to maintain their level of triglycerides.

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