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## Enriched nutrient diet shortens the developmental time –A transgenerational effect in *Drosophila sulfurigaster sulfurigaster.*

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### Abstract

Developmental time has a great relevance to fitness in all organisms. We set out to investigate the effect of parental larval diet on offspring development time, with relatively low amounts of sugar as a carbohydrate source and different concentrations of protein to assess the role of macronutrient balance on developmental time in *Drosophila sulfurigaster sulfurigaster* species. In the current study, the influence of larval diet experienced during just one generation extends into the next generation. Offspring reared on high protein and relative sugar concentration underwent metamorphosis significantly faster compared to the offspring of adults from low protein diet relative to sugar diets. A transgenerational effect of parental diet on offspring was found. Developmental time recorded was shortest in the case of offspring when compared to parents fed with high protein diet.

### Introduction

Deficiency or imbalance of fat, carbohydrate, or protein can affect characters such as growth and reproduction. Protein deficiency reduces fecundity and growth in *Drosophila melanogaster* (Wang *et al.*,1995), and in fruit-feeders protein is often limiting macronutrients (Markow *et al*., 2001). Many organisms face a challenge of meeting their optional nutritional requirement for somatic and reproductive growth under natural conditions (Raubenheimer *et al.*, 1991). During development, body tissues constantly require a specific quantity and proportion of nutrients in order to attain optimal growth and performance (Bauerfeind *et al.*, 2005). In contrast diet restriction on mild starvation can increase longevity as well as tolerance to stressors such as heat stress (Wenzel and Smith, 2006) demonstrating the complexity of organismal nutrient acquisition and utilization. A variety of factors may affect organismal stress tolerance. These include physiological as well as behavioral changes. The bulk of studies on physiological and evolutionary responses to nutrient deficiencies focus on reproduction and fecundity (Naya, 2007). On the other hand, parents may also respond to environmental cues in ways that enhance offspring performance under particular

environmental circumstances. Under this scenario, offspring will do best in an environment similar to that experienced by their parents (Cruz-Neto *et al.*, 2007).

Developmental time, a very important life history trait, is largely affected by environmental conditions (James and Partridge, 1995). Nutritional manipulation is one of the most 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; 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 most obvious way by which environmental variation may influence body condition and fecundity is via nutritional effects resulting from variability in food type availability. In general terms, diet effect can be classified as either quantitative (*i.e*., food availability) or qualitative (*i.e*., food composition). The quantitative effects are evident, since animals obtain energy and other nutritional requirements from food. Thus, under a natural range of conditions there is a positive correlation between food availability and body condition or fecundity. Qualitative effects often are divided into two categories: namely, nutritional deficiencies and inhibitory metabolites.

Parental genotype and environment often influence offspring fitness through non-genetically transmitted parental effects. Such effects may be maladaptive, *e.g*., malnourished parents may produce offspring of poorer quality (parental stress hypothesis). However, parents may also respond to environmental cues in ways that enhance offspring fitness. In particular, if the nutritional conditions experienced by the mother and offspring are positively correlated, mothers subject to nutritional stress would be favored to induce plastic changes in the offspring that make the latter more tolerant to nutritional stress. This adaptive hypothesis thus predicts that fitness of offspring on poor diet would be enhanced if their parents also experienced poor diet (Mousseau and Fox, 1998; Badyaev and Uller 2009). One potential mechanism of such adaptive parental effects involves adjustment of investment per offspring, which in organisms lacking parental care can be approximated by egg or newborn size (Azevedo *et al*., 1997). Life history theory predicts that under adverse conditions the optimal trade-off between offspring size and number is expected to shift towards fewer but better provisioned offspring (Roff, 1992).

The *Drosophila nasuta* subgroup, belonging to the *Drosophila immigrans* species group, includes more than ten species which are morphologically similar distributed in Pacific-Australasian and the pan-Indian Ocean areas. A number of these populations in different continental areas and islands of the Pacific were demonstrated to have diverged to the point of being separate sibling species (Wheeler and Takada 1964; Kitagawa *et al*., 1982). One of the species, *Drosophila sulfurigaster*, has the largest distribution among the *Drosophila nasuta* subgroup and consists of four subspecies. In light of the above investigation, a transgenerational effect has been sensed in the offspring fed with enriched nutrient diet with reference to developmental time in *Drosophila sulfurigaster sulfurigaster*.

## Materials and Methods

*Drosophila sulfurigaster sulfurigaster* stock was obtained from the *Drosophila* stock center, University of Mysore, Mysore, India. The stocks were maintained in an uncrowded culture condition at 22 ± 1˚C, 70% humidity, and 12h:12h light and dark cycles in standard wheat cream agar medium. From this stock about 200-250 eggs were collected and placed in culture bottles (about 10 to 50 eggs/bottle). Further, the eggs laid were recorded for hatchability and adult eclosion (Harini, 2011).

About 30 males and females were separated by gender and were transferred to the fresh media vials containing control and variable diet composition of proteins and carbohydrate. Two days after eclosion the adults ( F1) from each of the standard wheat cream agar medium and experimental diet were collected to record the effect of nutrition on developmental time. Developmental time was recorded daily from the egg to adult eclosion. The said experiments were carried out by feeding different concentrations of protein (Brewer's yeast), *i.e.*, 5g/L, 15g/L, 25g/L and glucose (30g/L), through the food media along with the control.

**Statistical Analysis**

Mean egg to adult development time (egg, larval hatchability, pupation, and adult emergence) were subjected to one-way ANOVA, Tukey's HSD by using SPSS 20.0.

Table 1. One way-Analysis of variance for developmental time testing for differences between groups of parental and F1 generation.

| Generations | Stages | df | Mean Square between groups | F | P-Value |
|---|---|---|---|---|---|
| Parental | Egg | 4 | .057 | .515 | .725 |
| | Larvae | 4 | 5.340 | 10.890 | .990 |
| | Pupae | 4 | 4.283 | 2.217 | .050 |
| | Adult | 4 | 2.224 | 6.143 | .000 |
| F1 | Egg | 4 | .057 | .515 | .725 |
| | Larvae | 4 | 5.640 | 12.170 | .990 |
| | Pupae | 4 | 8.717 | 5.799 | .000 |
| | Adult | 4 | 61.067 | 30.474 | .000 |
| Error | | 145 | | | |
| Total | | 149 | | | |

(Note: P < 0.05 significance values)

**Results**

Both egg to pupation and from pupa to adult eclosion (metamorphosis) time is considered for developmental time, but from pupa to adult eclosion showed a difference and was, therefore, analyzed further. Flies whose parents developed on the high protein diet had metamorphosed early. The data observed have shown that pupal to adult eclosion time lasted for 4 days and 4.50 days with low protein diet and only carbohydrate, when compared to control 5.50 days. The differences were insignificant from egg to larval hatchability, while it was significant from pupa to adult eclosion ($P < 0.05$) with that of control (Figure 1). The analysis of variance (Table 1) indicates significant difference for all the concentrations of protein and carbohydrate with that of control ($P < 0.000$), respectively. In addition to this the developmental time in F1 generation has significantly increased compare to parental the generation on exposure to high protein concentration (25 g Brewer'syeast) than in control and in low protein (5 g/L, 15 g/L ) and only carbohydrate (30 g/L) fed flies, *i.e.*, the pupal to adult eclosion time lasted for 2 days and 3.50 days low protein diet and only carbohydrate with that of the control, which lasted for 5.50 days in the parent generation as shown in Figure 2. Thus the overall data reveal that the higher the concentration of protein and low carbohydrate has shortened the developmental time in both parental and as well in F1 generation. Interestingly, developmental time (in days) has still more reduced in F1 generation than the parental stock. Thus the enriched media with protein and carbohydrate have a positive effect by reducing the egg to adult eclosion time significantly, which depicts the transgenerational effect found in *Drosophila sulfurigaster sulfurigaster*.

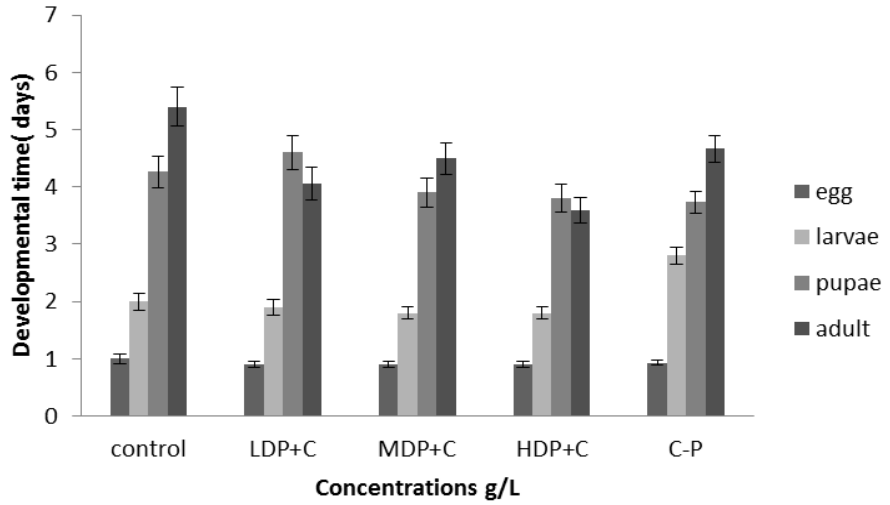


Figure 1. Mean developmental time (in days) of *Drosophila sulfurigaster sulfurigaster* parental stock (F0) fed with different concentrations of protein (Brewer's yeast) and carbohydrate (Glucose) diet. (Note: LDP = Low dose protein + Carbohydrate, MDP + C = Mid dose protein + Carbohydrate, HDP + C = High dose protein + Carbohydrate, C-P = Carbohydrate-Protein).

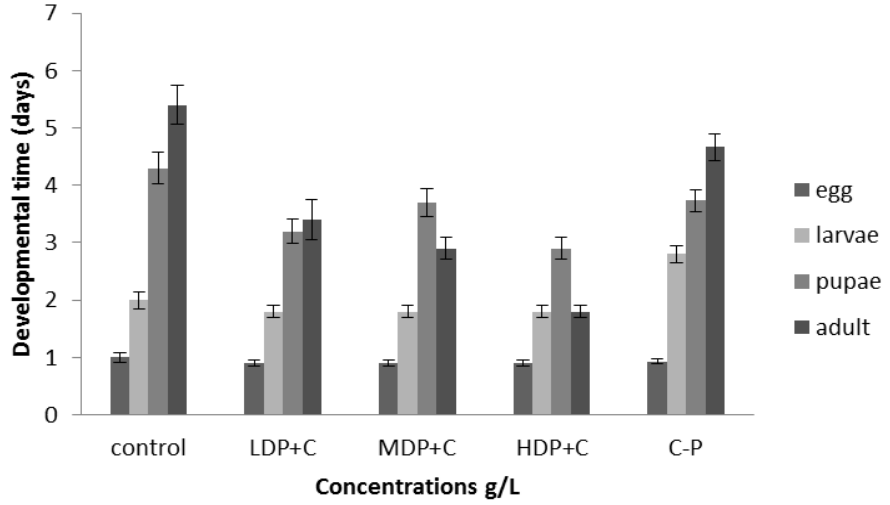


Figure 2. Mean developmental time (in days) of *Drosophila sulfurigaster sulfurigaster* offspring (F1) fed with different concentrations of protein (Brewer's yeast) and carbohydrate (Glucose) diet. (Note: LDP = Low dose protein + Carbohydrate, MDP + C = Mid dose protein + Carbohydrate, HDP + C = High dose protein + Carbohydrate, C-P = Carbohydrate-Protein).

## Discussion

According to life-history theory, natural selection could be expected to favor parents that produce fewer but better provisioned offspring in response to cues indicative that offspring will experience nutritional stress (Smith *et al.*,1974; Fox *et al.*,2000). The effects of DR have been investigated for more than 70 years in various organisms. Although DR is known to extend the lifespan of a wide range of organisms, species-specific effects of DR restriction have also been recorded (*e.g.*, Mockett *et al*., 2006). 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). 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. The

developmental rate of *Drosophila* is a function of numerous metabolic and developmental processes (Church and Robertson, 1966). By subjecting a wild-type population to fast and slow developmental rate selection, Robertson (1964) has shown that genetic variation exists in natural populations of *Drosophila melanogaster* for developmental rate. A significant interaction between the parental diets indicates that a parent's dietary effect on offspring development time was dependent upon the dietary source. The hatchability was significantly different ($P < 0.05$) between the groups of different concentrations in comparison to control, and insignificant between 5 g/L, 15 g/L of the protein concentration with ($P > 0.990$) and glucose (30 g/L). Experimental and controlled flies have not shown differences in all concentrations with that of control $P < 0.05$ and insignificant between 15 g/L and 25 g/L ($P > 0.755$) with respect to Table 1. Dietary restriction (DR) in *Drosophila* is often achieved by dilution of the food medium, and complete records of food intake are needed to determine if flies compensate for the reduced nutritional content of food by increasing the total amount of food they consume. Yeast has been shown as the most important compound of the food medium in *Drosophila* studies by several researchers. Life history traits like ageing, fecundity, viability, and development are directly affected by the levels of yeast used in the food medium. We emphasize the importance of carbohydrate and protein intake for developmental time. By comparing development times of offspring of parents with those of offspring parents it would appear that parents transferred their condition to their offspring. However, because the shortest development times were found among offspring of parent and F1 generation one can conclude that the complex interplay between nutrient balance and development time highlights the necessity of accurately measuring food intake when conducting development studies.

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