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The Effect of Mass Gainer on Pupation site Preference in Drosophila Melanogaster

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Abstract

Pupation site preference (PSP) is a vital component in *Drosophilamelanogaster* preadult development that involves habitat selection. The location chosen by larvae can significantly impact their survival as pupae. The larval pupation site preference (PSP) in *Drosophilamelanogaster* was analyzed in laboratory conditions which are treated with three different diets wheat-cream agar media (control), 10g Mass gainer media and 20g Mass gainer media. Our result showed that PSP differ significantly under different diets. Irrespective of diets, larvae preferred to pupate on walls of the culture bottle which considerably higher in all the three different diets. Across the media where fewer number of larvae selected their site for their pupation and it varied under different concentration of media. Results showed that larvae fed with the wheat-cream agar media showed maximum number of pupations at media, but larvae fed with 10 g Mass gainer media and 20 g Mass gainer media revealed a least PSP towards the media, On the other hand the same larvae supplied with the 10 g Mass gainer media and 20 g Mass gainer media showed a maximum number of pupation at walls of the culture bottle and those which are raised under wheat-cream agar media showed slightly lesser PSP when compared tothe 10 g Mass gainer media and 20 g Mass gainer media. Thus, our study suggests that nutritional component present in the 10 g Mass gainer media and 20 g Mass gainer mediaprovides the required amount of energy to the larvae to move at a maximum rate from the media to pupate on the walls of the culture bottle. Therefore, our results revealed that Mass gainer is capable of increasing the PSP in *D. melanogaster* and is that improves an overall fitness of an organism.

Keywords: Pupation site preference (PSP), Diet, *D. melanogaster*, Pupa, Larva

Introduction

Drosophila life cycle has four stages: egg, larva, pupa, and adult. During the larval stage, the insect goes to various surfaces to pupate (Moreno *et al*, 2007). Pupation site preference (PSP) is a critical step in *Drosophila* preadult development that involves habitat selection, because the location chosen by larvae can have a significant impact on their future survival as pupae (Sameoto and Miller, 1968). Studies on *Drosophila* species have revealed that both biotic (sex, density, locomotory path length, developmental period, and digging behavior) and abiotic (temperature, humidity, moisture, light and dark, and pH) variables have an important effect in pupation site selection (Zhang *et al*, 2019). Furthermore, research have showed that PSP is influenced by genetic factors, and the genes that control PSP are found on autosomes with little or no dominance (Bauer & Sokolowski, 1985).

It has been examined in various *Drosophila*sub groups,including *D.melanogaster*, *D.virilis*, *D.repleta*, and *D.immigrant*. The study foundthat*D. immigrants* prefer to pupate on wheat cream agar media, while *D. replete* prefers glass. The*Drosophila melanogaster* group has been the subject of recent breakthroughs. In this group, *D.simulans* and *D. mauritiana* favored wheat cream agar media, but *D. melanogaster* selected glass for pupation.Mostanalyzed species favored maximal wheat creamagar media for pupation, while only a few selected glass. *D. rajasekari* and *D.gibberosa* used cotton for pupation (Shirk *etal*., 1988; Shivanna*etal*.,1996;vandal*etal*.,2003).A genetic investigation of pupation found that the

distinction between larvae who prefer to pupate on media and those that prefer the bottom of the media bottle is primarily attributable to a single gene change(de Souza*etal*., 1970).

Mass gainer is a powdered supplement that combines carbohydrates and protein which is usually used to increase body mass (Campbell *etal*., 2008). Mass gainer, often known as a "weight gainer," is a powder that is intended to replace meals with the goal of gaining muscle mass. In order to encourage an energy surplus and the synthesis of muscle protein, the majority of mass gainers are high in fat, protein, and carbohydrates. In order to speed up recuperation, mass gainers may also include additional muscle-building components like beta-hydroxymethylbutyrate (HMB) and creatine monohydrate. Since most studies focus on how different species of *Drosophila* and their hybrids prefer PSP. This study aims to investigate the impact of Mass gainer on PSP utilizing *D. melanogaster* as a model organism.

Materials and Methods Material and methods:

The mass gainer was purchased through Flipkart App from A207, Lane No. 9, No. 4, Mahipalpur, Delhi, 110037, India. This mass gainer used to prepare the experimental media.

Establishment of stock:

Experimental Oregon K strain of *Drosophilamelanogaster* used in the study was collected from *Drosophila* stock center. Department of studies in Zoology, University of Mysore, Mysore and this stock was cultured in bottles containing wheat cream agar media (100g of jaggery, 100g of wheat cream rava,10g of agar was boiled in 1000 ml distilled water and 7.5 ml of propionic acid was added). Flies were maintained in laboratory conditions such as humidity of 70% and 12 hours dark 12 hours light cycles and temperature 22° C+ 1° C, where these were collected to conduct our experiment.

Establishment of experimental stock:

The flies obtained as above were used to establish the experimental stock with different diet media. [Wheat cream agar media: Wheat cream agar media was prepared from 100g of jaggery, 100g of wheat cream rava, 10g of agar boiled in 1000ml distilled water and 7.5 ml of propionic acid added to it.

10g of Mass gainer media: is prepared from100g of jaggery, 90 g of wheat cream rava, 10g of mass gainer powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it.

20g of mass gainer media: is prepared from 100g of jaggery, 80g of wheat cream rava and 20g ofmass gainer powder,10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it]. The flies emerged from the wheat cream agar media and other experimental treated media under the same laboratory conditions as mentioned above were used to study the pupation site preference experiment in *Drosophilamelanogaster.*

Experimental procedure:

To investigate the influence of Mass gainer on the pupation site preference approximately 20 flies, ten male and ten female flies grown in control media were collected. The gathered flies were placed in the culture bottles of control and 10g mass gainer media and 20g mass gainer media and left for 3 hours. Later, the flies were removed from the culture bottles, and the cultured bottles were left for 24 hours to produce 1st instar larvae from the flieseggs. Thirty 1st instar larvae were retrieved by scooping the medium from the culturebottles. The collected first instar larvae were transferred to respective nutrient media in group of ten larvae, namely control, 10g, and 20g Mass gainer media in the vials. The treated larvae in all vials were monitored up to their pupation. The number of larvae pupated at three different sites (cotton, glass wall and media of culture vials) were counted and tabulated.

Results and Discussion

Effect of media on pupation site preference in *Drosophila melanogaster*

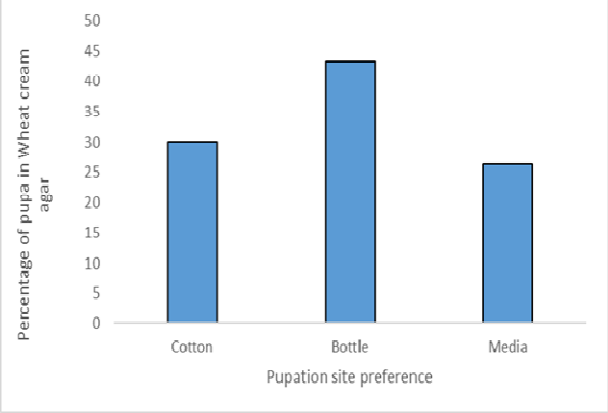

Figure 1 a

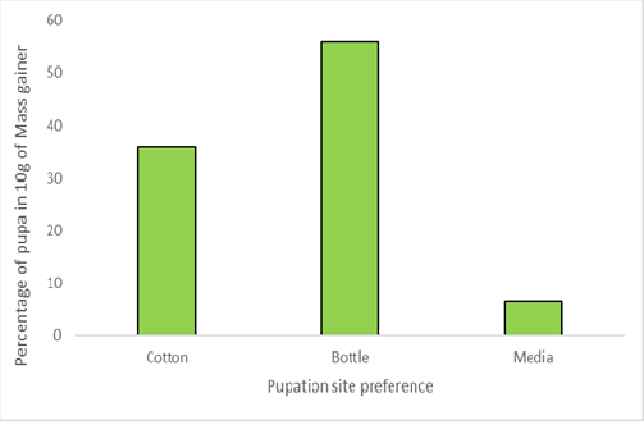

Figure 1 b

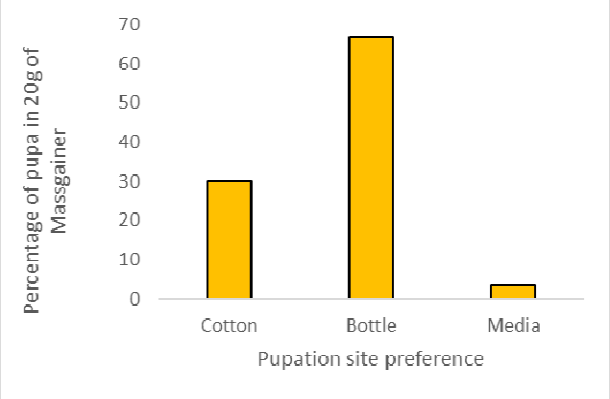

Figure 1 c

Figure 1a -Effect of control media on the pupation site preference in *Drosophila melanogaster* representing the percentage of pupation in respective sites of the culture bottle.

Figure 1b -Effect of 10g of mass gainer on the pupation site preference in *Drosophila melanogaster* representing the percentage of pupation in respective sites of the culture bottle

Figure 1c- Effect of 20g of mass gainer on the pupation site preference in *Drosophila melanogaster* representing the percentage of pupation in respective sites of the culture bottle

Discussion

An essential stage of the *Drosophila* life cycle is the larval stage, during which the insect migrates to different surfaces to become a pupa. The capacity of third-instar larvae to pupate on substratum is an essential part of the *Drosophila* life cycle because puparia stay immobile and are exposed to potentially hazardous environmental influences (Manning and Markow, 1981).

From figure 1a, figure 1b and figure 1c it was revealed that larvae preferred different sites for pupation in different media. In wheat cream agar media, 10g of mass gainer and 20g of mass gainer more pupae were formed on the bottle/wall of the culture bottle, followed by near/on cotton and less on the media. This is suggesting that nutrients found in mass gainer provide required energy for the larvae to move higher from the media and pupate on the wall or bottle of culture bottle.

Differentiating the nutrients has an impact on the number of pupae that grow, according to research

findings. Protein ingestion, not carbohydrate consumption, controls the growth of the body and tissues in larvae (Britton and Edgar, 1998). However, both proteins and carbohydrates influence the variance in the timing of development (Schwarz *etal*., 2013). Larvae raised in food with high yeast concentrations show greater fertility but decreased longevity and resilience to famine in comparison to those raised on a diet low in yeast (Chippindale *etal*., 1993).

In our present study, Larvae which are grown in control media tend to choose pupation sites in which they have a higher probability of surviving i.e., most of the larvae prefers to choose the walls of the bottle as their best pupation sites which are fed with wheat cream agar media. And this shows that *D. melanogaster* has higher probability of surviving at walls of the culture of bottle when compared towards the cotton and media site, that few numbers of larvae opted cotton of the bottle and media.

Our study also supported the experiment conducted by (Cleona and Krishna, 2018) effect of avocado and yogurt on pupal behavior of *Drosophila melanogaster*. Thus, the studies suggest that avocado and yogurt had significant influence on the pupation behavior of *Drosophila melanogaster.*

Pupation site selection was also significantly influenced by the salivary gland protein known as glue protein secretion. Immediately prior to pupariation, *Drosophila* larvae make a glue that enables the pupa to remain attached to a substrate during metamorphosis. These proteins are known as salivary gland secretion proteins (Sgs), and they have swiftly evolved across and within species. A few minutes after expectoration, the adhesive dries, spreading between the body and the substrate (Borne *et al*, 2021), In*D.melanogaster*the pupation site is determined by the amount of glue proteins released (Shivanna*et al*, 1996). Shivanna states that larvae that secrete more glue protein typically pupate on cotton, larvae that secrete half as much glue protein typically pupate on glass, and larvae that secrete very little glue protein typically pupate on media surfaces.

Based on this research we can conclude that nutrition with different ratios of protein and carbohydrates affects the pupation site in *Drosophila*, and because the pupal stage cannot move and cannot withstand environmental variations, the availability of food and its scarcity, the choice of the larva's preferred place to pupate is very important for survival for pupae.

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