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The Effect of Rolled Oats on Pupation Site Preference in *Drosophila Melanogaster.*

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ABSTRACT:

The life cycle of the holometabolous insect *Drosophila* consists of four stages: egg, larva, pupa, and adult. The ability of *Drosophila* larvae in their third instar to pupate on an appropriate substrate is an important aspect of their life cycle. *Drosophila melanogaster* larvae were reared on wheat cream agar, 25% oats, 50% oats, and 75% oats media in the current investigation. The findings indicate that in wheat cream agar media, the greatest number of larvae chose to pupate on the bottom region (media), followed by the average number in the middle region (glass) and the lowest number on the neck (cotton). In 25% oats media, the number of larvae pupated were seen to be increased in glass (middle)and cotton (neck)region, while there was decreased pupation percentage was observed in media (bottom)region. The similar trend was also observed in 50% and 75% concentrations of oats media. However, the larvae raised in wheat cream agar media pupated in greater numbers on the bottom region, indicating that the nourishment from varying oats concentrations supplied the energy needed to migrate up from the media to glass and cotton. The quantity and quality of nutrients available in the various media appear to be the main factors affecting the choice of pupation location. The presence of dietary fibre and quality protein supplemented with a regular meal gives the larvae support to go to higher regions for pupation. However, these investigations imply that oats had a major impact on *D. melanogaster* pupation behaviour.

Key words: *Drosophila melanogaster*, rolled oats, pupation site preference.

Introduction:

Numerous other species, including *Drosophila*, have been the subject of behaviour analysis, an intriguing field of study. The life cycle of the holometabolous insect *Drosophila* consists of four stages: egg, larva, pupa and adult. Foraging, burrowing, skipping, and selecting a pupation site are all examples of larval activity. A critical stage in the *Drosophila* life cycle is the larva's choice of suitable pupation site, which includes habitat selection (Cleona and Krishna, 2018). Pupation site preference, or PSP, is a crucial step in *Drosophila* development because the larvae's choice of place can significantly affect their chances of surviving as pups (Someoto and Miller, 1968). Since puparia, the larval skin that encloses the pupa, are immobile and susceptible to infections, desiccation, and predators, *Drosophila* must be able to pupate on a suitable substrate throughout the third instar larva's life cycle (Manning and Markow, 1981).

The percentage of larvae that pupate at different sites, such as media (bottom), cotton (neck), glass (middle), etc., is included in the Pupation Site Preference. PSP is often assessed based on two parameters. One is Pupation Site Preference, which measures the percentage of larvae that pupate on various surfaces such as cotton, glass, and media, and the other is Pupation Height, which measures how far the larvae have to move upward to pupate away from the surface of food (Shilpashree et al., 2024). When third instar larvae migrate to pupation sites, they are known to react differently to several types of extrinsic factors: light (Manning and Markow, 1981, Rizki and Davis, 1953), moisture (Sameoto and Miller, 1968), gravity (Markow, 1979), larval density (Singh & Pandey, 1993), larval developmental time (Markow, 1979), and the presence of other species (Rizki and Davis, 1953).It has been demonstrated that a wide range of abiotic conditions affect behavioural variations in pupae, both within and between species (Markow, 1979; Sokolowski and Hansell, 1983). Several parameters, including temperature, pH, humidity, medium water content, crowding, sex, duration of larval stage, and autoclaving time, influence the pupation site of *Drosophila melanogaster* larvae. There is a connection between PSP and the glue proteins, which are the secretions from the larvae's salivary glands. It is shown that the amount of sticky protein secreted by the salivary glands is proportional to the tendency of the larvae to move away from the surface of the media (Shivanna et al., 1996).

Nutrition is a significant environmental component that has an impact on animal physiology, metabolism, and characteristics like fitness and survival. Due to the intricate makeup of diet, it is still difficult in biology to comprehend how each nutrient affects the animal phenotype (Felipe Martelli et al., 2024). An organism's life history is impacted by food, which is a crucial external component because it supplies sustenance and energy (Sterner and Schulz, 1998; Taylor et al., 2005). Development, health, reproduction, longevity, and stress tolerance are all impacted by the quality and quantity of food (Hoffmann and Parsons, 1991; Rion and Kawecki, 2007; Lee et al., 2008). Numerous research had been demonstrated how nutrition affects *D. melanogaster* pupation site selection. Avocado and yogurt by Cleona and Krishna (2018), ensure by Shilpashree et al., (2024), mass gainer by Asniati Jabbar et al., (2024) also demonstrated how nutrition affects *D. melanogaster* pupation location choice. And also, there are many other research which demonstrates the pupation site preferences in different species of *Drosophila* (Shilpashree et al., 2024). Effect of fruits on different species of *Drosophila* by Nakul et al., (2009). Hence in all these experiments different nutritional supplements were provided to assess the pupation behaviour of *Drosophila*, where the nutrition supported the larvae to pupate in higher regions but none had done the experiment on rolled oats, so in this experiment the rolled oats was taken to demonstrate its effect on the pupation site preference in *D. melanogaster*.

Oats are the sixth most important cereal grain crop produced for food and feed for people. When compared to other cereal grains like corn, barley, and wheat, whole or rolled oats have a higher crude protein (CP) content (11%), ether extract (EE) content (5%), starch content (39%), and gross energy (GE) density (4,272 kcal/kg). The hull is composed primarily of lignin and insoluble dietary fibre (beta glucan), which reduces the use of nutrients and

energy. Additionally, it has lipids, trace minerals, macronutrients, and vitamins. But there is increasing evidence that oat-derived β-glucans and phenolics (such as avenacosylates) act as anti-inflammatory compounds (Bonjin Koo and Charles Martin Nyachoti, 2021).

Materials and methods:

The swiggy instamart app in Mysuru was used to buy rolled oats (gluten free). The experimental media was made using this oat product.

Establishment of stock:

The *Drosophila* stock center, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru, is where the Oregon K strain of *D. melanogaster* flies were obtained. The resulting flies were cultivated in bottles using wheat cream agar medium, which included 7.5 ml of propionic acid to inhibit fungal growth, 100 grams of jaggery, 100 grams of suji powder, and 10 grams of agar in 1000 ml of boiling distilled water. These flies were housed in a lab setting with a 12:12 light and dark cycle, a temperature of 22+1°C, and a relative humidity of about 70% RH. Using various diet medium, experimental stocks were cultivated from flies from the aforementioned stocks.

Establishment of experimental stock:

Wheat cream agar media: 100g of jaggery, 100g of suji powder, and 10g of agar were combined with 1000ml of boiling distilled water and 7.5ml of propionic acid.

25% oats media: 25g of ground rolled oats, 75g of suji, 100g of jaggery, and 10g of agar were combined with 1000ml of boiling distilled water and 7.5ml of propionic acid.

50% oats media: 50g of ground rolled oats, 50g of suji, 100g of jaggery, 10g of agar, and 1000ml of boiling distilled water were combined with 7.5ml of propionic acid.

75g oats media: 25g of suji, 75g of ground rolled oats, 100g of jaggery, 10g of agar, and 1000ml of boiling distilled water were combined with 7.5ml of propionic acid.

Flies obtained from the above media were used in the present experiments.

Experimental procedure:

Twenty flies (both virgin females and unmated males) were taken and placed into media bottles containing 25% oats media, 50% oats media, 75% oats media, and wheat cream agar media respectively, in order to examine the impact of rolled oats on the pace of development. A day later, the larva were taken out, by scooping out media from media bottles and transferring them to various media bottles with the appropriate media that were plugged with cotton, ten first-instar larvae were gathered. Following larval hatching into pupae, each pupa's location—either media (bottom), glass (middle), or cotton (neck)—was noted. These experiments were carried out in the laboratory conditions as described above, the larvae were monitored and their preference for pupation location was recorded for each of the following media: Wheat cream agar, 25% oats, 50% oats, and 75% oats.

Results and Discussion:

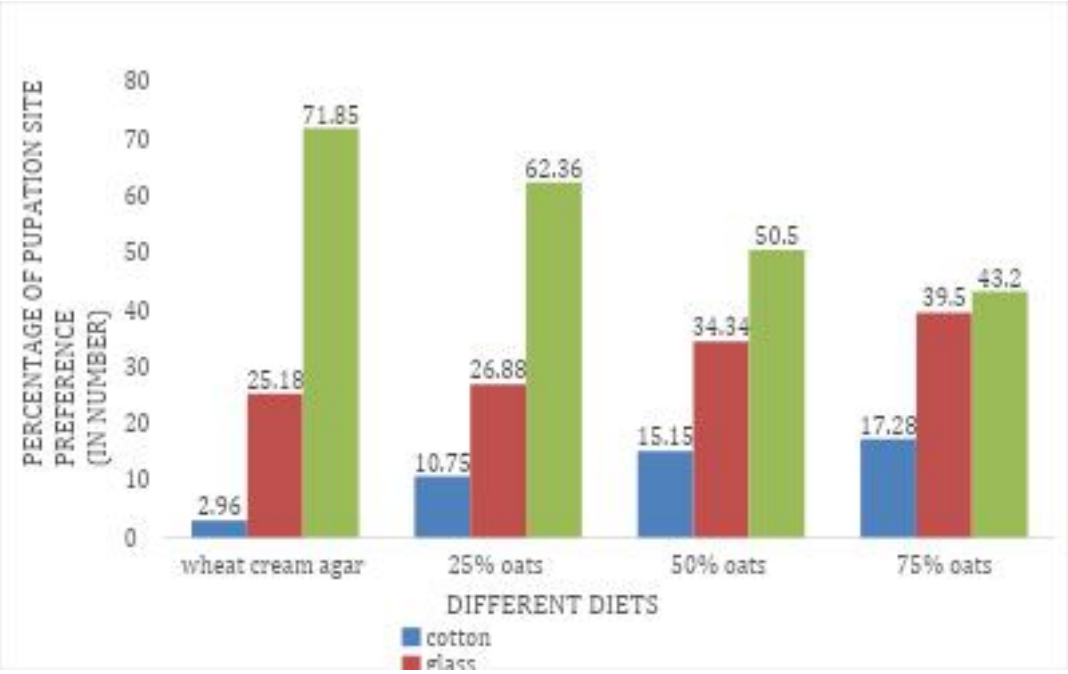

Figure 1: Effect of different diets on pupation site preference in *Drosophila melanogaster***.**

The life cycle of the holometabolous insect *Drosophila* consists of four stages: egg, larva, pupa, and adult. Searching for food, digging, skipping, and choosing a place to pupate are all instances of larval behaviour. The location of pupation, known as pupation site preference or PSP, is a critical stage in the development of *Drosophila* because the larvae's choice of site has a significant impact on their survival rate as pupae (Someoto and Miller, 1968). Numerous internal and external factors, including as the quantity of salivary gland protein (glue protein) secreted, various nutritional effects and environmental elements like light, temperature, moisture, and pH, were known to affect pupal behaviour (Shilpashree et al., 2024).

Impact of rolled oats on *Drosophila melanogaster* pupation location selection. The percentage of pupation in wheat cream agar media, 25% oats media, 50% oats media, and 75% oats media were shown in the chart (Figure 1). It showed that, in wheat cream agar media the highest larvae pupated in the media region and average in the glass and least number of pupae in the cotton. In contrast to this, in all the different concentrations of oats media such as 25%, 50% and 75% the number of larvae pupated were seen to be increased in glass and cotton region and decreased number of larvae pupated in media region as the concentration of oats increased. Compared to larvae fed with wheat cream agar media, those fed with oats-concentrated media grew noticeably in higher regions.

Dietary changes can improve fitness, as is widely accepted (Djawdan et al, 1998). They also showed that protein enrichment improved female survival and that eggs were more viable in sucrose-rich environments. Therefore, it shows that nutrition during the pre-adult years is essential to the overall well-being of adults and its behaviour. Different amounts of food available at these stages also affect how resources are processed. Additionally, they showed that lower food consumption leads to smaller body size (Zoltan and Gerdien, 2003), which is a frequent occurrence in ectotherms (Atkinson and Sibly, 1997) and seen in *D. melanogaster* and other species (Gebhardt and Stearns, 1998). Numerous research had demonstrated how nutrition affects *D. melanogaster* pupation site selection. Avocado and yogurt by Cleona and Krishna (2018), ensure by Shilpashree et al., (2024), mass gainer by Asniati Jabbar et al., (2024) also demonstrated how nutrition affects *D. melanogaster* pupation location choice. And also, there were many other research which demonstrated the pupation site preferences in different species of *Drosophila*, effect of fruits by Nakul B et al., (2009). Hence in all these experiments different nutritional supplements were provided to assess the pupation behaviour of *Drosophila*, where the nutrition supported the larvae to pupate in higher regions.

According to Manning and Markow (1981), species of *D. melanogaster* usually pupate in dark regions. As temperatures rise, larvae pupate in higher locations, according to Mensua (1967). A bigger feeding area and less digging behaviour were two further factors that lead to more pupation sites (Sokolowski & Hansell, 1983). Increased larval density is associated with high preference for pupation sites (Singh and Pandey, 1993). The presence of other species may lead to the establishment of pupation sites (Rizti and Davis, 1953). The correlation between pH and pupation distance had been investigated in numerous studies, and the findings show that larvae prefer to pupate close to the lowest pH resource as opposed to the highest pH. This implies that the development period is impacted by acidic resources and that larvae would go further to become healthier (Hodge et al., 1996; Vandal et al., 2008). However, in this experiment consistent light-dark phases and laboratory temperature was maintained. Each replication in all the media was limited to only to ten larvae, each larva had enough space to locate food, making digging easier and the foraging area was the same for all the larvae. We kept the pH constant throughout our study. Thus, all these factors could not account for any variations in pupation site selection.

One important factor that affected pupation site choice is the release of glue protein, a salivary gland protein. The quantity of glue proteins produce determines the pupation site (Shivanna et al., 1996). According to Shivanna, larvae with higher glue protein secretion tend to pupate on cotton (neck), those with half as much glue protein secretion choose to pupate on glass surfaces (middle), and those with very little glue protein secretion tend to pupate on media surfaces (bottom). The amount of glue protein that the larvae made was not measured in this study. Therefore, we were unable to conclude that the amount of glue proteins produced was the reason of the variance in pupation location choice.

Humidity has an impact on the PSP as well. In wet environments, larva pupate on the wall and nearby or on the cotton, according to Divya Singh et al., (2022), since the surrounding moisture may make it difficult to build pupal chamber. The aforementioned statement was supported by our experiment, which demonstrates that the oats media was viscous, allowing for a higher percentage of pupa to be seen on the glass and then on the cotton of the media bottle to shield from the moisture. Because of its capacity to create thick solutions, high dietary fibre, which is more prevalent in oats, has higher physiological functionality (Noora Makela et al., 2020).

In our experiment, the amount of nutrients varied in different media, which caused this variation in PSP, which was mostly impacted by protein concentration in the diet. In comparison to the wheat cream agar media, various concentrations of oats media have a high protein level (Bonjin Koo and Charles Martin Nyachoti, 2021). According to Krittika et al.'s 2019 research, which demonstrated that a low protein concentration reduces pupation height and a high protein concentration raises pupation height in *D. melanogaster*, our study corroborates their results**.**

In figure 1, shows that the larvae preferred the glass and cotton region more than the media region in different concentrations of oats when compared to the wheat cream agar medium. However, the solubility of dietary fibre contributed to the viscosity and the high amount of concentration of dietary protein in the rolled oats product that affected PSP across different media. As a result, differences in the quantity and quality of the nutrients found in various concentrations of oats media caused the observed variation in our experimental data.

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