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Exploring the Influence of Intermittent Heat Exposure on Spontaneous Mutations in *Drosophila melanogaster*: Assessing the Role of Vitamin C in Mitigating Heat Stress and Examining Inheritance Patterns of Induced Mutations

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ABSTRACT

Climate change poses a significant threat to the well-being of organisms. It has a detrimental impact on the survival of smaller organisms in response to climatic shifts, posing a substantial danger to biodiversity, which is already under stress due to habitat loss, emerging invasive species, and diseases. This study aimed to assess the influence of fluctuating temperatures on the physiology and behavior of *Drosophila melanogaster*, as well as to investigate whether such temperature fluctuations have any effect on phenotypic expression through potential spontaneous mutations. Genotypic changes were examined by observing cytological alterations in the salivary gland chromosomes. *Drosophila melanogaster* were exposed to intermittent heat conditions for a period of two weeks. The experimental setup was divided into four groups: a control group maintained at room temperature (25±2°C), a group at room temperature supplemented with vitamin C, a group exposed to heat at 38±2°C, and a group exposed to 38±2°C with vitamin C supplementation. Revival of the flies was noticeably better in the vitamin C supplemented group. These flies exhibited a higher revival rate

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even after exposure to the heat stress. Salivary gland chromosome analysis provided intriguing insights. More balbiani rings were observed, indicating elevated mRNA production during the heat exposure. Furthermore, an increase in the number of puffs in polytene chromosomes was noted, suggesting an overall increase in mRNA production in the heat-exposed flies. Additionally, the evaluation of wing mutants yielded important findings. It became evident that these mutations were not related to vestigial or curly wing traits. Instead, they indicated that heat exposure was damaging wing formation, resulting in abnormal wing patterns. These results suggest a substantial impact of temperature fluctuations on insect behavior, which can even lead to the induction of mutations. Generational studies further indicate that these mutations can be inherited.

1. INTRODUCTION

Drosophila melanogaster, commonly referred to as the fruit fly, is a diminutive insect belonging to the *Drosophilidae* family. Despite its unassuming size, ranging from 2 to 4 millimeters, this unassuming creature has played a pivotal role in advancing biological research. It has propelled our understanding of genetics, development, behavior, and various other scientific disciplines to unprecedented heights (Bier, 2016).

Diving into the distinctive characteristics of *Drosophila melanogaster* reveals a recognizable morphology (Otto and Hardwick, 2007 a). Their rapid life cycle facilitates swift experimentation (Stearns and Ashburner, 2004), and they exhibit a penchant for consuming fermented foods (O'Malley and Ashburner, 2016). Moreover, their ecological tolerance makes them valuable indicators of environmental conditions (Toth and McDonald, 2015). They display a degree of heat resistance (Ashburner and O'Malley, 2004) and have adapted mechanisms to survive in cold environments, such as entering diapause (Otto and Hardwick, 2007 b). The stress response in lower organisms is well-documented (Rasgon and Otto, 2017).

Vitamin C, also known as ascorbic acid, is an essential nutrient with a vital role in numerous biological functions, including free radical scavenging (Appel, 2003). Vitamin C is believed to aid in the fruit flies' ability to withstand both heat and cold stress (Khan and Khan, 2013; Das et al., 2014). Many fruits, such as cantaloupe (muskmelon), oranges, and strawberries, are rich sources of Vitamin C (McMillan and Fairweather-Tait, 2007). For many eukaryotic organisms, Vitamin C is a critical nutrient (Khan et al., 2017; Khan et al., 2015).

The primary focus of this study is to investigate the impact of vitamin C on the stress tolerance of *Drosophila melanogaster* under conditions of heat stress. By subjecting fruit flies to controlled temperature conditions and observing their survival rates and physiological responses, researchers aim to elucidate whether vitamin C supplementation can effectively mitigate the adverse effects of temperature stress.

1.1. OBJECTIVES OF THE STUDY

The objectives of this study are as follows:

1. To investigate the impact of fluctuating temperatures on the physiology and behavior of *Drosophila melanogaster*.

2. To assess whether temperature fluctuations, particularly heat stress, influence the phenotypic expression of *Drosophila melanogaster* through potential spontaneous mutations.

3. To examine genotypic changes in *Drosophila melanogaster* by observing

cytological alterations in the salivary gland chromosomes in response to heat exposure.

4. To evaluate the revival and survival rates of *Drosophila melanogaster* under heat stress conditions, particularly in the group supplemented with vitamin C.

5. To analyze and characterize the observed physiological and behavioral changes in *Drosophila melanogaster* in response to intermittent heat exposure.

2. MATERIALS AND METHODS

2.1. SPECIMEN ACQUISITION AND MAINTENANCE

The study involved the culturing and induction of mutations in *Drosophila melanogaster*. *Drosophila melanogaster* specimens were obtained from the *Drosophila* stock center at Mysore University and were maintained at a controlled temperature of 25±2°C.

2.2. PREPARATION OF SIMPLIFIED DROSOPHILA MEDIUM

To create a medium for culturing *Drosophila melanogaster*, a recipe utilizing only four essential ingredients was employed. These ingredients included agar (15-20 grams) as the solidifying agent, brewer's yeast (50 grams) as the primary source of protein, jaggery (100 grams) to provide carbohydrates and sweetness, and approximately 1 liter of water.

2.3. GROUPING AND TREATMENT ASSIGNMENT

The flies were organized into four distinct groups, including a control group (Group 1), a group exposed to heat (Group 2), a control group supplemented with vitamin C (Group 3) (1g per 100ml of media), and a group exposed to heat with vitamin C supplementation (Group 4) (1g per 100 ml of media).

2.4. INTERMITTENT HEAT EXPOSURE

Intermittent heat exposure was administered to Group 2, and Group 4, subjecting them to temperatures of 38**±2**°C for one hour daily. Regular observations were conducted, including fly counts and assessments for behavioral changes.

2.5. ASSESSMENT OF THERMOTOLERANCE

In the thermotolerance assessment, a protocol inspired by Morgan and Mackay (2006) was adopted. *Drosophila melanogaster* flies from Group 2 and Group 4 were placed in a water bath for 38**±2**°C for one hour daily. After each exposure, detailed observations were conducted to assess behavioral changes indicative of thermotolerance or susceptibility, with specific criteria for categorization. Daily monitoring and data recording enabled the determination of the proportion of flies exhibiting thermotolerance or susceptibility. Statistical analyses were performed to assess significant differences between experimental groups. This method allowed for the investigation of how heat stress influenced fly thermotolerance and whether vitamin C supplementation played a role in modulating this response.

2.6. CHROMOSOME PREPARATION AND CYTOLOGICAL EVALUATION

Chromosome preparation was carried out using salivary glands, and staining techniques were employed to evaluate cytological changes, including the presence of Balbiani rings and puff formation. For *Drosophila melanogaster* larval salivary gland chromosome staining, slides were rinsed prepared in phosphate-buffered saline (PBS), applied Giemsa stain, incubated for 5-15 minutes, rinsed again, and air-dried. Microscopic examination revealed chromosome patterns.

2.7. EVALUATION OF HEAT-INDUCED WING MUTANTS

Furthermore, the study included the evaluation of wing mutants induced by heat exposure. Wing morphology was meticulously examined in both the controls and heat-exposed groups, with and without vitamin C supplementation, to understand the effects of heat stress on wing development.

3. RESULTS AND DISCUSSION

The heat-exposed flies exhibited a significant induction of wing mutations, leading to visible challenges in flying, along with a reduction in wing width (Figure 1). Notably, the administration of vitamin C supplementation proved beneficial in reviving the flies, as depicted in Figure 2.

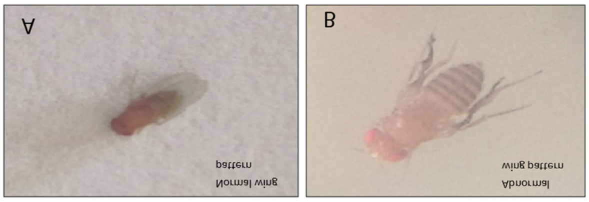

Figure 1: Wing pattern observation in the control (A) and heat-exposed (B) flies

The supplemented group displayed notably improved revival rates, even following exposure to heat stress, as shown in Figure 2.

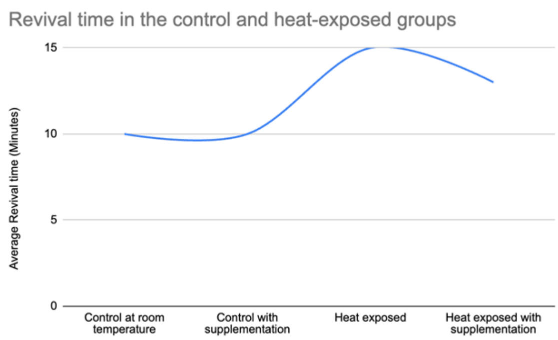

Figure 2: Revival time in the control and heat-exposed groups. 1-Control at room temperature. 2-Control with supplementation. 3-Heat exposed. 4-Heat exposed with supplementation

In terms of thermotolerance, the control group exhibited superior resistance to heat stress in comparison to the heat-exposed group. Notably, supplementation with vitamin C played a significant role in enhancing thermotolerance levels, as indicated in Figure 3.

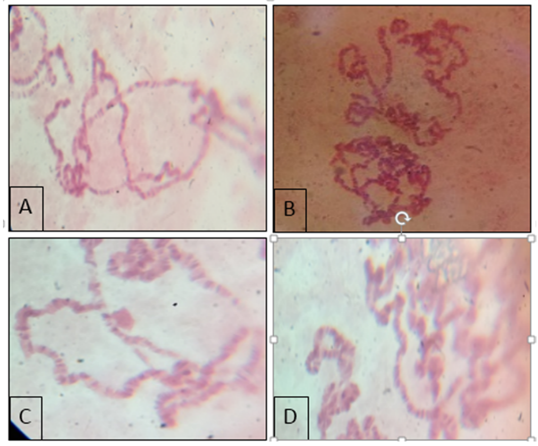

Figure 3: Salivary gland chromosomes of A- Control. B- Supplemented group at room temperature. C- Heat exposed. D- Supplemented group at heat exposure.

Salivary gland chromosome analysis unveiled an increase in Balbiani rings during heat exposure, signifying heightened mRNA production, potentially associated with stress response mechanisms. Furthermore, an increase in the number of puffs in polytene chromosomes suggested an overall rise in mRNA production.

The evaluation of wing mutants ruled out vestigial or curly wing mutations. Instead, it indicated that heat exposure inflicted damage upon wing formation, resulting in the observation of abnormal wing patterns, as depicted in Figure 4. It is noteworthy that the abnormal wing formation was less pronounced in group 4 (group exposed to heat with vitamin C supplementation) when compared to group 2 (group exposed to heat).

Figure 4: Abnormal wing pattern observed in heat-exposed flies.

These findings underscore the significant impact of temperature fluctuations on *Drosophila melanogaster's* physiology and behavior. They also highlight the potential benefits of vitamin C supplementation in enhancing thermotolerance. Additionally, the cytological changes observed in salivary gland chromosomes shed light on the molecular responses to heat stress, while the presence of abnormal wing patterns provides insights into the consequences of heat exposure on wing development.

4. CONCLUSION

The study's results shed light on the significant impact of temperature fluctuations on *Drosophila melanogaster's* physiology and behavior. Heat exposure induced wing mutations in these insects, leading to observable challenges in their flying abilities and alterations in wing morphology. Notably, vitamin C supplementation played a crucial role in enhancing thermotolerance and improving fly revival rates after heat exposure.

These findings offer a captivating avenue for scientific exploration into the intricate interplay between *Drosophila melanogaster* and temperature stress. The potential benefits of vitamin C as an antioxidant intervention highlight its role in bolstering stress resilience in these organisms. This research contributes valuable insights into the mechanisms of stress responses and provides a foundation for future studies aimed at understanding how various organisms, including humans, can better adapt to environmental challenges.

In conclusion, the study underscores the importance of considering temperature stress and potential interventions, such as vitamin C supplementation, when exploring the physiological responses of *Drosophila melanogaster*. This knowledge not only enriches the understanding of stress resilience mechanisms but also opens possibilities for innovative strategies to enhance stress adaptation across diverse species, ultimately advancing our comprehension of the complex relationship between biological systems and environmental stressors.

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