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The Effect of Cerelac on the Cold Resistance in *Drosophila melanogaster*

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Abstract

Stress resistance in an organism may be influenced by various factors including morphological, physiological, behavioural changes and quality and quantity of diet. The current study demonstrates the effect of the different concentration of cerelac media on the cold resistance of *D.melanogaster* in Wheat cream agar media, (10%, 20%, and 30%) cerelac treated media. The results suggest that flies fed with different concentration of cerelac media had greater cold resistance than Wheat cream agar media. Cerelac media provides sufficient nutrition to withstand cold. The study also shows that female flies had more starvation resistance than that of males (virgin and mated) and unmated female flies had greater cold resistance than those of mated flies. Thus, this implies that carbohydrates and proteins in cerelac may play major role in increasing the cold resistance in *D.melanogaster*.

Keywords: *D.melanogaster*, cold resistance, cerelac diet, stress resistance.

Introduction

Because of its broad range of genetic variation and high thermal tolerance, *Drosophila* is widely used as a model organism. It serves as a valuable tool to study how temperature influences biochemical processes and ecological patterns (Angilletta, 2009).

Cold resistance significantly affects insect abundance and distribution, and acclimation to temperature changes greatly contributes to how organisms cope with stress. For cold-adapted ectotherms such as *Drosophila*, the nature and level of resistance vary extensively both across and within species (Stanley *et al*., 1980; Kimura, 1988). Life history strategies tend to allocate energy between reproduction and survival, and the weight of each is shaped by internal and external influences (Hoffmann, *et al* 2003).

However the effect of cerelac on cold resistance was studied, hence present study was undertaken in *D.melanogaster.* Cerelac Ragi (Nachni) Apple Flavour is a complementary food product commonly used for infants and young children due to its balanced nutritional profile. Ragi (Eleusine coracana), also known as finger millet or nachni, is a nutrient-rich grain, valued for its high calcium, iron, and fiber content. It is gluten-free and provides essential amino acids such as methionine, lysine, and threonine, which are vital for growth and development (Black *et al* 2013).

Method and Material

"The Cerelac (Nestle, Ragi Nachni Apple flavor) was purchased from a Shree medicals store #14, shop no.1, 6th main, Vinayakanagara, MYSURU-570012 and used as a dietary component in the experimental setup.

Establishment of Stock

The *Drosophila melanogaster* flies used in this study were obtained from the *Drosophila* stock center, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysuru. The collected flies were cultured in glass containers containing wheat cream agar media composed of 100g jaggery, 100g rava powder, and 10g agar dissolved in 1000ml boiling distilled water, with 7.5ml propionic acid added to inhibit fungal growth. The flies were maintained under laboratory conditions at a temperature of 22 ± 1°C, and a 12:12 hour light-dark cycle and humidity level of approximately 70% RH.

Establishment of Experimental Stock

Wheat Cream Agar Media (Control Media): This was made using 100g of jaggery, 100g of rava powder, and 10g of agar in 1000ml of boiling distilled water, along with 7.5ml of propionic acid.

Treated Cerelac Media

- 10% of cerelac media was prepared using 100g of jiggery, 90g of rava powder, 10g of cerelac powder, 10g of agar powder in 1000ml of boiling distilled water, along with 7.5ml of propionic acid.
- 20% of cerelac media was prepared using 100g of jiggery, 80g of rava powder, 20g of cerelac powder, 10g of agar powder in 1000ml of boiling distilled water, along with 7.5ml of propionic acid.
- 30% of cerelac media was prepared using 100g of jiggery, 60g of rava powder, 30g of cerelac powder, 10g of agar

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powder in 1000ml of boiling distilled water, along with 7.5ml of propionic acid. These flies were maintained under the laboratory condition mentioned above and used to study cold resistance in *D.melanogaster*.

The flies obtained from above 3 concentrated media were used to conduct cold resistance experiment.

Experimental Procedure

To study the cold resistance, 5 days old virgin and mated male and female *Drosophila* flies from the wheat cream agar, 10%, 20%, and 30% cerelac media were used. Five flies from each media were transferred to separate empty vials (plugged with cotton) and kept in refrigerator at 5°C. For every one hour interval, the vials were observed until the death of each fly. A total of sixty flies were observed for each of wheat cream agar media, 10%, 20% and 30% cerelac media. Separate experiments were carried out for unmated and mated flies.

Result

Figure 1: Showed the mean and standard error value of cold resistance in unmated female and unmated male flies cultured in Wheat cream agar media, 10%, 20% and 30% cerelac media. According to this data, cold resistance was greater in 20% and 30% cerelac media, more or less similar in wheat cream agar media and 10% cerelac media. Further, this data also showed that unmated female flies had more cold resistance than unmated male flies in 20% of cerelac diets.

The above cold resistance data subjected to two-way ANOVA followed by Tuckey's Post hoc test showed significant variation in time taken by unmated female and male flies cultured wheat cream agar media, 10%, 20% and 30% cerelac media in to survive in cold temperature. However, insignificant variation in cold resistance was noticed in interaction between treatment and sex. Tukey's post hoc test showed significant variation in cold resistance in unmated female flies of 20% cerelac media and unmated male flies of wheat cream agar, 10% and 30% cerelac media.

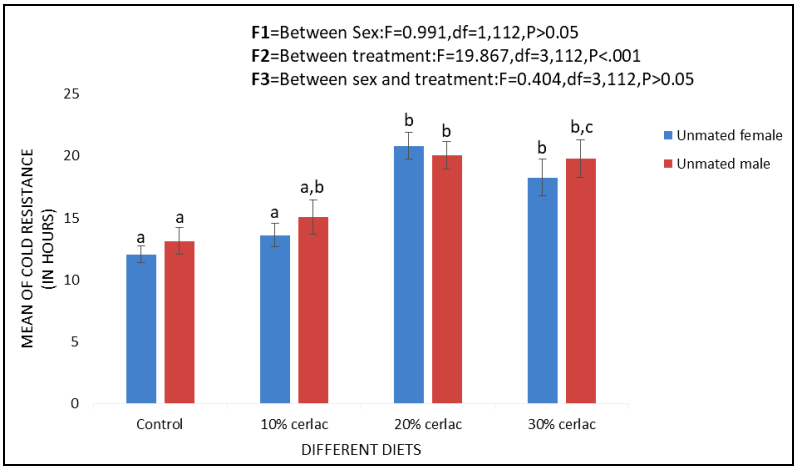

Fig l: Effect of cerelac on cold resistance in unmated female and unmated male of *D. melanogaster*

The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test.

Figure 2: Showed the mean and standard error value of cold resistance in mated female and mated male flies cultured in Wheat cream agar media, 10%, 20% and 30% cerelac media. According to this data, cold resistance was greater in 20% and 30% cerelac media, more or less similar in wheat cream agar media and 10% cerelac media. Further, this data also showed that mated female flies had more cold resistance than mated male flies in 20% of cerelac diets.

The above cold resistance data subjected to two-way ANOVA followed by Tuckey's Post hoc test showed significant variation in time taken by mated female and male flies cultured wheat cream agar media, 10%, 20% and 30% cerelac media in to survive in cold temperature. However, insignificant variation in cold resistance was noticed in interaction between treatment and sex. Tukey's post hoc test showed significant variation in cold resistance in mated female flies of 20% cerelac media and mated male flies of wheat cream agar, 10% and 30% cerelac media.

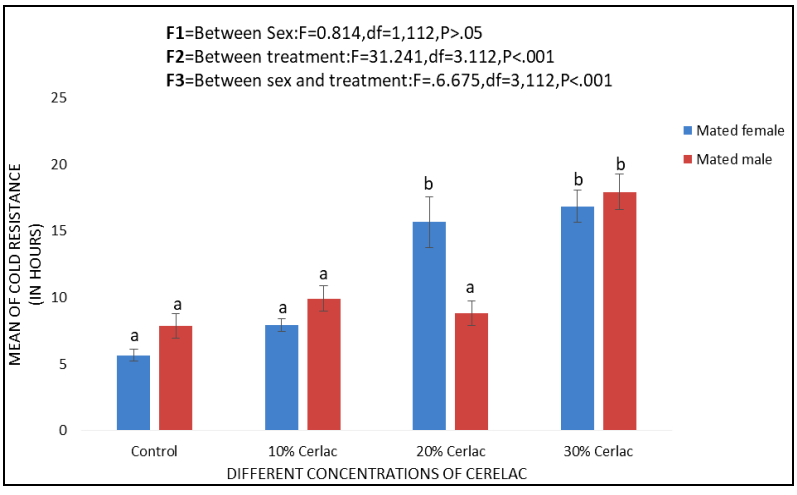

Fig 2: Effect of cerelac on cold resistance in mated female and mated male of *D. melanogaster*

The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test.

Figure 3: showed the mean and standard error value of cold resistance in unmated female and mated female flies cultured in Wheat cream agar media, 10%, 20% and 30% cerelac media. According to this data, cold resistance was greater in 20% and 30% cerelac media, more or less similar in wheat cream agar media and 10% cerelac media. Further, this data also showed that unmated female flies had more cold resistance than mated male flies in all diets.

The above cold resistance data subjected to two-way ANOVA followed by Tuckey's Post hoc test showed significant variation in time taken by unmated female and mated male flies cultured wheat cream agar media, 10%, 20% and 30% cerelac media in to survive in cold temperature. However, significant variation in cold resistance was noticed in interaction between treatment and sex. Tukey's post hoc test showed significant variation in cold resistance in unmated female flies of wheat cream agar media, 10%, 20% and 30% cerelac media.

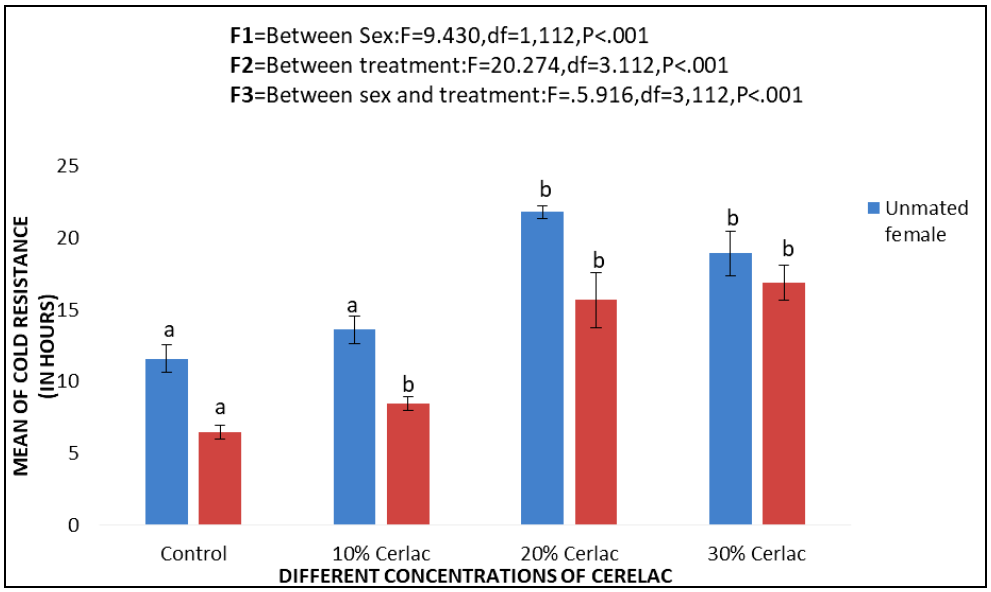

Fig 3: Effect of cerelac on cold resistance in unmated female and mated female of *D. melanogaster*

The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test.

Figure 4: The mean and standard error value of cold resistance in unmated male and mated male flies cultured in Wheat cream agar media, 10%, 20% and 30% cerelac media. This data showed highest cold resistance in flies fed with 20% and 30% cerelac media compared to other two diets. The result showed that unmated male flies had greater cold resistance than mated male flies in 20% and 30% cerelac media and more or less equal in wheat cream agar media and 10% of cerelac media.

The above data was subjected to two-way ANOVA followed by Tuckey's Post hoc test showed significant variation in cold resistance in between diets. However, insignificant variation in cold resistance was noticed in interaction between diet and sex and sexes. Tukey's post hoc test showed significant variation in cold resistance in unmated male and mated males flies of wheat cream agar and 10% of cerelac media and mated flies of in other two diets.

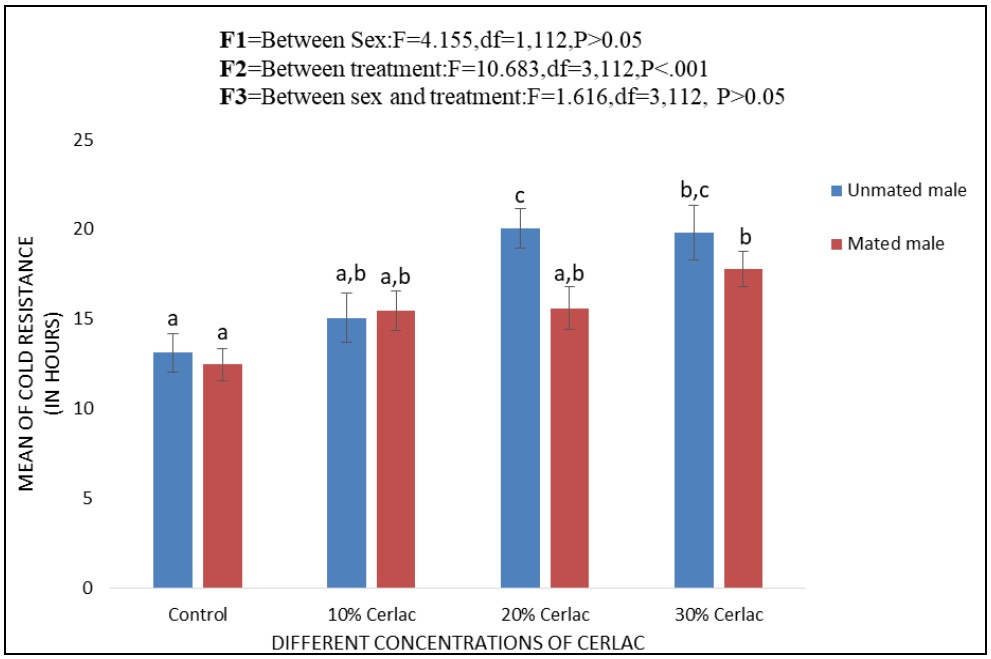

Fig 4: Effect of cerelac on cold resistance in unmated male and mated male of *D. melanogaster*

The different letters on the bar graph indicates the significant variation at 0.05 levels by Tukey's Post Hoc test.

Discussion

Stress-related traits vary widely among insects and other organisms, particularly due to insects' remarkable adaptability to climatic changes. The ability to tolerate cold stress in organisms can depend on several factors, including nutrition, sex, and genetic makeup. In insects, diet quality and composition are known to influence cold resistance.

In this study, results (Figures 1–4) demonstrate that *D.melanogaster* fed with 20% and 30% cerelac media exhibited significantly higher cold tolerance. Notable differences in cold resistance were also observed among flies raised on wheat cream agar and 10% cerelac media, suggesting that both the quality and quantity of dietary nutrients impact cold stress resistance. The high amino acid and nutrient contents cerelac powder in likely supplies energy that enhances survival under cold conditions. Previous studies have shown that selection for cold tolerance increases lipid content (Chen & Walker, 1994) and that accumulation of haemolymph sugars supports cold resistance (Kimura, 1982). However, excessive dietary sugar may reduce thermal tolerance.

Our findings also highlight sex-based differences in cold resistance, with female flies consistently outperforming males across all diets (Figures 1 and 3). This sexual dimorphism may reflect differences in gene regulation and physiological trade-offs. Several studies indicate that nutritional composition affects cold tolerance differently in males and females.

Factors such as age, condition, and reproductive roles may further influence this variation (Grath & Parsch, 2016; Mank, 2017). Additionally, we observed that mating status influences cold tolerance. Unmated males showed greater cold resistance than mated flies (Figure 4), possibly due to physiological changes induced by exposure to female pheromones. However, contrasting studies suggest that mating depletes male energy reserves, increasing vulnerability. Unmated females, on the other hand, showed enhanced cold tolerance compared to mated flies (Figure 3), aligning with findings from Goenga *et al*. (2012). Increased food intake post-mating (Ravi Ram & Wolfner, 2007; Carvalho *et al*., 2006) and transfer of accessory gland proteins (Acps) during copulation may explain this improved stress resistance (Wolfner, 2002; Gillot, 2003).

Thus, this implies that carbohydrates and proteins in cerelac may play major role in increasing the cold resistance in *D.melanogaster.* Further female flies had more starvation resistance than that of males (virgin and mated) and unmated female flies had greater cold resistance than those of mated flies.

Conclusion

Hence our experimental study we can conclude that, the flies developed with cerelac supplemented diet had the greater resistance to the cold than the flies fed with wheat cream agar media further cold resistance increased with increasing the concentration of cerelac. Thus these studies suggesting that quality and quantity of nutrients present in cerelac affects cold resistance in *D. melanogaster.*

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