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Research Article



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Prebiotics supplement increases heat and cold resistance in Drosophila melanogaster

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Abstract

Prebiotics are a specific type of nutrients that can endure the enzymatic and acidic digestion that occurs in the small intestine while still being broken down by the gut flora. Prebiotic dietary supplements are produced for several kinds of health beverages with high fibre and vitamin contents. To grow, develop, reproduce, and resist the impacts of the environment, organisms require energy. In the present study flies of *D. melanogaster* cultured in different concentration of prebiotic supplement (2.5%, 5%, 10%) and control media. Flies cultured in different concentrations of prebiotics supplement showed significant influence on heat and cold resistance. From the results obtained in experiment, heat and cold treatment was greater in 10% concentration prebiotic supplement. Whereas, the heat and cold resistance showed significantly lesser in flies cultured from control media. As the concentration of prebiotics supplement, mated females had significantly greater cold and heat resistance than males and similar trend was also noticed in unmated flies. Further the female flies were more resistance to cold and heat resistance than males in control media and different concentrations of prebiotic supplement. Thus, these studies suggests that prebiotics supplement increases heat and cold resistance.

Keywords: Prebiotic supplement, Cold and heat resistance, *D. melanogaster*

Introduction

Environmental stress resistance, life-history features, and reproduction are all strongly influenced by the quantity and quality of nutrients that organisms consume. Numerous variables may influence an organism's ability to withstand stress through behavioural and physiologic changes. It was also demonstrated that climatic changes experienced by an organism may also cause physiological changes such as, hardening process, coma, production of metabolites, and making an organism tolerant to temperature extremes (Srensen; Nielson *et al.*, 2005; Lalouette; Kostal *et al.*, 2007). These changes in turn affect life history traits such as fecundity, fertility, longevity, and stress resistance. Increased tolerance to stress, such as thermal stress, can also be achieved through further dietary restriction or mild fasting (Wenzel, 2006; Smith, Hoi *et al.*, 2007). According to recent research (Prasad *et al.*, 2003; Jenki *et al.*, 1997; Bijlsm *et al.*, 1996), organisms' life histories and features like illness susceptibility, fertility, reproduction, longevity, and stress resistance are strongly influenced by the quantity and quality of nutrients they consume.

Evolutionary theory can be used to characterize biological stress (Hoffmann and Parsons, 1991). According to (Sibly and Calow; Koehn and Bayne,1989) stress is widely defined as any environmental change that lowers an organism's fitness. The degree to which genetic variations in stress tolerance led to adaptive change depends on how frequently an organism is exposed to its environment and the physiological costs involved (Hoffmann and Parsons, 1991). Environmental stress is defined as the lack of acceptable or sufficient food supplies, which deprives a population of normal nutrients (White, 1993). It has been stated that stress related to few resources has an impact on the populations of most species. Selection probably influences resistance traits either directly or indirectly since stress resistance traits in Drosophila frequently differ across latitudinal clines (Sisodia and Singh, 2010). Many species' individuals must endure times of a food crisis or exposure to unsatisfactory nutrition. In areas where food is likely to be less plentiful or momentarily less consistent, positive selection for resistance to hunger stress is anticipated. As is frequently observed when insects are restricted to food low in protein relative to carbohydrate (Raubenheimer and Simpson, 1999). compensatory feeding for the limiting nutrients results in the over-ingestion of other nutrients when faced with nutritionally imbalanced diets. This may increase lipid storage and reduce fitness (Simpson et al., 2004; Warbrick-Smith et al., 2006).

In *D. ananassae* Sisodia and Singh, (2010) discovered that stress resistance varied widely among populations. The ratio of protein to carbohydrate in India naturally varies with latitude as do eating habits and food composition. Along with clinal impacts, food accessibility in that area also plays a significant role in variations

in stress resistance. In consideration of this, the current study's objective was to assess the physiological adaptations and the impact of changes in energy allocation under various dietary regimes on life histories. Thus, we looked into how significant two macronutrients are in relation to desiccation, starvation, thermal tolerance (hot and cold), and life-history traits.

Prebiotics, as defined by Gibson (2004), are nondigestible foods that benefit the host by encouraging the growth of a certain strain of bacteria in the colon. Later, in 2004, this was revised. Prebiotics are polysaccharides in the small intestine that can withstand acidic and enzymatic digestion and are used by probiotics that come next. The two categories of saccharides are "oligosaccharides" and "galacto oligosaccharides." Prebiotics such as inulin, oligofructose, and lactulose oligolactose are naturally present in a variety of plants. A class of substances known as prebiotics is broken down by the gut flora. The connection between human health and the recent increase in interest in this topic is shown. Functional meals, pills, tablets, powders, and nutraceuticals all contain prebiotics as an ingredient. Prebiotics are a natural way to provide individuals nourishing ingredients in diet. Most prebiotics are easily absorbed and give food the necessary properties. Prebiotics with short chains, for instance, behave like sugars and aid in the final product's crisping and browning. As a replacement for fat, long-chain prebiotics such soybeans, artichokes, asparagus, and bananas. So effect of prebiotic supplement far. on environmental stress such as heat and cold resistance has not been studied therefore present study has been undertaken in D. melanogaster.

Materials and Methods

Establishment of stock

Experimental Oregon K strain of *D. melanogaster* used in the study was obtained from Drosophila stock center, Department of zoology, University of Mysore, Manasagangothri, Mysuru, used to establish experimental stock. The stock was cultured in bottles containing wheat cream agar

media (100g of wheat powder, 100g of jaggery, 10g Agar the contents were boiled in 1000ml of distilled water and 7.5 ml propionic acid). Flies were maintained in laboratory conditions such as humidity of 70% and 12 hours dark: 12 hours light cycles and temperature of $22^{\circ}c \pm 1^{\circ}c$. The flies were used to conduct the experiments.

Establishment of experimental stocks

Flies cultured in normal wheat cream agar media were used as control flies. And flies were obtained from above stock used to establish the experimental stock with different concentrations of dietary Prebiotic supplement (2.5%, 5%, 10%) per 100 ml of wheat cream agar media. Flies obtained from control and prebiotic treated flies were used in the present experiment.

Effect of prebiotic supplement on heat and cold resistance in *Drosophila melanogaster*

Heat resistance experiment of control and treated flies mated (male/ female) and unmated (male/

female) were used to study heat resistance. Twenty flies (control/treated flies), in vials were kept in incubator for 36°c. the flies were observed for every 5 minutes of interval until the death of each fly. The heat resistance was observed in minutes, total replicates of five were run each of control and prebiotic treated media. Separate experiment was run for both male and female.

Cold resistance was measured using 5 days old mated (male and female) and unmated (male and female) flies from control and treated media. To study cold resistance. twenty flies (control/treated) were transferred to empty vials. These vials were kept at 5°c in refrigerator for constant cold. The flies were observed for every 12 hours interval until the death of each fly. The cold resistance was observed in hours and the total replicates of 3 were run each of control and treated media. Separate experiment was run for both male and female. The data obtain was subjected to Two-way ANOVA followed by Tukey's post hoc test using SPSS package 29 version.

Results

Effect of prebiotic supplement on heat resistant in *D. melanogaster*

Figure 1: The effect of different concentration of prebiotic supplement on heat resistant mated flies (male and female) of *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test.

Both control mated male and female flies had significantly lesser heat resistance compared to prebiotic treated mated males and females **Fig 1**. Further heat resistance increased with increasing concentration of prebiotic supplement in both mated males and females. The data subjected to Two-way ANOVA followed by Tukey's post hoc test showed significant variation in heat resistance of mated male and female raised in prebiotic and control media, between sexes and also interaction between treatment and sex. The mated male was significantly lesser heat resistance than females in both control and prebiotic treated media. Further among prebiotic treated flies, 5g prebiotic treated flies had significantly high heat resistance compared to 2.5g and 10g prebiotic treated flies by Tukey's post hoc test.

Figure 2: The effect of different concentration of prebiotic supplement on heat resistant unmated flies (male and female) of *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test.

Prebiotic treated unmated males and females were significantly greater heat resistance compared to unmated males and females of control media **Fig 2**. Further heat resistance increased with increasing concentration of prebiotic treatment in both unmated males and females. The data subjected to Two-way ANOVA followed by Tukey's post hoc test showed significance variation in heat resistance between treatments, between sexes and also interaction between treatment and sex. The unmated male was significantly lesser heat resistance than females in both control and prebiotic treated media. Further among prebiotic treated flies 10g flies had taken significantly high heat resistance compare to 2.5g and 5g prebiotic treated flies by Tukey's post hoc test.

Figure 3: The effect of different concentration of prebiotic supplement on heat resistant of unmated and mated male in *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test.

Figure 3. shows heat resistance mean ± standard error of control and prebiotic treated flies (2.5%, 5%, 10%). From the data obtained it was noticed that the lowest heat resistant was found in control flies and highest heat resistance in (10%) treated media. Further among prebiotic treated flies, the resistance increased with increasing as the prebiotic powder. The concentration of comparison between unmated male and mated male showed that more heat resistance was observed in unmated males of control and prebiotic treated flies than mated flies. The data

subjected to two-way ANOVA followed by Tukey's Post hoc test showed that significance variation in male heat resistance between control and prebiotic treated flies, between sexes and also interaction between treatment and condition (mated V/S unmated). The Tukey's post hoc test between control and 10% prebiotic treated flies was found to be significant. Further among prebiotic treated flies 10% flies had taken significantly more heat resistant compared to 2.5g and 5g prebiotic treated flies by Tukey's post hoc test.

Figure 4: The effect of different concentration of prebiotic supplement on heat resistant of unmated and mated female in *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test.

Figure 4, shows mean \pm standard error of heat resistant of control and prebiotic treated flies (2.5%, 5%, 10%). From the data obtained it was noticed that in both mated and unmated female the lowest heat resistant noticed in control flies and highest heat resistant was seen in (10%) treated flies. Further among prebiotic treated flies, the heat resistance increased with increasing as the concentration of prebiotic supplement. The comparison between unmated female and mated female showed that more heat resistant was found in unmated females in both control and prebiotic treated flies. The data subjected to two-way ANOVA followed by Tukey's Post hoc test showed significance variation in heat resistance between treated flies of mated and unmated condition and also interaction between treatment and control diets. Further among prebiotic treated flies 10% flies had taken significantly more resistant compared to 2.5g and 5g prebiotic treated flies by Tukey's post hoc test.

Effect of prebiotic supplement on cold resistant in *D. melanogaster*

Figure 1: The effect of different concentration of prebiotic supplement on cold resistant mated flies (male and female) of *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test.

Both control mated male and female flies had significantly lesser cold resistance compared to prebiotic treated mated males and females **Fig 1**. Further cold resistant increased with increasing concentration of prebiotic supplement in both mated males and females. The data subjected to Two-way ANOVA followed by Tukey's post hoc test showed that significance variation in cold resistance of mated male and female raised in prebiotic and control media, between sexes and also interaction between treatment and sex. The mated male was significantly less heat resistant than females in both control and prebiotic treated media. Further among prebiotic treated flies, 10g prebiotic treated flies had significantly high cold resistant compared to 2.5g and 5g prebiotic treated flies by Tukey's post hoc test.

Figure 2: The effect of different concentration of prebiotic supplement on cold resistant unmated flies (male and female) of *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test.

Prebiotic treated unmated males and females were significantly greater cold resistant compared to unmated males and females of control media **Fig 2**. Further cold resistant increased with increasing concentration of prebiotic treatment in both unmated males and females. The data subjected to Two-way ANOVA followed by Tukey's post hoc test showed significance variation in cold resistant between treatments, between sexes and also interaction between treatment and sex. The unmated female was less cold resistant than males in both control and prebiotic treated media. Further among prebiotic treated flies 10g flies had taken significantly high cold resistance compared to 2.5g and 5g prebiotic treated flies by Tukey's post hoc test.

Figure 3: The effect of different concentration of prebiotic supplement on cold resistance of unmated and mated male in *D. melanogaster*. [Control media- wheat cream agar media; prebiotic supplement (2.5%, 5%, 10% concentration)].



Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test.

Figure 3. shows cold resistant mean \pm standard error of control and prebiotic treated flies (2.5%, 5%, 10%). From the data obtained it was noticed the lowest cold resistance in control flies and highest cold resistant in 5g and 10g treated media. Further among prebiotic treated flies, the resistance increased with increasing concentration of prebiotic supplement. The comparison between unmated male and mated male showed that more cold resistant was observed in unmated males of control and prebiotic treated flies.

The data subjected to two-way ANOVA followed by Tukey's Post hoc test showed that significance variation in male cold resistant between control and prebiotic treated flies, between conditions (mated V/S unmated) and also interaction between treatment and sex. The Tukey's post hoc test between control and 10% prebiotic treated flies was found to be significant. Further among prebiotic treated flies 5% and 10% flies had taken significantly more cold resistant compared to 2.5g prebiotic treated flies by Tukey's post hoc test





Different letters on the bar graph indicate the significant variation between the different diet at 0.05 levels by Tukey's post hoc test.

Cold resistant data of control and prebiotic treated flies (2.5%, 5%, 10%) were provided in **Figure 4**. From the data obtained it was noticed that in both mated and unmated female the lowest cold resistant was noticed in 2.5g flies and highest cold resistance was seen in (10%) treated flies. Further among prebiotic treated flies, the cold resistance increased with increasing concentration of prebiotic supplement. The comparison between unmated female and mated female showed that more cold resistant was found in mated females in

both control and prebiotic treated flies. The data subjected to two-way ANOVA followed by Tukey's Post hoc test showed significance variation in cold resistance between treated flies of mated and unmated condition and also interaction between treatment and control. Further among prebiotic treated flies 10% flies had taken significantly more resistance compared to 2.5g and 5g prebiotic treated flies by Tukey's post hoc test.

Discussion

For growth, development, physiology, starvation resistance, and survival, food is a crucial component that all organisms need. The quantity and quality of nutrients that an organism consumes have a significant impact on life history processes like reproduction and stress tolerance (Sisodia and Singh, 1988) Additionally, the quantity and quality of nutrients available in various diets vary. The ability of insects to adapt to and reduce the effects of changing climatic conditions is based on variation in stress-related traits. For example, in *Drosophila*, a high level of desiccation resistance is linked to adaptation to and habitats while a high level of cold resistance is linked to adaptation to high latitudes

Prebiotics' influence on D. melanogaster's ability to withstand heat and cold was therefore the subject of the current investigation. The heat and cold resistant of prebiotics-treated flies was found to be substantially higher than that of control flies. This indicates that adding prebiotics to meals may improve D. melanogaster's ability to withstand heat and cold (Fig 1-4). This confirms the earlier studies of dietary effect, cold and heat resistance in Drosophila. They also demonstrated that the amount and quality of nutrients consumed had a significant impact on the ability to withstand cold and heat. Physiological changes are required for increased cold and heat tolerance, which are likely to impair other fitness-related traits. By reducing the amount of food, notably protein (yeast), available to adult flies, caloric restriction increases their resilience to cold and heat, with up a twofold difference between females to previously fed ad labium yeast and those given no yeast. (Chown and Nicolson, 2004; McCue, 2010; Laparie et al., 2012; Ribeiro et al., 2010; Burger et al., 2017). Additionally, Sisodia and Singh (2012) found that south Indian D. ananassae groups that eat fruits high in carbohydrates were less susceptible to a food crisis than north Indian tribes that eat fruits high in protein.

In the present study also found that females were stronger heat and cold resistance than males in both control and treated flies (**Fig 1-4**). This is because females in species of *Drosophila* are larger and heavier than male flies in contrast to them. In many organisms, males and females were likely to respond to resistance in different mostly because ways. their nutritional requirements and methods of usage differ (Hoyenga et al., 1982). It has been shown that strains. even within D. melanogaster, of sexual considerably affect the pattern dimorphism in cold and heat resistance.

When exposed to ecologically significant temperature variations, adults of *D. melanogaster*, like many other insects, can quickly increase their thermal tolerance. lower the thermal threshold for survival by 1-2°c, and the improved cold resilience may also be crucial for other fitnessrelated features including sustained activity and reproductive behavior in colder climates (Chen et al., 1987). In the present study, the cold and heat resistance of mated and unmated males and females of D. melanogaster was also examined. It was found that unmated males considerably outperformed mated males in both the control and prebiotic-treated flies in terms of cold and heat resistance. The most likely reason for this is that mated females consumed more food and built up more lipids than men (Carvalho et a., 2006; Lee et al., 2013). It has also been demonstrated in more recent research that female D. melanogaster can dramatically increase their midgut size during mating. Mated females are able to meet their heightened energy requirements for egg production by increasing their post-ingestive nutrient consumption.

Thus, these studies suggests that prebiotic supplement in the diet enhances cold and heat resistance in *D. melanogaster*. Further cold and heat resistance increased with increasing concentration of prebiotic supplement.

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