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RESEARCH ARTICLE

THE EFFECT OF THE ENSURE® NUTRITIONAL SUPPLEMENT ON THE SEX RATIO IN *DROSOPHILA MELANOGASTER*

Yashaswini. S.P., Aishwarya.K. C., Ashwini.M., Tanmayi Kishore,
Suma.S., Shilpashree B.M and *M. S. Krishna

Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru-560006, Karnataka, India

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ABSTRACT

A The nutritional diet is one of the important external environmental factor that affects on the growth and development, stress resistance, survival ability, reproduction and also alters the sex ratio. In the present study the flies of *Drosophila melanogaster* were cultured in the wheat cream agar media, mixed and Ensure® media, to understand the effect of ensure nutritional supplement on the sex ratio in *Drosophila melanogaster*. The results revealed that, female offspring were more than the male offspring in all three diets. The Ensure® media flies produce more male and female offspring compared to control and mixed diets. Thus it suggests that nutritional dietary components in the food alters the offspring sex ratio in *Drosophila melanogaster*.

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INTRODUCTION

More people are realizing the value of using the fruit fly *Drosophila melanogaster* as an organism model in nutrition research. Nutritional studies on fruit flies require careful consideration of the composition of the experimental diets. In addition to balanced diets that often includes agar, wheat, sugar, and yeast. Furthermore, *Drosophila* can be fed diets that have been chemically created. Further research is needed to determine the specific macro and micronutrients that make up "holistic meals," as the quantitative requirements of flies remain unknown. (Thomer Roder *et al.*, 2019)

One important extrinsic factor that can affect an organisms survival, growth and development is, its diet (Sisodia and Singh, 2012). It can be shown that food restrictions that do not result in famine impact. The longevity and procreative capacity of a wide range of species, including nematode worms and mammals. The studies demonstrated that diets significantly affected both longevity and fertility (Piper *et al.*, 2011). There are two categories how an organism diet affects its life history traits: quantity which is determined by the availability of food and quality, which is determined by the nutritional content of food (Sisodia and Singh, 2012). Its being shown that changing an animals food can lengthen its life span including that of flies.

The Sex ratio in animals is known to be influenced by a number of factors, both genetic and environmental. Deviation from a theoretical 1:1 ratio of male and female caused by factor-producing changes in the primary sex ratio or by factors

operating subsequent to fertilization and causing changes in the secondary sex ratio. Surprisingly little work has been designed to examine the effect of the environment on the sex ratio. It has been well-published for many species that physical factors such as temperature, humidity, light, and crowding may produce differential effects on the viability of different genotypes. The goal of the current study was to investigate how temperature and genotyping interact to alter the sex ratio in *Drosophila melanogaster* (Donald J. *et al.*, 1980). The study of sex ratios in insects has been a significant area of research in evolutionary biology (Leigh *et al.*, 1985). In insects with separate sexes, the ratio of females indicates the reproductive potential of a population. Normally, in a population that mates randomly, the sex ratio varies around 1:1 due to the segregation of sex chromosomes during gametogenesis (Rawlings and Maudlin, 1984), (Werren and Godfray, 1995), (Hoy, 2004). This 1:1 sex ratio typically indicates stabilizing selection on males and females (Schowalter, 1996).

In numerous organisms where the sexes are distinct, females and men are generated in roughly equal quantities. (Hardy, 2002). Consequently, the sex ratio is 1:1 in many organisms, including insects (Prakahs, 2008). This ratio maximizes genetic variety by optimizing the availability of males to females (Schowalter, 2016). However, sex ratios are ultimately influenced by a number of environmental factors. Physical, chemical, or biological environmental elements can influence the sex ratio (Schowalter, 2016), (Hardy, 2002). Numerous studies have examined the impact of various physical factors on the sex ratio of *Drosophila melanogaster*. The three

*Corresponding author: **M. S. Krishna**

Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru-560006, Karnataka, India

physical factors are electromagnetic field radiation, minimum light conditions, and ambient temperature. One of the main external environmental elements controlling population increase is dietary nutrition.

Sex ratio changes in insects, reptiles, and birds in response to food availability and other environmental conditions have long been documented. For example, the dramatic skewing of the sex ratio in the Samoan butterfly, *Hypolimna spolina*, caused by male selective killing by *Wolbachia* infection.

Male- or female-based sex ratios are observed in the wild because a variety of factors in nature cause various species to produce biased offspring sex ratios. Both male and female life history traits are impacted by this. When a biased sex ratio develops, organisms adapt their mating behavior to survive in unfavorable conditions. This has an impact on how well individuals perform gender roles in both male and female, which they adjust to maintain fitness under challenging circumstances.

In this work, *Drosophila melanogaster* served as the model organism. The organism used in this investigation was selected for a number of reasons. First of all, *Drosophila* is an organism that regularly produces a large number of progeny. Second, due to its short life cycle, this creature is known to reproduce quickly, producing large numbers of eggs in a short period of time. Thirdly, this organism has frequently served as a model organism for numerous studies looking into different biological issues (Neethu, *et al.*, 2014)

Make sure Ensure® powder is primarily recommended for low-residue diets, weight loss, muscular hypertrophy, and immunological function. by providing malnourished individuals with a full, balanced diet that includes all the nutrients they need to enhance their body weight and nutritional status. 32 nutrients offer high-quality protein and support people's continued strength and activity. Make sure the ratio of macronutrients is adjusted to enable the best possible use of protein. We get 80% of the recommended daily allowance (RDA) of calcium to maintain strong bones. It has no gluten by nature, and once reconstituted, it can be drunk with milk or water.

MATERIALS METHOD

The Ensure® protein powder was purchased from the Medplus pharmacy shop, in Srirampura, Mysuru, Karnataka, India. This Ensure® protein powder is used to prepare the experimental media.

Establishment of Stock

The experimental stock of *Drosophila melanogaster* was established using the Oregon K strain, which was obtained from the *Drosophila* Stock Center at the Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysuru. To cultivate the flies, wheat cream agar media (100g jaggery, 100g wheat powder, 10g agar boiled in 1000 ml distilled water, and 7.5ml propionic acid added to prevent fungal growth) was used. The flies were kept in a laboratory setting with 70% humidity, a 12:12 photoperiod (dark and light cycles), and a temperature of $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$. We carried out our experiment with these flies.

Establishment of experimental stock

The experimental stock of flies was established using various diet media using above flies.

Wheat cream agar media: 100 grams of jaggery and 100 grams of semolina (powder made from wheat rava) were used to make the medium. 100ml of distilled water, 10 grams of agar, and 7.5ml of propionic acid;

Ensure® powder media: 7.5 ml of propionic acid was added to 100g of jaggery, 100g of Ensure® powder, and 10g of agar that had been boiled in 1000ml of distilled water to create the Ensure® powder media;

The Mixture or Intermediate media, (wheat cream + Ensure® powder media) (1:1): A mixture of 100 grams of jaggery, 50 grams of semolina (wheat cream powder), 50 grams of Ensure® powder and 10 grams of agar are cooked in 1000 ml of distilled water with the addition of 7.5 ml of propionic acid.

The Sex ratio experiments in *Drosophila melanogaster* were studied using the flies that emerged from the Wheat Cream Agar medium and other experimentally treated media that were previously discussed.

SexRatio Experiment: The virgin female and male flies were taken out of the control, mixed, and treatment. After allowing them to mate, the mated pairs were moved to a vial with the control medium. Record the number of male and female progeny that emerge from each vial of food once every seven days for 30 days. Twenty pairs in total, one for each of the test, mixed, and control media were made independently.

RESULTS

Figure 1: The graph showed the comparison between male and female adult flies number in *D.melanogaster* raised on different diets (wheat cream agar media, mixed and Ensure® media). The figure 1 suggests that the female offspring are produced more than the male offspring in all three diets. When the data subjected to the Chi Square analysis, showed significant variation between the male and female sex ratio in control and mixed and non-significant in test media.

Table 1: The effect of wheat cream agar, mixed, and Ensure® diet on the male offspring sex ratio of *D.melanogaster*.

Treatment (diets)	No. of adults	No. of females	No. of males	F: M ratio
Control	1417	744	673	1: 0.904
Mixed	1192	643	549	1: 0.853
Test	1547	844	703	1: 0.832

Table 2 The effect of wheat cream agar, mixed, and Ensure® diet on the female offspring sex ratio of *D.melanogaster*.

Treatment (diets)	No. of adults	No. of females	No. of males	M:F ratio
Control	1417	744	673	1: 1.105
Mixed	1192	643	549	1: 1.171
Test	1547	844	703	1: 1.200

Table 3 The effect of wheat cream agar, mixed, and Ensure® diet on the male and female offspring sex ratio of *D.melanogaster* and Chi-square analysis value.

Treatment	F: M ratio	Chi-square value	Significant
Control	1.105: 0.904	1.778	P< 0.05
Mixed	1.171: 0.853	3.706	P< 0.05
Test	1.200: 0.832	6.425	P> 0.05

DISCUSSION

Numerous studies have shown that physical elements like temperature and photoperiod have an impact on insects' sex ratios, including *Drosophila melanogaster*. However, to the best of our knowledge, this research is the first report on the Effect of Ensure[®] nutritional supplement on the sex ratio in *D.melanogaster*. Nutritional diet is one of the key external environmental factors that influences, growth and development, stress tolerance, survival ability, reproduction, and sex ratio. Insect sex ratio are influenced by temperature and photoperiod, particularly in *Drosophila melanogaster* (Eppley and Sharpe, 2006). Temperature affects insects' sex ratio in a number of different ways. In certain species, higher temperatures produce more females, while lower temperatures produce more males (Eppley and Sharpe, 2006). The phrase "temperature-depend sex determination" applies to this. (Sharkey and Eppley, 2006) have revealed that temperature indirectly influences the sex ratio in other species by influencing the survival rate of males and females. For example, in *Drosophila melanogaster*, higher temperatures cause more males to develop near the end of their development, but more females in the beginning. This is due to the fact that temperature affects the expression of genes that determine the sex of the individual (Sharkey and Eppley, 2006). However, because the flies in this study were kept in a laboratory, the temperature had no effect on the sex ratio. Therefore, the obtained result can only be attributed to the effect of Ensure[®] nutritional supplement and its varying concentrations. Since the result of the current investigation showed that the control, mixed and test media produced more female offspring than male offspring (fig 1). This is because the quantity and quality of diet is influenced on the variation in the sex ratio of the offspring.

Numerous studies have shown that the quantity and quality of a mother's diet affect her ability to reproduce and the sex of the organism. According to Yazgon (1972), the number of female *Pimple thirunallar* (endoparasitoid) individuals rise with the increased amount of amino acids in the diet. The creation of a specific sex in offspring is preferred by parent flies as it enhances the chances of survival for the species by aiding in reproduction or reducing competition for mates, resources, and habitat. In addition to these three variables, the mother's health can occasionally affect the sex of the offspring (Trivers and Willard, 1973).

Oviposition rate, maternal nutrition, and their combination had a significant impact on Offspring sex ratio. The quality of the food consumed by the females who were reproducing affected not only how many offspring the mother produced, but also the size and gender of the offspring. In general, those who consumed high-quality diets had female-biased sex ratios, while those who consumed low-quality diets had male-biased sex ratios. In our study, according to the obtained male and female ratio, (Table A to C) the flies fed with the Ensure[®] media produce more male and female offspring than the control media (Agar cream media) as well the mixed media the Ensure[®] nutritional supplement that is diet rich with protein and carbohydrate content with the same amount of minerals. This may alter the physiology of the flies and result in a variation in the sex ratio compared to the control and mixed diet. Our study also supported by the maternal diet quality also influenced on the clutch sex ratios. The females raised on the inferior diet gave birth to clutches that were skewed toward male. Furthermore, reduced female *A. quadridentata* offspring than

male offspring were produced as a result of the nutrient-poor diet (Fater *et al.*, 2014). Additionally, in laboratory mice, a diet high in fat and low in carbohydrates produces more male offspring than female offspring. Similarly, additional calories to the diet in the form of carbohydrates rather than fat produce more female offspring than males. According to Wiebe and Bortolotti (1992), a large number of female offspring are produced in American kestrels (*Falco sparverius*) whenever food resources are limitless. The variation in the dietary source-carbohydrates (sucrose), lipids, vitamins, proteins (amino acids) results in the variation in the sex ratio of the *Pimple turionellae*, (Mustafa *et al.*, 2005).

Numerous studies have demonstrated that changes in temperature, light, age, and other environmental conditions also affect the sex ratio of a variety of insects, including *Drosophila melanogaster*. However, in our experiment, we used the same aged flies raised on different diets, which were maintained under the same environmental conditions. We are used to studying the sex ratio, hence the observed variation in the experimental results is due to the variation in the quality and the quantity of the nutrients in the diet. In the present study, it was discovered that one of the major factors affecting the sex ratio of the organism is diet. The current study found that nutrition is one of the main elements influencing the organism's sex ratio. Compared to the control media, the Ensure[®] media boosts the production of female progeny.

CONCLUSION

Hence from our study in *D.melanogaster* we can conclude that the nutrition is one of the key factors influenced on the sex ratio of the organism. The Ensure diet increases the production of the female offsprings than the male offsprings than the wheat cream agar and mixed diet.

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