

Effect of Spirulina Supplement on Sex Ratio in Drosophila Melanogaster

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Abstract

Diet, as another environmental component, the quantity and availability of nutrients in the food has a strong impact on resistant to environmental stress. The nutritious diet is a significant external environmental component that influences growth and development, stress resistance, survival, reproductive fitness, and sex ratio. Environmental factors influencing sex ratio can be physical, chemical, or biological. Here we demonstrate the effect of spirulina on Sex ratio in *Drosophila melanogaster*. Sex ratios have been shown to be affected by a variety of environmental conditions. Animal sex ratio is known to be altered by a variety of genetic and environmental factors. Environmental influences on sex ratios might be physical, chemical, or biological in nature. Nutrition is a significant environmental component that has been shown to influence life span, aging rate, and reproductive potential. Nowadays, spirulina is more popular, however the quality and quantity of spirulina used has either a negative or positive effect on organism fitness. The present study explored the impact of a few morphological characteristics on the sex ratio in *D. melanogaster*. In the present study the flies of *D. melanogaster* flies are cultured in wheat cream agar media and Spirulina treated media to understand the effect of spirulina on the sex ratio. Our result reveals that female offspring were produced more in number than compare to males in both control and spirulina treated media. Thus suggests that spirulina dietary nutrient increases the female offspring. Thus suggests that nutrition alters offsprings ratio in *D. melanogaster*.

Keywords: Sex ratio, offsprings, spirulina, nutrition, Drosophila melanogaster

Introduction

Females emerge in approximately the same quantity as male in many organisms when the sexes separated (Hardy, 2002) ^[10]. As a result, the sex ratio is 1:1 in many organisms, such as insects (Prakahs, 2008) ^[19]. This ratio maximizes variation in genes by increasing the availability of male compared to females (Schowalter, 2016) [24]. The sex ratio is healthy in absence of altering (Cherian et al., 2016)^[3]. Many biological research regularly explore the sex ratio as an issue. The term "sex ratio" itself frequently implies the proportion of men to females in a population (Skalski, 2005; Bailey, 2004) ^[25, 1]. The number of men per 100 or 1000 women, or the percentage of the population that is female (or male), are frequent ways to represent the ratio (Skalski et al., 2005; Qazi and Qazi, 2006; Prakahs, 2008) [25, 21, 19]. However, it is still largely unknown what factors and underlying mechanisms cause these alterations.

Sex ratios are subsequently affected by a number of environmental variables (Schowalter, 2016; Hardy, 2002; Rosenfeld and Roberts, 2004) ^[24, 10, 23]. Physical, chemical, or biological elements can all impact sex ratios in the environment (Skalski *et al.*, 2005; Schowalter, 2016; Hardy, 2002; Rosenfeld and Roberts, 2004; Wajnberg *et al*, 2008) ^[25, 24, 23, 28]. The current investigation studied at how several physical factors affected the sex ratio in *D. melanogaster*. Environmental temperature, minimum light levels, and

electromagnetic field (EMF) radiation are those physical factors. One of the main factors affecting population increase is temperature (Miller and Spoolman, 2009; Price *et al*, 2013) ^[16, 20]. One of the most crucial variables controlling how organisms live is this one (Bhatnagar, 1996) ^[2]. Light can also be a limiting factor in addition to temperature (Miller and Spoolman, 2009; Roberts *et al*, 2000) ^[16, 22]. Light has an impact on how organisms reproduce (Inyang and Daniels, 2009) ^[11]. Recent reports indicate that EMF field radiation may have an impact on the population of organisms (Fauzi *et al*, 2016) ^[7].

Modifications in sex ratio due to changes in food availability and other environmental conditions have been seen in insects, reptiles, and birds (Pienaar and Greeff, 2003; Thuman *et al.*, 2003; Freedberg and Wade, 2001; Komdeur *et al.*, 2002; Dyson and Hurst, 2004) ^[18, 26, 8, 13, 5]. The ratio's female representation shows a population's capacity for reproduction (Schowalter, 2016) ^[24]. Furthermore, the relevance of the sexual mating system and other details about the past, present, and future of a population are additionally expressed in the sex ratio (Skalski *et al.*, 2005; Schowalter, 2016) ^[25, 24].

In the present study, *D. melanogaster* served as a model organism. The organism used in this investigation was selected for a number of reasons. First of all, *Drosophila* is a species that regularly produced large numbers of eggs (Neethu *et al*, 2014) ^[17]. Second, this organism is noted to

reproduce quickly, produce a large number of eggs, and have a brief life cycle (Neethu *et al*, 2014; Fauzi *et al*, 2016) ^[17, 7]. Third, this organism has frequently used as a model organism in several studies exploring at different biological issues (Neethu *et al*, 2014; Jennings, 2017) ^[17, 12].

The purpose of the present research was to examine the effect of spirulina and how genotype interact to affect the sex ratio in *Drosophila melanogaster*.

A small filamentous, spiral-shaped blue-green alga known as spirulina (Arthrospira platensis). This cyanobacterium provides a good source of proteins (55 and 70%), antiinflammatory compounds, and antioxidants such carotenoids,carotene, phycocyanin, and phycocyanobilin. Both humans and other animals ingest spirulina due to its wide variety of nutritional qualities and pharmacological effects (Kumar et al., 2017) ^[15]. "Superfood" spirulina is a widespread concept. Spirulina is becoming more and more popular as a dietary supplement due to the health advantages that are promised; it may either be used as a powder or taken as capsules (Grosshagauer and colleagues 2020)^[9]. Spirulina has become widely used by people as a dietary supplement due to its many health advantages. Numerous studies show that spirulina intake is beneficial for lowering triglycerides and bad cholesterol. It also helps in the treatment of diseases linked to the metabolic syndrome. In reality, several studies have demonstrated that spirulina supplements can aid in the treatment of a range of carcinomas (Konickova et al., 2014) ^[14]. As a consequence, the present study was conducted on D. melanogaster.

Result

Materials and Methods

Establishment of Stock: The Oregon K strain of *D. melanogaster*, procured from the *Drosophila* stock centre, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysuru, was used to establish the experimental stock. Wheat cream agar media (100g of jaggary, 100g of wheat powder, 10g of Agar was boiled in 1000ml of distilled water and 7.5 ml of propionic acid was added to avoid the fungal growth.) was used to culture the flies and were maintained in laboratory conditions such as humidity of 70% and 12:12 Photoperiod (dark and light cycles) and temperature of $22^{\circ}C \pm 1^{\circ}C$. These flies were utilized to conduct our experiment.

Establishment of Experimental Stocks: Flies cultured in wheat cream agar media were considered as control flies. Spirulina treated flies were obtained by using different concentration of spirulina i.e., 2.5g,5g and 10g was mixed thoroughly with 100ml of wheat cream agar media. Both control and spirulina flies were maintained in afore mentioned laboratory conditions.

Sex Ratio Experiment: The virgin male and female flies were collected from the control and spirulina treated media. Allow to mating, after mating, these mated pairs were transferred to vial containing their respective media. Once in seven days until their death, and note down the males and females offsprings emerged from each vial of all diet. A total of twenty pairs were made separately for each of the control and spirulina treated media.

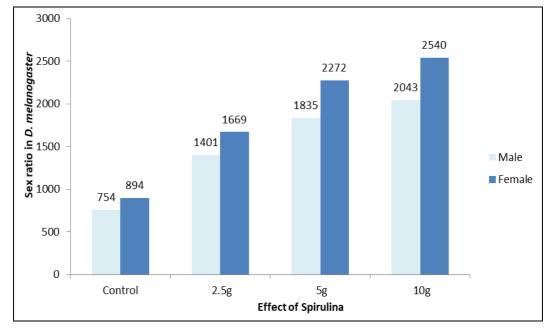


Fig 1: The graph shows that control and spirulina treated (2.5g, 5g, 10g concentration) offsprings sex ratio (Male and female) in *D. melanogaster*.

 Table 1: Effect of control and spirulina treated flies on offsprings sex ratio in D. melanogaster.

Treatment	No. adults	No. females	No. males	F:M ratio
Control	1648	894	754	1:0.82
2.5g	3070	1669	1401	1:0.83
5g	4107	2272	1835	1:0.80
10g	4583	2540	2043	1:0.80

Table 2: Effect of control and spirulina treated flies on offsprings				
sex ratio in D. melanogaster.				

Treatment	Total off spring	No. females	No. males	M:F ratio
Control	1648	894	754	1:1.18
2.5g	3070	1669	1401	1:1.17
5g	4107	2272	1835	1:1.20
10g	4583	2540	2043	1:1.20

sex fatto in D. metanoguster.								
Treatment	Sex ratio F:M	Chi square value	Significant level					
Control	1.18:0.82	0.0648	P>0.05					
2.5g	1.17:0.83	0.0578	P>0.05					

1.20:0.80

1.20:0.80

0.08

0.08

P>0.05

P>0.05

Table 3: Effect of control and spirulina treated flies on offsprings sex ratio in *D. melanogaster*.

Discussion

5g

10g

The several studies reveals that the physical factors temperature, photoperiod effects on the Sex ratio in insects including Drosophila melanogaster as per our information this is first report on effect of nutritional diet on the sex ratio D. melanogaster. Nutritional diet is external environmental factor that regulates growth and development, stress tolerance, longevity, reproduction, and also affect sex ratio. Spirulina is rich with Proteins (60-70%), vitamins, minerals, and bioactive compounds are all abundant in spirulina. This major nutritional content has antioxidant, anti-inflammatory, and immunomodulatory properties, as well as a positive effect on a number of diseases. Therefore present study has been undertaken in D. melanogaster to study the effects of Spirulina supplementary diet on Sex ratio in D. melanogaster. In the present study, the (fig 1) results revealed that the females offsprings are produced more than the male offsprings in both control and spirulina treated media ie., 2.5g, 5g, 10g spirulina concentration. This suggests that the quality and quantity of the diet is influenced on the variation in the sex of the offspring. Several studies have been demonstrated that quality and quantity influences maternal reproductive output and sex ratio in organisms. According to the Yazgan 1972 [31], the increase in aminoacid in the diet increases the number individuals of Pimple turionella. Flies parents support the development of a certain sex in their offspring because it boosts the chances of survival for that species by increasing reproduction or reducing competition for resources and mates. In addition to these variables, the mother's health can occasionally affect the sex of the offsprings. (Trivers and Willard, 1973)^[27].

Offspring sex ratios were strongly affected by maternal diet. In general, females fed the high-quality diet produced femalebiased sex ratios and those on the low-quality diet produced male-biased sex ratios (Warner et al., 2007)^[29]. In the present study shown that variation in the sex ratio between the different sex and also in different diets. The flies which fed in both control and spirulina treated flies had more female offsprings ratio than compared to male offsrings. The lack of food results the significant reduction in the number of males compared to female offsrings of A. quadridentata. Wiebe and Bortolotti (1992)^[30] reported that in American krestels, Falco sparverius, whenever, food resources are unlimited, high numbers of females progeny are produced. The variation in the sex ratio of the *pimple turionellae*. (Coskun et al., 2005) ^[4]. From this experiment we observed that, The importance of this study lies in its identification of the presence of significant variation in production of sons and daughters by parents of different ages and of factors that were responsible for the variation. And also there are several reaserch showed that the variation in the sex ratio in insects including D. melanogaster.

Hence from our study in *D. melanogaster* we can conclude that the nutrition is one of the key factor influenced on the sex ratio of the organisms. The spirulina treated media increases

production of the female offsprings than the male offsprings than the control media.

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References

- 1. Bailey J. The Facts on File Dictionary of Ecology and the Environment. New York: Facts on File, Inc, 2004, 214.
- 2. Bhatnagar VS. Chandigarh, the City Beautiful: Environmental Profile of a Modern Indian City. New Delhi: A. P. H. Publishing Corporation, 1996, 61.
- Cherian A, Rose A, Gupta D, Minz S, Prasad J, George K. Whither Sex Ratios in a Low Mortality Setting. IJWHR [Internet]. 2016 April [cited 2017; 4(2):64-67.
- 4. Coskun M, Ozalp P, Sulanc M, Emre I. Effects of various diets on the oviposition and sex ratio of Pimpla turionellae L. *RNA*. 2005; 75:75-00.
- Dyson EA, Hurst GDD. Persistence of an extreme sexratio bias in a natural population. Proc Natl Acad Sci U S A 2004; 101:6520-6523.
- Fauzi A, Corebima AD, Zubaidah S. The Fluctuation of Adult Filial Number and Eclosion Time of *Drosophila melanogaster* that Exposed by Mobile Phone in Multiple Generations. In: the 6th Annual Basic Science International Conference [Internet], 2016, 2-3; Atria Hotel and Conference, Malang, Indonesia; Faculty of Mathematics & Sciences, Brawijaya University, 2016, 124-128
- Fauzi A, Corebima AD, Zubaidah S. The Utilization of Drosophila melanogaster as a Model Organism in Genetics I and Genetics II Courses in Faculty of Mathematics and Natural Science, State University of Malang. In: International Conference on Education and Training 2016 [Internet]; 2016 Nov 4-6; Malang; Faculty of Education, State University of Malang; 2016, 51-56.
- 8. Freedberg S, Wade MJ. Cultural inheritance as a mechanism for population sex-ratio bias in reptiles. *Evol Int J Org Evol* 2001; 55:1049-1055.
- 9. Grosshagauer S, Kraemer K, Somoza V. The true value of Spirulina. Journal of agricultural and food chemistry. 2020; 68(14):4109-4115.
- Hardy ICW, editor. Sex Ratios: Concepts and Research Methods. Cambridge: Cambridge University Press, 2002, 2.
- Inyang HI, Daniels JL, editors. Environmental Monitoring. Oxford: Eolss Publishers Co. Ltd.; 2009; 1:22.
- Jennings BH. Drosophila-a versatile model in biology & medicine. Materialstoday [Internet]. 2011, 2017; 14(5):190-195.
- Komdeur J, Magrath MJ, Krackow S. Pre-ovulation control of hatchling sex ratio in the Seychelles warbler. Proc R Soc Lond B Biol Sci 2002; 269:1067-1072.
- Konícková R, Vanková K, Vaníková J, Vánová K, Muchová L, Subhanová I, Vítek L. Anti-cancer effects of blue-green alga Spirulina platensis, a natural source of bilirubin-like tetrapyrrolic compounds. Annals of Hepatology. 2014; 13(2):273-283.
- 15. Kumar A, Christian PK, Panchal K, Guruprasad BR, Tiwari AK. Supplementation of spirulina (Arthrospira

platensis) improves lifespan and locomotor activity in paraquat-sensitive DJ-1 β Δ 93 flies, a Parkinson's Disease model in *Drosophila melanogaster*. Journal of Dietary Supplements. 2017; 14(5):573-588.

- 16. Miller GT, Spoolman SE. Essentials of Ecology. Belmont: Brooks/Cole Cengage Learning, 2009, 224.
- 17. Neethu BK, Babu YR, Harini BP. Flavors supplemented in diet regulate the hatchability and viability in *Drosophila*. Dros. Inf. Serv [Internet], 2013(2013):24-28.
- Pienaar J, Greeff JM. Maternal control of offspring sex and male morphology in the Otitesella fig wasps. *J Evol Biol* 2003; 16:244-253.
- 19. Prakahs M. Insect Behaviour. New Delhi: Discovery Publishing House Pvt. Ltd., 2008, 245.
- 20. Price MF, Byers AC, Friend DA, Kohler T, Price LW, editors. Mountain Geography: Physical and Human Dimensions. Berkeley: University of California Press; 2013, 224.
- 21. Qazi SA, Qazi NS. Population Geography. New Delhi: A. P. H. Publishing Corporation, 2006, 95.
- 22. Roberts M, Reiss M, Monger G. Advanced Biology. Chetenham: Nelson; 2000. 199.
- Rosenfeld CS, Roberts RM. Maternal Diet and Other Factors Affecting Offspring Sex Ratio: A Review. Biology of Reproduction [Internet]. 2004; 71(4):1063-1070.
- 24. Schowalter TD. Insect Ecology: An Ecosystem Approach, Fourth Edition. London: Academic Press; 2016, 147.
- 25. Skalski RS, Ryding KE, Millspaugh JJ. Wildlife Demography: Analysis of Sex, Age, and Count Data. Burlington: Elsevier Academic Press; 2005, 49.
- 26. Thuman KA, Widemo F, Griffith SC. Conditiondependent sex allocation in a lek-breeding wader, the ruff (*Philomachus pugnax*). Mol Ecol 2003; 12:213-218.
- 27. Trivers RL, Willard DE. "Natural selection of parental ability to vary the sex ratio of offspring". Science. 1973; 179(4068): 90-92.
- Wajnberg E, Bernstein C, Alphen JV. Behavioural Ecology of Insect Parasitoids: From theoretical approaches to Field Applications. Malden: Blackwell Publishing; 2008, 254.
- 29. Warner DA, Lovern MB, Shine R. Maternal nutrition affects reproductive output and sex allocation in a lizard with environmental sex determination. *Proceedings of the Royal Society B: Biological Sciences.* 2007; 274(1611):883-890.
- 30. Wiebe KL, Bortolotti GR. Facultative sex ratio manipulation in American kestrels. *Behavioral Ecology and Sociobiology*. 1992; 30:379-386.
- 31. Yazgan S. A chemically defined synthetic diet and larval nutritional requirements of the endoparasitoid, *Itoplectis conquisitor J. Insect Physiol*, 1972.