



Effect of Prebiotics supplement on Sex ratio in *Drosophila melanogaster*

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Abstract

The diet is one of the key factors influence the reproductive success and offspring sex ratio of an organism. In the present study the flies of *Drosophila melanogaster* were cultured in the wheat cream agar media, prebiotic supplement (2.5%, 5%, 10%) were used to understand the effect of prebiotic supplement on offspring sex ratio in *D. melanogaster*. The results revealed that the female's offspring were produced more than the male's offspring in all concentrations of prebiotic supplement along with control diet. This ratio deviating from expected offspring sex ratio 1:1. Thus this study suggests that nutrition alters offspring sex ratio in *D. melanogaster*.

Keywords: *Drosophila melanogaster*, prebiotic supplement, sex ratio.

Introduction

The majority of earlier research on this subject concentrated on the amounts of food available to reproducing females and found strong maternal-allocation responses to experimental manipulations of resource quantity in ways that were likely to affect both maternal and offspring fitness (Meijer and Langer, 1995; Selman and Houston, 1996; Williams, 1996; Rutstein *et al.*, 2005). Food availability can affect patterns of sex allocation relative investment into males and females; (Rosenfeld and Roberts, 2004; Robertson *et al.*, 2006), in addition to more visible features like offspring size and number. When the fitness returns from producing males

are different from those from producing females, theoretical models indicate that parents will allocate resources differently to the sexes (Trivers and Willard, 1973). Parents in such circumstances frequently give more resources to the sex that benefits most from the current circumstances (Komdeur, 1996; Nager *et al.*, 1999; Kalmbach *et al.*, 2001). These relative fitness returns frequently rely on environmental circumstances. Parents can either (i) produce biased offspring sex ratios (Clutton-Brock *et al.*, 1984; Dittus, 1998; Kalmbach *et al.*, 2001) or (ii) they can produce balanced sex ratios, but invest more energy into specific offspring of one sex than the other (Velando, 2002).

Numerous biological research frequently focuses on the sex ratio. The word "sex ratio" itself often refers to a population's male to female ratio (Skalski *et al.*, 2005; Bailey, 2004). Males per 100 or 1000 females or the proportion of the population that is female (or male) are two frequent ways to express the ratio (Skalski *et al.*, 2005; Qazi and Qazi, 2006; Prakahs, 2008). A population's ability for reproduction is indicated by the ratio's female gender composition (Schowalter, 2016). The significance of the sexual mating system and other information related to the past, present, and future of a population are also reflected in the sex ratio, along with other life history qualities (Skalski *et al.*, 2005; Schowalter, 2016)

Animal sex ratio is known to be altered by a variety of genetic and environmental factors. Male: female ratios may deviate from the theoretical 1:1 ratio due to variables affecting the primary sex ratio or mechanisms at work after fertilization that affect the secondary sex ratio. Surprisingly little study has been done to investigate how the environment affects the sex ratio. As a result, the sex ratio is 1:1 in many organisms, such as insects (Prakahs, 2008). This ratio maximizes genetic variability by increasing the availability of males relative to females (Schowalter, 2016). The sex ratio is constant in the absence of altering (Cherian, 2016). Sex ratios are eventually influenced by a number of environmental factors (Schowalter, 2016; Cherian, 2016; Rosenfeld and Roberts, 2004). 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, 2008).

Prebiotics are indigestible foods that benefit the host by promoting the growth of a certain species of bacteria in the colon. Later, in 2004, this was changed. The carbohydrates known as prebiotics, which can endure enzymatic and acidic digestion in the small intestine, are used after probiotics. There are two types of saccharides: galacto oligosaccharides and oligo saccharides. Examples of prebiotics found naturally in many plants are inulin, oligofructose, and lactulose oligolactose

(Gibson *et al.*, 2004). The gut flora digests a group of chemicals known as prebiotics. It illustrates the link between human health and the rise in interest in this subject over the past few years. Nutraceuticals, functional foods, capsules, tablets, and powders all include prebiotics as an ingredient. More naturally occurring compounds can be found in foods including soybeans, artichokes, onions, and asparagus.

Materials and Methods

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°C ± 1°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.

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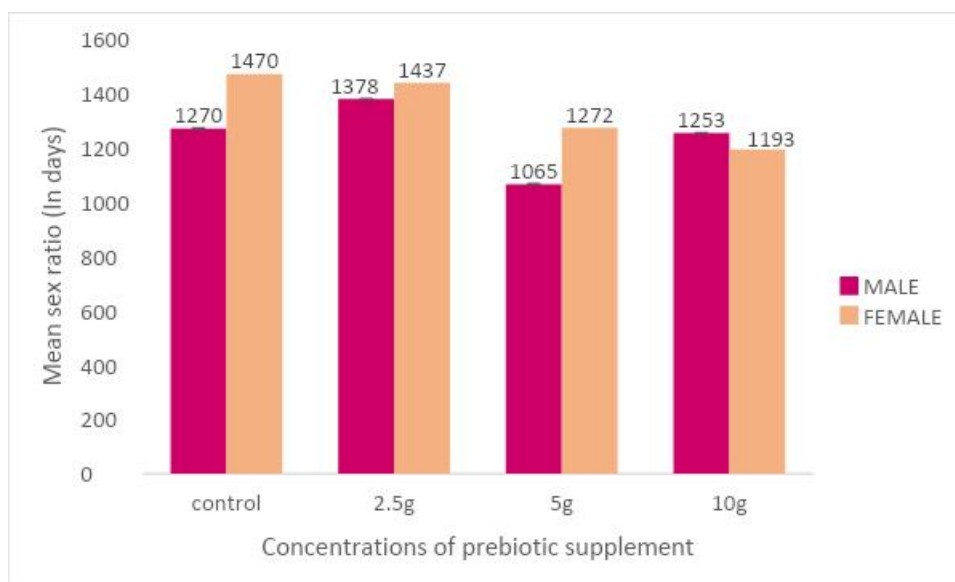
Five-day-old virgin females and unmated males were isolated from control and prebiotic supplement treated media within three hours of their emergence. One male and one female of these flies were put into the mating chamber individually, and we watched them for an hour. The pair was discarded if mating didn't happen in one hour. These mated pair were put into their

respective media, and once every seven days, they were changed into a fresh vial into the same media. Total number of female and male offspring were recorded. Sex ratio is defined as

the proportion of females to males in a population. If there are exactly equal numbers of males and females, the sex ratio is 1:1, or 1000 females to every 1000 males.

Results

Figure1: Effect of control and prebiotic diet on offspring sex ratio in male and female of *D. melanogaster*.



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

Table1A. Effect of control and prebiotic diet on offspring sex ratio (M: F) ratio alteration on *D. melanogaster*.

Treatment	Male	Female	Total	M: F ratio
Control	1270	1470	2744	1: 1.1
2.5g	1378	1437	2815	1: 1
5g	1065	1272	2623	1: 1.1
10g	1253	1193	2456	1: 0.9

Table1B. Effect of control and prebiotic diet on offspring sex ratio (F: M) ratio alteration on *D. melanogaster*.

Treatment	Male	Female	Total	F: M ratio
Control	1270	1470	2744	1: 0.86
2.5g	1378	1437	2815	1: 0.95
5g	1065	1272	2623	1: 0.83
10g	1253	1193	2456	1: 1

Table 1C. Effect of chi-squares and p value on sex ratio of *D. melanogaster*.

Treatment	Sex ratio M:F	Chi-square	P- value
Control	0.86: 1.1	0.029	P>0.05
T1	0.95: 1	0.0025	P>0.05
T2	0.83: 1.1	0.0389	P>0.05
T3	1:0.9	0.01	P>0.05

Chi-square shows non significance values

Figure 1 and table (1A-1C) shows the sex ratio in control and prebiotic treated flies (2.5%, 5%, 10%). From the data obtained it was noticed the flies raised in control media had significantly high sex ratio compared to the flies fed with prebiotic treated media. The comparison between males and females in each group were close to 1:1. The present results indicate the sex ratio in females were higher than male flies. Further among prebiotic treated flies, sex ratio decreased with increasing the concentration of prebiotic supplement. In control media females have high sex ratio and lowest sex ratio seen in 10g prebiotic supplement, whereas males with high sex ratio in 2.5g and low sex ratio in 5g prebiotic supplement. Thus male: female sex ratio is more than female: male sex ratio in *D. melanogaster*.

Discussion

Compelling studies have shown that according to environmental factors were known to alter sex ratio in *D. melanogaster* (Yander, 1965) the results, while environmental temperatures have some influence on appropriate sex ratios, they are mostly controlled by genetics. At each temperature, each genotype appeared to have its own usual sex ratio, which frequently deviated greatly from a 1: 1 ratio. Further the aging of *Drosophila* males led to a shift in the sex ratio in favor of a higher incidence of female. Temperatures have the potential to change the sex ratio by affecting male or female survival rates from the larval to imago phases (Precht, 1973).

This confirm earlier studies of nutrition effect on offspring sex ratio they shown that food quantity (i.e., maternal feeding rate) affects maternal reproductive output in reptiles, but the effects of

dietary quality have largely gone unnoticed. (Ballinger 1977; Seigel and Fitch 1985; Du 2006). Thus, these studies reveal that reproductive females' diet quality affects not just their ability to reproduce, but also the size and sex of their offspring. Therefore, the reaction norms that relate the quality of a maternal nutrition to her ability to reproduce may have significant fitness implications. The low-quality diet's detrimental effects on maternal health and the quantity of clutches generated imply that the females receiving this treatment had little energy available for reproduction. Furthermore, females on the low-quality diet started having offspring almost a month after those on the high-quality diet had already given birth to their initial generation. This is likely because the females on the low-quality diet needed more time to gather adequate energy for transmission production in the face of low nutrient availability. Despite the fact that they laid less eggs overall, the females who consumed the poor diet did so at a substantially higher egg size than those who consumed the high-quality diet. Even after studies were adjusted for clutch size, it is interesting to note that this pattern remained proving that investment per egg increased without a decrease in egg number. This pattern shows that the way reproductive females devote their energy to reproduction is influenced by the quality of their nutrition. Our findings are comparable to those that were previously published for insects (Fischer *et al.*, 2006).

Therefore, present study has been undertaken in *D. melanogaster* to know the effect of prebiotic supplement on sex ratio. It was noticed that in both control and treated flies female offspring were produced greater than those of male offspring. (**Figure 1; Table 1A-1C**) this shows

the nutrition is an important factor affecting sex ratio variation in *D. melanogaster*. Further studies have also shown that offspring sex ratios were strongly affected by maternal diet, the date of oviposition and their interaction (Table 1A-1C). In general, females fed the high-quality diet produced female-biased sex ratios and those on the low-quality diet produced male-biased sex ratios. Thus, this study suggests nutrition alter sex ratio in *D. melanogaster*.

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