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Effect of fruit body extract of *Pleurotus ostreatus* (Oyster) mushroom on offspring sex ratio in *D. melanogaster*

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Abstract
The effects of nutrition on physiology and behavior vary substantially depending on an individual's age, gender, and mating status. However the present study the flies of *D. melanogaster* was cultured in wheat cream agar media and *Pleurotus ostreatus* (oyster) mushroom diet media, to understand the effect of *P. ostreatus* mushroom diet on the offspring sex ratio of *D. melanogaster*. The result revealed that in both control and *P. ostreatus* mushroom treated flies produced greater number of female offspring than those of male offspring. Further the flies raised in *P. ostreatus* mushroom had greater production of female offspring compared to control media flies. Thus this study suggests that nutrition alters the offspring sex ratio in *D. melanogaster.*

Keywords: *Pleurotus ostreatus*, offspring sex ratio, male and female offspring

Introduction
The study of sex ratio in insects has been one of the important topics in evolutionary biology (Leigh *et al*., 1985) [15].In many organism with the sexes are separate, females are produced in approximately equal numbers with males (Hardy, 2002) [11]. Therefore, in many organism, such as insects, the sex ratio is 1:1 (Prakash, 2008). This ratio maximizes the availability of males to females, and hence maximizes genetic heterogeneity (Schowalter, 2016) [25, 26]. In the absence of manipulation, the sex ratio are constant (Cherian *et al*., 2016) [2].
In insects with separate sexes, the sex ratio is the proportion of male or female progeny produced. The proportion of females indicates the reproductive potential of a population. Normally, in a randomly mating population, sex ratio varies around 1:1 because of the segregation of sex chromosomes during gametogenesis (Rawlings and Maudlin, 1984; Walker 1984; Werren and Godfray, 1995; Sheldon and West, 2002; Hoy, 2004) [14, 21, 28, 32, 33] and this 1:1 sex ratio generally indicates stabilising selection on males and females (Schowalter, 1996) [25, 26].
Sex ratio, however, is often affected by both intrinsic and extrinsic factors. The intrinsic factors include genetics, behaviour, physiology, and the presence of intracellular endosymbionts; whereas extrinsic factors include biotic and abiotic factors, such as temperature, photoperiod, humidity, light condition and type, quantity and quality of host or prey (Wrensch, 1993; Schowalter, 1996) [25, 26, 34]. Among abiotic factors, temperature and photoperiod are the most important environmental factors that can affect many aspects of biology and ecology of an insect species, e.g., development, reproduction, survival, longevity and diapause, as well as sex ratio.
Sex ratio varies within and between populations (Walker, 1984) [32] and male-biased or female-biased sex ratios may be found in many insects (Martin *et al.* 1999; Greeff 2002; Santolamazza-Carbone and Rivera, 2003) [9, 16, 24], including species in Hemiptera (Groeters, 1996; Ambrose, 1999; Shahayarag and Sathiamoorthi, 2002; Cullen and Zalom, 2005) [1, 3, 10, 27]. *D. melanogaster* was used as a model organism in this study.
The organism was choosen in this study because of some reasons. First, *Drosophila* is an organism that produced consistently high numbers of offspring. Second, this organism is recorded as rapid breeder with a lot of eggs and short life cycle. Third, this organism has often been as model organism in many study examining various problems in biology.

Materials Method
Collection of fruit body extract of *Pleurotus ostreatus* (oyster) mushroom
The fruit body extract of oyster mushroom powder was purchased from the ROOTED
(Active naturals). Delivered by Amazon app by online.

Establishment of stock
Oregon K strain of *D. melanogaster* provided by *Drosophila* stock center, Department of Zoology, University of Mysore, Mysuru. was used in the investigation. This stock was used in the present study, was cultured in bottles containing wheat cream agar medium (100g jaggery, 100g wheat powder, 10g agar, 1000ml distilled water, and 7.5 ml propionic acid were added). Flies were kept in a lab environment with 70% humidity, 12-hour cycles of darkness and light, and a temperature of 22°C ±1°C.

Experimental media preparations
Experimental media was obtained by using the different concentrations (2.5g/ 5g/10g) of *P. ostreatus* (oyster) mushroom powder was mixed thoroughly with 100ml of wheat cream agar media. Flies cultured in wheat cream agar media were considered as control. These experimental flies were also maintained in same laboratory conditions as described above. Flies obtained from control and experimental flies were used in the present experiment.

Effect of *P. ostreatus* (oyster) mushroom on sex ratio (Male and Female offspring)

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 recorded total number of males and females offspring from each diet. A total of twenty pairs were made separately for each of the control and *P. ostreatus* (oyster) mushroom treated media.

Result

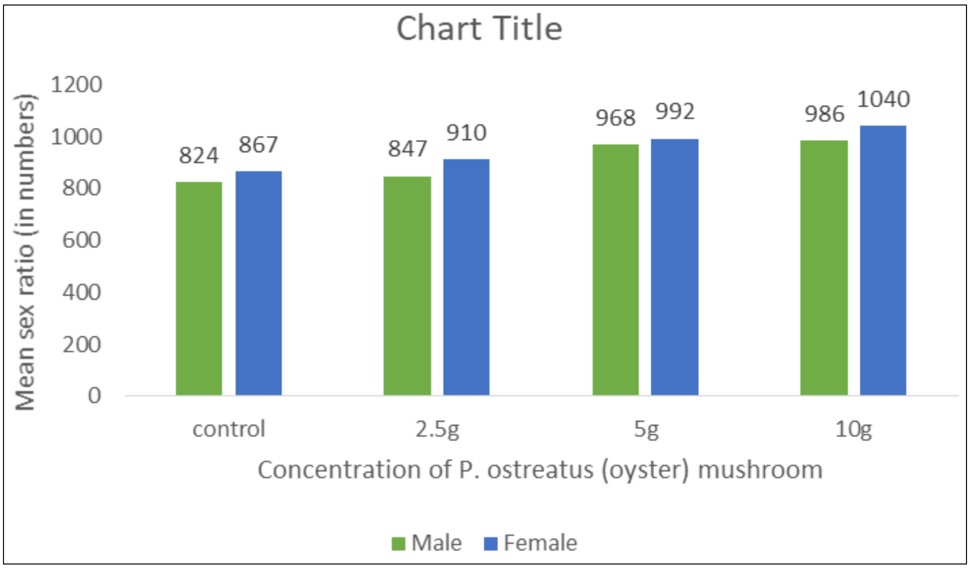

Fig1: The graph showing offspring sex ratio in control and *P. ostreatus* (oyster) mushroom treated media in *D. melanogaster* Different letters on the graph indicates significance at 0.05 levels by Tukey's post hoc test.

Table 1 [A]: Effect of control and *P. ostreatus* (oyster) mushroom treated flies on male offspring sex ratio in *D. melanogaster*.

Treatment (diet)	No. of adults	No. of females	No. of males	F:M ratio
Control	1691	867	824	1:0.95
2.5g	1757	910	847	1:0.93
5g	1960	992	968	1:0.97
10g	2026	1040	986	1:0.94

Table 1 [B]: Effect of control and *P. ostreatus* (oyster) mushroom treated flies on female offspring sex ratio in *D. melanogaster*.

Treatment (diet)	No. of adults	No. of Females	No. of Males	M:F ratio
Control	1691	867	824	1:1.05
2.5g	1757	910	847	1:1.07
5g	1960	992	968	1:1.03
10g	2026	1040	986	1:1.06

Table 1 [C]: Effect of control and *P. ostreatus* (oyster) mushroom treated flies on male and female offspring sex ratio using Chi square test in *D. melanogaster*.

Treatments	F:M ratio	Chi square value	Significant
Control	1.05:0.95	0.005	P<0.05
2.5g	1.07:0.93	0.0098	P<0.05
5g	1.03:0.97	0.0018	P<0.05
10g	1.06:0.94	0.0072	P<0.05

Discussion

In species where animals lay eggs in food resources, such as fruit flies, females must choose between maximizing their own nutrition and providing a high-quality nutritional environment for the development of their offspring (Reaume and Sokolowski, 2006) [22]. One environmental component that can influence sex determination in the adult phase is temperature (Precht, 1973; Egna, 1997) [5, 19]. Temperatures can impact the survival level of male or females from larval to imago periods, causing the sex ratio to shift (Precht, 1973) [19]. Furthermore, temperature has been shown to impact numerous genes in *Drosophila* that are involved in defining sex (Feldmeyer, 2009) [7].

In the present study (Table 1 A and B)) revealed that the flies reared in *Pleurotus ostreatus* (oyster) mushroom had greater reproduction (offspring) than compared to control wheat cream agar media flies. This suggest that the macronutrients and rich protein content had positive impact on offspring production. However the flies reared in control wheat cream agar media had lower reproduction (offspring). May be due to less protein content than compared to oyster mushroom flies. The effects of nutrition on physiology and behavior vary substantially depending on an individual's age, gender, and mating status. Mated females and larvae, for example, balance their intake of protein (P) and carbohydrates (C) to achieve P:C ratios that maximize growth and reproduction [mated females P:C 1:1.5 (Lee *et al.*, 2008, 2013) , larvae P:C 1:2 (Rodrigues *et al.*, 2015)] [13, 14, 23].

The present study (Table 1 A, B, C) shows between the male and female offspring reared in *P. ostreatus* (oyster) mushroom and control media. The female offspring greater than male offspring reared in *P. ostreatus* (oyster) mushroom diet and control media. However it is also noted that the increasing the concentration the male and female offspring ratio also increased. This is because the quality and quantity of diet is influence on the variation in sex of the offspring and also maternal reproductive output and sex of the organism.

Sex ratio is an important characteristic of hermaphroditic organisms (Fei *et al.*, 2011; Qin *et al.*, 2001) [6, 20] and affected by internal genetics and external environment (Dong *et al.*, 2001) [4]. The 1:1 sex ratio is genetically stable because it ensures the parents make an equal genetic investment between females and males of the offspring (Simon, 1994) [29]. However, there is a significant difference of sex ratio among dioecious organisms because of biological diversity in nature (Zhang *et al.*, 1990; Sundstrom, 1994; Zhao *et al*., 2010) [31, 36, 37, 38], leading to a distinct female or male dominance of the population.

(Zhang *et al.,* 2008) [36, 37] conducted a study on the biological characteristics of *B. difformis* in the mushroom tunnels, and found that there were more females than males so that it was difficult to collect males, and the ratio of female to male was generally greater than 1:1. Different sex ratio structure will affect the mating and spawning of insect (Fjerdingstad, 2002) [8].

Mating and spawning ability are linked to the female-male ratio structure, which influences insect population expansion. Under a given sex ratio, it is evident that the higher an adult's fecundity, the faster population growth (Yao *et al.*, 2008) [35]. Recent studies using nutritional geometry (Simpson and Raubenheimer, 1993, 2012; Simpson *et al.,* 2015b) [30], a conceptual approach to dissecting the nutritional interactions between animals and their environment, have shown how fruit flies actively balance their acquisition of macronutrients (protein and carbohydrates) to trade off fitness traits such as development time, reproduction, and survival e.g. *D. melanogaster* (Lee, 2015; Lee *et al*., 2008, 2013; Piper) [13, 14, 17].

Hence from our study in *D. melanogaster* found that nutrition also affects offspring sex ratio in *D. melanogaster.*

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