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Research Article

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Fruit body extract of *Pleurotus ostreatus* (Oyster) mushroom increases reproductive fitness in *D. melanogaster*

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Abstract

Dietary nutrient present in the food is responsible for animal overall fitness. However the availability of nutrient and its ratio in the food may not be stable. Further, it is not entirely clear how dietary enrichment of specific macronutrients (fat, protein, or sugar) influences behavior, mate selection and reproductive fitness of an organism. In the present study flies obtained from control media and oyster mushroom treated media were used to study reproductive fitness such as mating latency, copulation duration and fertility in pair wise mating experiment. It was noted that the mating latency varied significantly between control and oyster mushroom based media. Time taken for initiating mating was lowest in control flies compared to oyster mushroom treated media. Further copulation duration was highest in the flies reared in control wheat cream agar media than those of oyster mushroom treated flies. Progeny production was least in control flies where as progeny production was increased with increasing concentration of oyster mushroom. Flies raised in control media mated faster, copulated longer but produced least progeny than those of flies grown in oyster mushroom treated media. Thus in *D. melanogaster* oyster mushroom increases reproductive fitness.

Keywords: *Pleurotus ostreatus*, Nutrients, Reproductive fitness, D. *melanogaster*.

Introduction

All biological processes directly associated to reproduction play an essential role in determining fitness. Many factors contribute to fitness, including mating latency, mating time, mating duration, fertility, fecundity, production, viability, and longevity. Collectively, these processes determined the fertility component of fitness, where fertility is defined broadly (Turner and Andersson, 1983).

The sexual behaviors of various *Drosophila* species, particularly their basic courtship patterns, genetic control, role of stimuli, and contributions of the sexes to variations in mating activity and

repeated mating, have been extensively studied (Parsons, 1973; Banerjee and Singh ,1977; Gromko and Pyle, 1978; Casares *et al.*,1998). The role of fertility differences in selection has been proven repeatedly in many Drosophila species (Turner and Andersson, 1983; Partridge *et al.*, 1987 and Santos *et al.,* 1988). Demonstrated the effect of body size on mating success. Body size also effects mating latency, fertility, and other fitness components (Ewing, 1961; Moncls and Prevosti, 1971; Partridge and Farquhar, 1983; Partridge *et al.,* 1987; Santos *et al.,* 1988; Hegde and Krishna, 1997; Krishna and Hegde, 1999). Male age is another factor known to influence female mating preferences, mating latency, copulation duration, fertility and other fitness traits in species of *Drosophila* (Santos *et al*., 1988). Diet is one of the key environmental factor known to affects sexual attractiveness and individual fitness which are inextricably related as animals learn to perceive features that indicate high fitness and reproductive potential in potential mates. This is not surprising given that different physiological and sex-specific tasks demand different nutrients, and certain allocation decisions, such as survival versus reproduction, are frequently optimized by different diets (Fricke *et al*., 2008; Maklakov *et al*., 2008; Vargas *et al*., 2010; Gosden and Chenoweth, 2011). Because diet composition can vary greatly over an individual's lifetime, natural selection is likely to favor biological mechanisms that rapidly alter allocation decisions in response to nutrient availability, as well as mechanisms in individuals of the opposite sex to evaluate such decisions in potential mates.

Unstable conditions can lead to shifting mate preferences, hence it is critical to understand how constantly changing environmental parameters, such as nutrition availability, influence reproductive success (reviewed by Miller and Svensson, 2014). Animal fitness is condition-dependent and can be impacted by environmental factors such as nutrition, and nutrient availability has been demonstrated to alter sexual selection and mate choice (Janicke *et al.,* 2015; Kunz and Uhl, 2015; Xue *et al.,* 2016) .The optimal macronutrient ratio (fat, protein, and sugar) varies according to sex and species, but an ideal diet enhances lifelong fertility (Lee *et al*., 2008; Maklakov *et al.,* 2009; Pirk et al., 2010; Solon-Biet *et al.,* 2015). If imbalanced diets reduce fertility, we assume that good-condition animals will find these mates less appealing and will change their behavior, whereas poor-condition individuals will not. Disentangling these variables is a difficult challenge given the complexity and amount of unanswered questions surrounding mate selection. However, the genetically tractable *D. melanogaster* is an excellent animal model for studying how nutrition influences mating selection and individual beauty. Mushrooms are fungus fruiting structures that often grow on top soil and wood as feeding sources. They can be produced or taken from the wild for human consumption and are either edible or poisonous. There are about 2000 mushroom species in nature, with just around 25 of them acceptable for human eating (Barros *et al*., *2007*).This nutritional information was provided by the USDA.3Oyster mushrooms are a high-fiber, low-calorie food that is also high in phosphorus, copper, and niacin, among other vitamins and minerals. Ethnopharmacologically, mushrooms are used to cure illnesses such as cancer, diabetes, obesity, heart disease, hyperacidity, and hypertension (Choudhury *et al*., 2013). Mushrooms have been shown to have anticancer (Blagodatski *et al.,* 2018; Lindequist *et al.,* 2005), antiviral (Lindequist *et al.,* 2005; Ellan *et al.,* 2019), antithrombotic (Ellan *et al.,* 2019; Islam and Uddin, 2015), antioxidant (Boonsong *et al.,* 2016) and immunomodulatory properties. The presence of bioactive compounds such as polysaccharides, proteins, and lipids, as well as a variety of low molecular weight metabolites such as lectins, lactones, terpenoids, alkaloids, sterols, and phenolic acids, is what gives medicinal mushrooms their therapeutic capabilities. Low carbohydrate levels, high protein, vitamin, and mineral levels, and low lipid and cholesterol levels (Barros *et al*., 2007). However its effects on reproductive fitness has not been studied therefore present study has been under taken in *D. melanogaster* to study the effect of fruit body extract of *Pleurotus ostreatus* (Oyster) mushroom

on mating and fertility production of *D. melanogaster*.

D. melanogaster eats rotting fruit infected by yeast in the wild. Fruit macronutrients composition changes depending on genetics, environment, and season. *Drosophila melanogaster*, a fruit fly, is utilized as a model organism in studies ranging from fundamental genetics to tissue and organ developmental and nutrition. The *Drosophila* genome is 60% more identical to human DNA than human DNA is redundant, with fly homologs for 75% of human disease-causing genes. Because of these qualities, the fruit fly is well suited to investigate complicated processes relevant to scientific inquiry, such as cancer. They also contribute to the fruit fly's rapid generation time, low maintenance costs, and easy access to powerful genetic tools.

Materials and Methods

Establishment of stock:

Experimental stock of the *Drosophila melanogaster* K strain used in the study was provided by the *Drosophila* Stock Center. Department of Zoology, University of Mysore, Mysore; this stock was grown in bottles with wheat cream agar medium (added 100g jaggery, 100g wheat powder, 10g agar, 1000ml distilled water, and 7.5ml propionic acid). In a lab setting with a humidity of 70%, 12-hour cycles of darkness and light, and a temperature of 22°C ± 1°C, flies were housed. These flies were used to obtain experimental flies.

Collection of fruit body extract of Oyster (*P. ostreatus)* mushroom:

The fruit body extract of oyster mushroom powder was purchased from the ROOTED (Active naturals). Delivered by Amazon app by online.

Experimental media preparations:

Wheat cream agar media was prepared by using 50g of jaggary, 50g of wheat powder, 5g of agar boiled in 500ml of distilled water and 3.8ml of propionic acid was added to avoid the growth of fungus. Experimental flies were obtained by adding 2.5g, 5g, 10g of Oyster mushroom powder respectively to the 100ml of wheat cream agar media. Whereas, flies cultured in wheat cream agar media were considered as control.

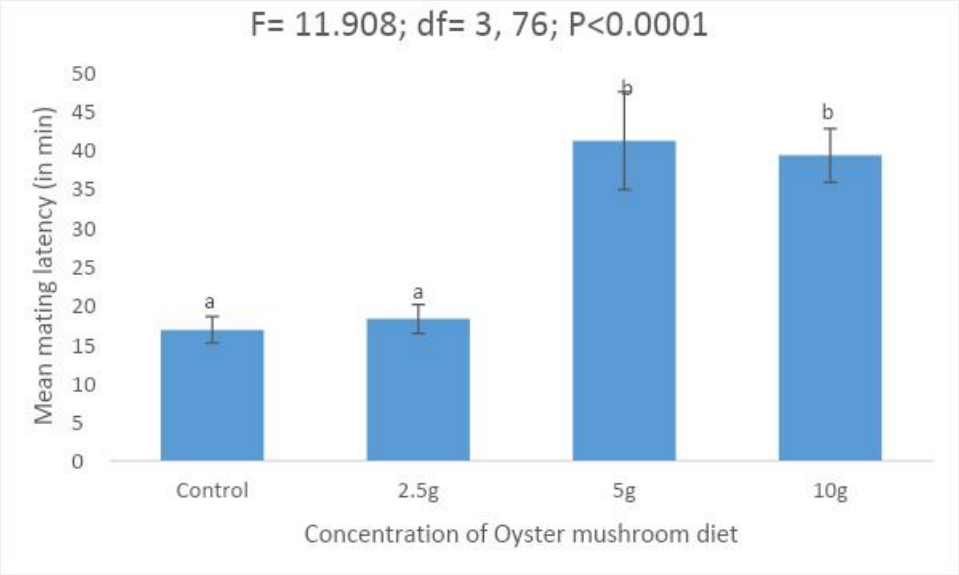
Effect of oyster mushroom on mating latency, copulation duration and Fertility rate of *D. melanogaster:*

From the control media and different concentration of *P. ostreatus* (oyster) mushroom treated *Drosophila* culture bottles, virgin females and unmated males were isolated within 3 hours of their eclosion from their respective media. These flies were aged for 5 days. Virgin female and unmated males were individually aspirated in to mating chamber and observed one hour. If mating does not occurs within 1 hour the flies were discarded. If mating occurs we have recorded their mating latency (the time elapsed between introduction of male and female into the mating chamber until initiation of copulation), copulation duration (time elapsed between the initiation to termination of copulation). These mated pairs were transferred to vials containing their respective media once in 7 days until to the death of the flies. Total number of progeny produced by each mated pairs was recorded as fertility. A total of twenty trials were made separately for each of the control, and oyster mushroom treated media.

Results

Effect of the fruit body extract of *P. ostreatus* (Oyster) mushroom on the mating latency in the *D. melanogaster:*

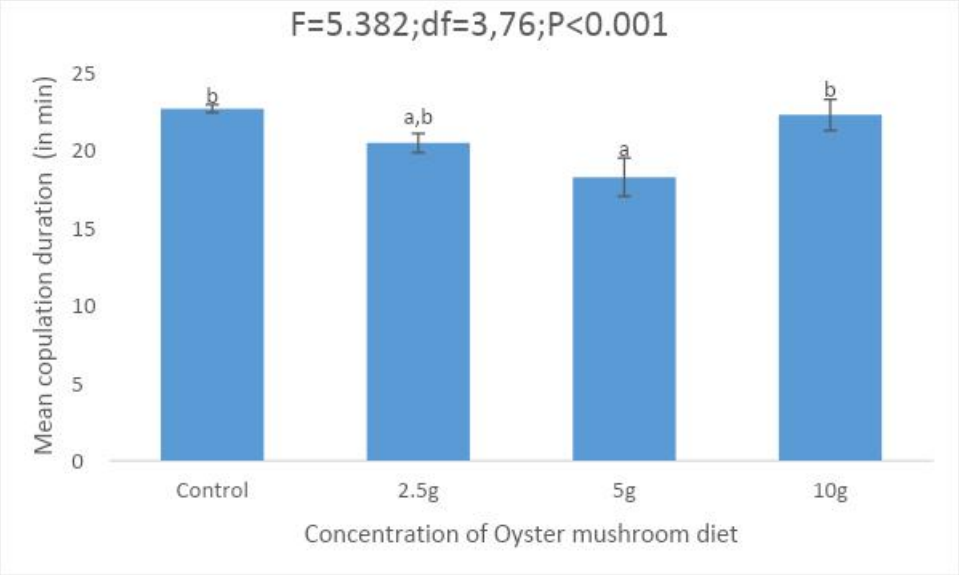
Figure 1: The effect of different concentration of *P. ostreatus* (Oyster) mushroom diet on the mating latency of *D. melanogaster*. [Control diet- wheat cream agar media; Oyster mushroom diet (2.5g, 5g, 10g concentration).



The different letters on the bar graph indicate the significant variation between the different diets by Tukey's post hoc test at 0.05 level.

Figure (1) shows that the mean value of mating latency of control and mushroom treated diet *D. melanogaster*. According to the data obtained showed that flies raised in the 5g concentration of Oyster mushroom diet media had highest mating latency than compared to control and 2.5g 10g concentration of Oyster mushroom diet respectively. Control flies had lowest mating latency than compare to other treated different concentration (2.5g, 5g, 10g) of oyster mushroom diet. Figure (1) experimental data was subjected to One way ANOVA followed by the Tukey's Post hoc test revealed the significant variation in the mating latency between the flies of different diet concentration and control flies. Tukey's post hoc test showed that that the *D. melanogaster* flies fed with 5g concentration of oyster mushroom diet had greater significant longer time for mating compared to the control and 2.5g, 10g, concentration of oyster mushroom diet fed flies. However non significant variation in mating latency was observed, between the flies which was fed by control diet and 2.5g concentration of Oyster mushroom diet and between the flies which had fed by 5g, 10g concentration of Oyster mushroom diet in *D. melanogaster* by Tukey's post hoc test.

Figure 2: The effect of fruit body extract of *P. ostreatus* (Oyster) mushroom on the copulation duration of *D. melanogaster*. [Control diet- wheat cream agar media; Oyster mushroom diet (2.5g, 5g, 10g concentration).

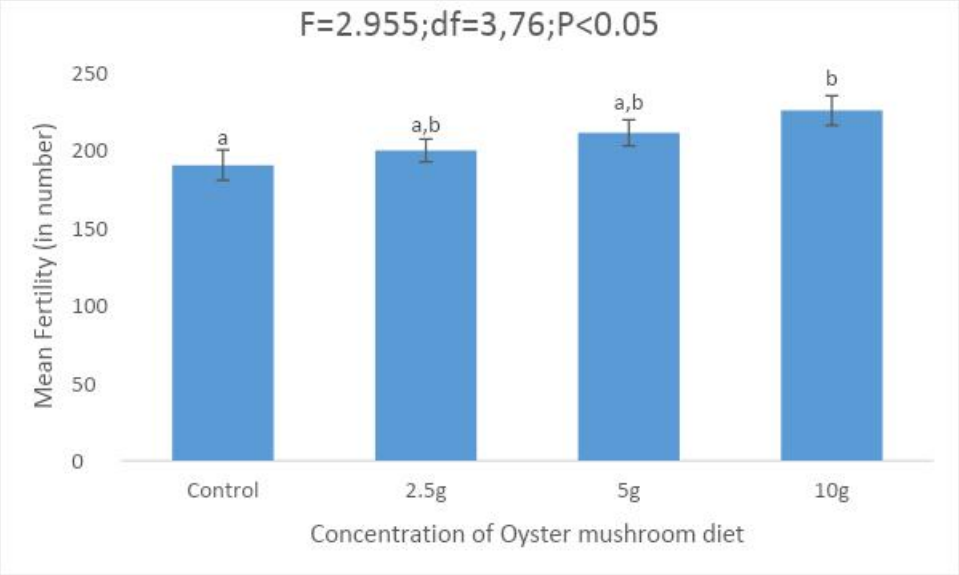


The different letters on the bar graph indicates significant variation between the different diet by Tukey's post hoc test at 0.05 level.

The Figure (2) shows that the mean value of the copulation duration varied between flies raised in control diet and different concentration, 2.5g, 5g, 10g, of Oyster mushroom diet. According the data obtained the flies raised in the control media had greater copulation duration when compared to Oyster mushroom diet and lowest copulation duration in 5g oyster treated mushroom treated flies.

The Figure (2) experimental data was subjected to the One way ANOVA followed by the Tukey's post hoc test revealed significant variation in the copulation duration between control compared to the oyster mushroom diet fed flies. Tukey's post hoc test showed that the *D. melanogaster* flies fed with control media had significantly greater copulation duration between 10g concentration of Oyster mushroom diet and 5g concentration of Oyster mushroom diet. However there was non significant variation in copulation duration were observed between control and 10g concentration of Oyster mushroom diet and also between flies grown in 5g and 10g concentration of Oyster mushroom diet of *D. melanogaster* by Tukey's post hoc test.

Figure 3: Effect of fruit body extract of *P. ostreatus* (oyster) mushroom diet on Fertility of *D. melanogaster*. [Control diet- wheat cream agar media; Oyster mushroom diet (2.5g, 5g, 10g concentration)



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

The Figure (3) shows the mean value of fertility rate varied between the flies which were raised in different diets (control and different concentration (2.5g, 5g, 10g) of oyster mushroom). According to the data obtained showed that the fertility rate increases with increased concentration of oyster mushroom diet, and the control diet treated flies had lower fertility.

The experimental data was subjected to One way ANOVA followed by Tukey's post hoc test revealed significant variation in fertility between the flies grown in control diet and Oyster mushroom diet (Figure 3). The flies grown in 10g concentration of Oyster mushroom had greater significant than those flies grown in control wheat cream agar media. However there is non significant variation between control diet flies and the flies which grows in 2.5g, 5g concentration of Oyster mushroom diet by Tukey's post hoc test.

Discussion

Most compelling studies in *Drosophila* have shown that male traits such as male size, male age were known to affect reproductive to fitness in *Drosophila.* Diet is one of the most important environmental elements known to influence organism growth and development .The quality and quantity of food consumed by an organism affects its health and reproductive fitness. (Sisodia and Singh, 2012). In the present study the influence of fruit body extract of oyster mushroom diet on reproductive fitness parameters such as mating latency, copulation duration and fertility rate was examined in *D. melanogaster*. In species of *Drosophila* during courtship males and females exhibit series of courtship act, here successful mating depends on male activity and female receptivity (Manning, 1961), this is because female is usually discriminating partner in mating act. Flies which have greater activities during mating shows mating success further courtship activity of male and female culminate in copulation.

During this act males transfers accessory gland protein and sperm to the female reproductive tract which in turn affects fecundity and fertility (Anitha and Krishna, 2020). In the present study (Figure 1, 2 and 3) control diet flies had mated faster (as speed is reverse of time), copulated longer, produce least progeny size. This is because control male flies had transferred lesser accessory gland protein and sperm to the mated female resulting least progeny size. Flies raised in 10g oyster mushroom had mated slower copulated longest and produced highest number of progeny size because these flies during their copulation duration had transfer significantly greater quantity of accessory gland proteins and sperm to the mated females therefore they produce highest number of progeny size. Whereas the flies fed with 2.5g and 5g oyster mushroom had taken average mating latency, shorter copulation duration and produce average progeny size. Further (Figure 3) among the mushroom treated flies progeny size increased with increasing concentration of mushroom. These finding suggest that oyster mushroom treated flies had greater reproductive fitness over control diet. The present study also confirms earlier studies of dietary effect on reproductive fitness in species of *Drosophila* (Janna *et al.*, 2007). They also found that variation in protein carbohydrate ratio affects mating latency, copulation duration and fertility. Further they also found that flies fed with protein rich diet have greater reproductive success than those of flies fed with carbohydrate rich diet. Even in the presence of the mushroom treated flies have greater reproductive success than flies fed with control diet. This is because the protein content in the mushroom diet was significantly greater than those of control diet. In species of *Drosophila* have shown that many male traits such as male size, male age, and laboratory conditions were known to affect reproductive fitness. However in the present study we have used same aged flies and were cultured in same laboratory condition among the only difference was only diet therefore observed variation in the mating latency, copulation duration and fertility. Were due to variation in the nutrients present in the control diet and mushroom diet. Thus from all these studies we found that oyster mushroom treated flies had greater reproductive success than control diet flies suggesting mushroom with greater protein and other nutrients increases the reproductive fitness of the flies.

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