

distribution of the above species in the Western Ghats of south eastern Karnataka, at its border with Tamil Nadu (Erode District) district, is given in Table 1.

Table 1. *Drosophila* fauna of B.R Hills wildlife sanctuary in Chamarajanagar District of south eastern Karnataka, India.

S. No	Name of the species	Frequency distribution at B.R Hills wild life sanctuary							
		B.R Hills Forest		Near Temple (3/4) of the Hill		K. Gudi Forest		Total No. of Flies	
		(F)	(M)	(F)	(M)	(F)	(M)	(F)	(M)
1.	<i>D. ananassae</i>	70	78	30	40	80	98	180	216
2.	<i>D. bipectinata</i>	30	20	30	20	-	-	60	40
3.	<i>D. kikkawai</i>	40	32	18	13	-	-	58	45
4.	<i>D. malerkotliana</i>	65	67	38	40	-	-	103	107
5.	<i>D. takahashii</i>	30	45	15	25	60	78	105	148
6.	<i>D. neonasuta</i>	90	98	-	-	-	-	90	98
7.	<i>D. varians</i>	23	28	-	-	-	-	23	28
8.	<i>D. anomelani</i>	60	88	35	48	72	60	167	196
9.	<i>D. sampangiensis</i>	30	40	-	-	-	-	30	40
10.	<i>D. nigra</i>	30	45	-	-	-	-	30	45
Grand Total		468	541	166	186	212	233	846	963

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### Effect of nutritional regime on reproductive performance in *Phorticella straiata*.

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### Introduction

It was widely suggested that nutrition is one of the environmental variations that can affect body condition and reproduction. This is because energy required to perform each and every process of life of an organism comes from nutrition; thus, the balance depends on the interplay between matter intake, digestion, and allocation of acquired energy to various functions such as maintenance, growth, and reproduction (Karasov, 1986; Sterner and Schulz, 1998; Taylor *et al.*, 2005). Experimental modifications of animal diets have played a key role in the study of how organisms adjust their energy allocation (Chown and Nicolson, 2004; Cruz-Neto and Bozinovic, 2004). Deficiency or imbalance of fat, carbohydrate, or protein can affect characters such as growth and reproduction. Protein deficiency reduces fecundity and growth in *Drosophila melanogaster* (Wang and Clark, 1995), and in fruit-feeders protein is often a limiting macronutrient (Mattson, 1980; Adams and Gerst, 1991; Hendrichs *et al.*, 1991; Markow *et al.*, 1999, 2001). When faced with nutritionally imbalanced diets, compensatory feeding for the limiting nutrients results in over ingestion of

other nutrients, as is often seen when insects are confined to food low in protein relative to carbohydrate (Raubenheimer and Simpson, 1999). This may result in increased lipid storage and reduced fitness (Simpson *et al.*, 2004; Warbrick-Smith *et al.*, 2006). Therefore, more studies are required in different species of the same genera to have general concept. Hence the present study has been undertaken in *Phorticella straiata* to study effect of carbohydrate and protein enriched diets on reproductive performance.

## Materials and Methods

### *Establishment of experimental stock*

The experimental stock of *Phorticella straiata* was obtained from the progenies of 50 isofemale lines collected from Chamundi Hills, Mysore, India. In each generation flies obtained from these culture bottles were mixed together and redistributed to 20 different culture bottles containing wheat cream agar media (100 g of jaggery, 100 g of wheat powder, 8 g of Agar was boiled in 1000 ml of double distilled water, and 7.5 ml of Propionic acid was added) each with 20 flies (10 males and 10 females). These flies were maintained at  $22^{\circ}\pm 1^{\circ}\text{C}$  with a relative humidity of 70% in a 12 hr dark: 12 hr light cycle. This procedure was carried out for three generations to acclimatize flies to lab condition. At the fourth generation, eggs were collected using Delcour's procedure (1969). Eggs (100) were seeded to each culture bottle containing carbohydrate, protein, and carbohydrate-protein enriched media. Carbohydrate enriched media (20%) was prepared by mixing sucrose and wheat cream media in 1:4 ratio. The protein enriched media (60%) was prepared by mixing casein and wheat cream agar media in 3:2 ratio. The carbohydrate and protein enriched media (30% carbo + 30% protein) was prepared by mixing sucrose:casein:wheat cream agar media in 2:2:1. When pupae were formed, females and males were isolated within three hours of their eclosion and aged for five days to test for virginity. These flies were used for present experiments.

### *Effect of diet alteration on larval feeding in P. straiata*

Third instar larvae obtained from eggs collected ( $\pm 2$  hours) from wheat-cream agar media grown flies using Delcour's procedure (1969) were used to study feeding behavior. Each larva was placed in a vial containing carbohydrate enriched / protein enriched / carbohydrate and protein enriched media and observed under a stereomicroscope. The back and forth movement of the proboscis was recorded for a minute. A total of 50 replicates were run separately for each of the altered diets.

### *Effect of diet alteration on reproductive performance in P. straiata*

Four-day-old virgin female and unmated male from the carbohydrate enriched / protein enriched / carbohydrate and protein enriched media were aspirated into an Elens-Wattiaux chamber (Elens and Wattiaux, 1964). Each pair was observed for an hour and the pairs which did not mate within this time limit were discarded. Mating latency (time between introduction of a pair of male and female flies into the Elens-Wattiaux chamber until the initiation of copulation between each pair) and copulation duration (time between initiation to termination of copulation of each pair) were recorded. Mated flies were transferred once in 24 hr to new vials containing 5 ml of wheat cream agar media until death of females. Total number of eggs laid was also recorded. A total of 50 replicates were performed separately for flies grown on carbohydrate enriched / protein enriched / carbohydrate and protein enriched media.

### *Effect of diet alteration on ovariole number in P. straiata*

Four-day-old virgin females were etherized and killed. The thorax of these flies was individually dissected out using a pair of fine dissection needles in physiological saline under a binocular stereomicroscope. The ovaries were separated and the total number of ovarioles in either the right or the left ovary was noted following the procedure of Hegde and Krishna (1997).

## Results

Figure 1 provides the larval feeding rate in different diets. It was found that highest larval feeding rate occurred in flies grown in protein rich diet compared to carbohydrate rich and carbohydrate and protein rich

diets. One-way ANOVA followed by Tukey's *Post Hoc* test carried out using SPSS version 10.0 on the above data showed significant variation in feeding rate between different diets (Table 1). Tukey's *Post Hoc* test also showed that feeding rate was significantly greater in flies grown in protein enriched media than those flies grown in the other two altered diet media.

Table 1. One way ANOVA on feeding rate, mating latency, copulation duration, fecundity, and ovariole number in *P. straiata*.

Parameter	Source	Type III Sum of Squares	df	Mean Square	F value
Feeding rate (in no.)	Diet	35184.053	2	17592.027	531.816***
	Error	4862.640	147	33.079	
	Total	2090122.000	150		
Mating latency (in min)	Diet	81.413	2	40.707	321.176***
	Error	18.631	147	.127	
	Total	2857.057	150		
Copulation duration (in min)	Diet	46.053	2	23.027	88.686**
	Error	38.167	147	.260	
	Total	1489.434	150		
Fecundity (in no.)	Diet	26429.293	2	13214.647	242.235***
	Error	8019.300	147	54.553	
	Total	873555.000	150		
Ovarioles (in no.)	Diet	1957.213	2	978.607	63.271**
	Error	2273.620	147	15.467	
	Total	137139.000	150		

\*\*\*Significant at 0.0001 level ( $P < 0.001$ ); \*\*Significant at 0.001 level ( $P < 0.001$ ).

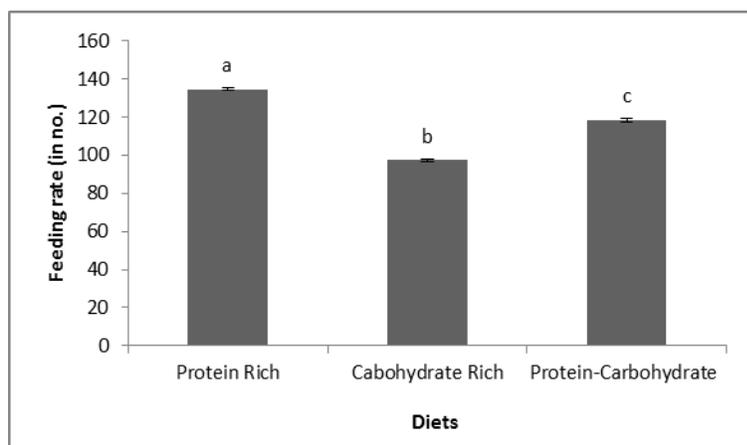


Figure 1. Diet effect on feeding rate in *P. straiata*. (Different letters on the bar graph indicate significance at 0.05 level by Tukey's *post hoc* test).

Mating latency of flies grown in altered diets is provided in Figure 2. It was observed that flies grown in protein rich media had taken least time to initiate copulation when compared to the other two altered media. One way ANOVA followed by Tukey's *Post Hoc* test carried out on the above data using SPSS version 10.0 showed significant variations in mating latency in different diets (Table 1). Flies grown in protein enriched media had taken significantly greater time to initiate copulation compared to flies grown on carbohydrate and protein enriched media by Tukey's *Post Hoc* test.

Copulation duration data of flies reared in different altered diets is provided in Figure 3. It was noticed that flies grown in protein rich media had copulated longest compared to other two altered media. One way ANOVA followed by Tukey's *Post Hoc* test carried out on the above data using SPSS version 10.0 showed significant variation in copulation duration in different diets (Table 1). Flies grown in protein enrich

media had copulated significantly longer than compared to flies grown on carbohydrate and carbohydrate and protein enriched media by Tukey's *Post Hoc* test.

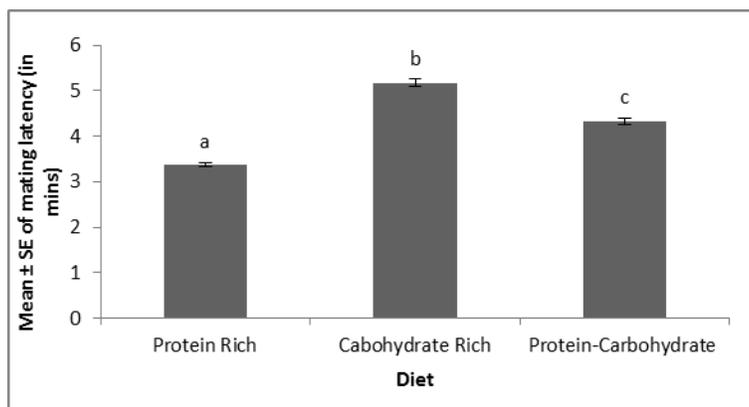


Figure 2. Diet effect on mating latency in *P. straiata*. (Different letters on the bar graph indicate significance at 0.05 level by Tukey's *post hoc* test).

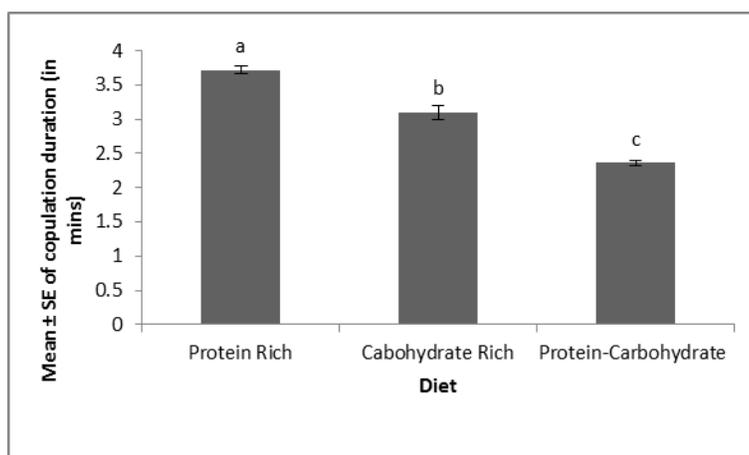


Figure 3. Diet effect on copulation duration in *P. straiata*. (Different letters on the bar graph indicate significance at 0.05 level by Tukey's *post hoc* test).

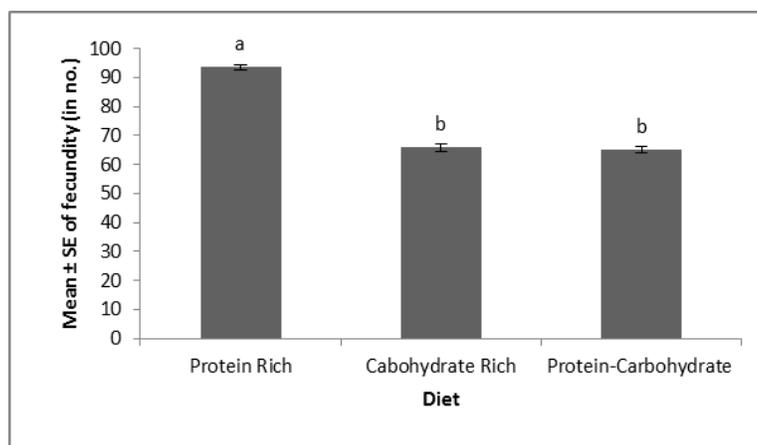


Figure 4. Diet effect on fecundity in *P. straiata*. (Different letters on the bar graph indicate significance at 0.05 level by Tukey's *post hoc* test).

Mean fecundity data of flies reared in different altered diets are given in Figure 4. It was found that flies grown in protein rich media had greater fecundity than those flies grown in carbohydrate and carbohydrate and protein enriched media. One way ANOVA followed by Tukey's *Post Hoc* test carried out on the above data using SPSS version 10.0 showed significant variation in fecundity between different altered

diets (Table 1). Flies grown in protein rich media had significantly greater fecundity compared to flies grown on carbohydrate and carbohydrate and protein enriched media by Tukey's *Post Hoc* test.

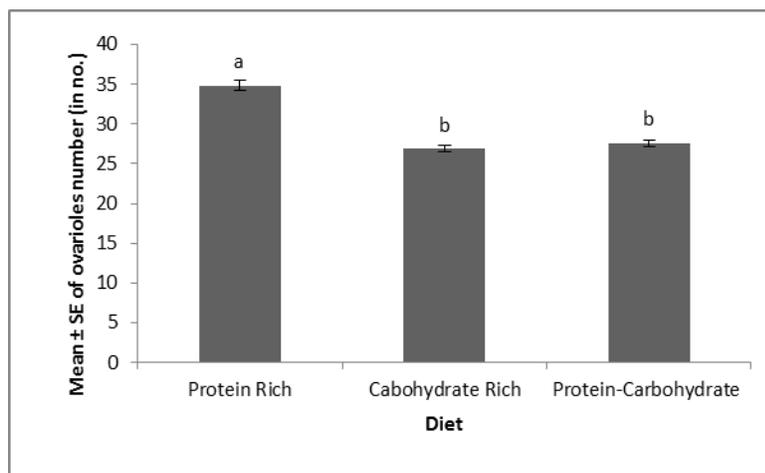


Figure 5. Diet effect on ovarioles in *P. straiata*. (Different letters on the bar graph indicate significance at 0.05 level by Tukey's *post hoc* test).

Figure 5 shows ovariole number data of flies reared in different altered diets. It was found that flies grown in protein rich media had greater ovariole number than those flies grown in carbohydrate and carbohydrate and protein enriched media. One way ANOVA followed by Tukey's *Post Hoc* test carried out on the above data using SPSS version 10.0 also showed significant variation in ovariole number between different altered diets (Table 1). Flies grown in protein rich media had significantly greater ovarioles number than those flies grown on carbohydrate and carbohydrate and protein enriched media by Tukey's *Post Hoc* test.

## Discussion

*P. straiata* is known to feed and breed on ripe or rotting fruits where protein/carbohydrate ratios vary temporarily and spatially. Therefore, it is possible that quantities of carbohydrate and proteins in the diet of an organism have a direct effect on reproduction of an organism (Sisodia and Singh, 2012). In species of *Drosophila* experiments on feeding rate have suggested that diet type had a significant influence on larval feeding. Such differences may be due to inhibition threshold. *D. melanogaster* larvae feed almost continuously, accompanied by a massive increase in mass. However inhibition threshold exist for feeding on new or foul tasting foods (Melcher *et al.*, 2007). Such inhibition threshold is not observed in larvae fed on protein enriched diet when compared to other two altered diets, since the rate of larval feeding was highest among larvae fed on protein diet.

Reproductive performance of an organism is a good index of fitness in organisms that go through repeated cycles of rapid population growth, and it has evolved as a way for species to maximize their fitness and is known to be influenced by various factors, such as body size, age, diet, and so forth (Partridge *et al.*, 1987; Krishna and Hegde, 1997; Somashekar and Krishna, 2010). In the present study it was noticed that in *P. straiata* flies grown on protein rich media had taken less time to initiate copulation compared to flies grown on carbohydrate rich and carbohydrate-protein rich media.

As the time is reverse of speed, flies which took less time to initiate copulation were fast maters, while flies which took greater time to initiate copulation were slow maters (Hegde and Krishna, 1997). Therefore in the present study flies grown on protein rich media were fast maters. This suggests that quality of diet had significant influence on mating latency.

Copulation duration is another important fitness trait. Flies which copulated longer can receive greater quantity of accessory gland proteins and sperm than those flies which copulate shorter (Krishna and Hegde, 1997). Figure 3 shows that flies grown on protein rich media had copulated significantly longer than flies grown on carbohydrate rich and carbohydrate-protein rich media. Further it was also noticed that flies grown on protein rich media had greater egg production compared to egg production in other two nutritional regimes

(Figure 4 and Table 1). A high protein requirement when producing eggs might reflect that synthesis of the egg-yolk protein vitalize in females is dependent on the incorporation of amino acids (Adams and Gerst, 1991; Markow *et al.*, 1999). This confirms the work of Sisodia and Singh (2012) in *D. ananassae*. They also found that flies reared in protein rich media had greater fecundity.

In the present study, altered diet effect on ovariole number was also studied in *P. straiata*. This is because both ovarioles number and fecundity are positively related. It was noticed that flies grown on protein rich diet had a significantly greater number of ovarioles than on the other two diets (Figure 5 and Table 1). This suggests that diet has significant effect on fecundity and ovariole numbers. Thus these studies in *P. straiata* suggest that altered diet had significant influence on reproductive performance.

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### ***Drosophila suzukii* larvae suppress *Aspergillus nidulans* growth particularly at high densities of larvae.**

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## **Introduction**

*Drosophila suzukii* Matsumura is an Asian species distributed from northern Japan southwards to the semi tropics and westwards at least as far as India. It has recently been introduced by human agency to the Pacific, North and South America, and Europe (Asquith and Messing, 2012; Calabria *et al.*, 2012; Cini *et al.*, 2012; Hauser, 2011) and is now quite widely spread in these areas. Its arrival has caused great concern in the soft fruit industry, because it lays eggs in fruit that are still on the tree or bush and are not decaying.

Several moulds kill insects including *Drosophila* larvae (Courtney *et al.*, 1990; Hodge *et al.*, 1999; Rohlf *et al.*, 2005). But, because it lays in fresh fruit, the larvae of *D. suzukii* might not be able to defend themselves against the moulds that occur in fruit decaying on the ground. We, therefore, tested this ability by challenging *D. suzukii* larvae with the mould *Aspergillus nidulans*. Wild type mould of this species produces a number of compounds toxic or fatal to insects including *Drosophila* larvae. But, in addition to the wild type mould, we also challenged larvae with a transgenic strain deficient in toxins. The transgenic strain ( $\Delta$  laeA ) cannot express the gene LaeA that regulates secondary metabolism. Blocking LaeA expression suppresses