Volume 69, Issue 2, 2025
Journal of Scientific Research
of
The Banaras Hindu University

Genotype and protein-rich diet leave their substantive influence on reproduction as well as biochemical parameters in *Drosophila ananassae*

Ankita Das[1], Shweta Upadhyay[2],Runa Boro[3] and A. K. Singh*[4]

[1]Ms. Ankita Das,Genetics laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India, 221005.Email id- ankitadas1712@bhu.ac.in

[2]Ms. Shweta Upadhyay,Genetics laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India, 221005.Email id- shwetau657@gmail.com

[3]Ms. Runa Boro, Genetics laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India, 221005. Email id- erahona41@gmail.com

[4*]Prof. A. K. Singh, Genetics laboratory, Department of Zoology,Institute of Science,Banaras Hindu University,Varanasi, India, 221005. Email id- ak.singh2@bhu.ac.in

Abstract: **The effect of food on insect reproduction and survival is a critical aspect of their ecology and biology. In this study, genetically varied isofemale lines of *Drosophila ananassae* were reared on the normal food ingredients and food containing a high content of protein (10% yeast) to observe the impact of elevated doses of yeast on the four major aspects, i.e., mating propensity, fecundity, pupation height preference, and biochemical parameters. The results suggest that flies reared on a high dose of protein were significantly more successful in mating and fecundity than the control lines. Pupation height preference is one of the genetically as well as environmentally influenced pre-adult traits that may determine the survival chance of the fly. The third instar larvae reared on elevated content of protein always preferred to pupate significantly away from the food surface, a behavior indicating to search drier areas for pupation. Flies reared on high protein diets show increased protein and other bio-molecules, potentially converting excess protein into glucose and triglyceride to meet body energy needs. An increase in glucose and triglyceride levels suggests that excess dietary protein is converted into glucose and fatty acids. This indicates the presence of biochemical pathways in insects like *Drosophila* that regulate nutrient balance by transforming surplus nutrients to maintain biochemical homeostasis.**

The investigation suggests that *Drosophila*, a semelparous insect, requires a high protein dose for optimal reproductive activities, a key characteristic of organisms exhibiting r-selection.

Index Terms: **Nutrition, development, survival, mating propensity, fecundity, pupation height preference, and biochemical parameters.**

I. Introduction

Nutrition can influence various life history traits of insects such as their longevity, fecundity, fertility, development time, and overall survival rates (Schmidt *et al.,*1919;Kritika *et al.,*2019). In honeybees (*Apis mellifera*), limited food supply can reduce longevity and colony survival (Paoli *et al.,* 2014). The amount and type of food available during larval stages can affect the development and future reproductive success of adult insects. In the case of the mosquito *Aedes aegypti*, larval nutrition influences adult size, which in turn affects their reproductive capacity (Fuchs *et al.,* 2014). Food scarcity or poor-quality food can lead to behavioral adaptations that impact survival and reproduction. This is evident in the Colorado potato beetle (*Leptinotarsa decemlineata*) in which a diet lacking essential nutrients can lead to changes in feeding behavior and reduced reproductive output (Alyokhin *et al.,* 2013).

Dietary macronutrients are important to the survival and propagation of insects and a slight deviation from an optimal protein to carbohydrate ratio can have a dramatic physiological effect on their fitness (Aguila *et al.,* 2013). Studies have suggested that an optimal protein to carbohydrate ratio can vary within species depending on the developmental stage and even sex as a result of different physiological needs (Canato & Zucoloto, 1998). Harrison *et al.,* (2014) reported that a high

protein diet causes an increase in the adult weight and egg production in field crickets (*Gryllus veletis*), whereas, their lifespan is maximized on high carbohydrate diets. The quality of food directly impacts reproductive success. Studies on the *Drosophila melanogaster* have shown that a protein-rich diet enhances its fecundity, while protein deficiency leads to reduced egg production (Lee *et al.,* 2008).

Drosophila flies have a dietary liking for the yeast content in the food. Yeast is a primary source of nutrition for *Drosophila* flies which provide essential nutrients such as amino acids, vitamins, and minerals, and influence their growth and development (Broderick & Lemaitre 2012). The quality and quantity of yeast in the diet of *Drosophila* directly affects their development rates and reproductive success (Anagnostou *et al.,* 2010). It has been observed that *Drosophila* fed on yeast-rich diets produce more offspring compared to those on yeast-deficient diets (Anagnostou *et al.,* 2010; Newell and Douglas 2014). *D. melanogaster* serves as an excellent model for studying the effects of diet on metabolism due to their genetic tractability and similarity to human metabolic pathways (Musselman *et al.,* 2011). The balance of proteins, carbohydrates, and fats in the diet is crucial for maintaining optimal health and longevity in *Drosophila*. Studies show that an imbalanced diet can lead to various metabolic disorders. While a high-protein diet may support reproduction and growth, it can negatively affect longevity and health. High sugar and fat diets tend to promote obesity, insulin resistance, and other metabolic issues (Musselman, *et al.,* 2011; Heinrichsen& Haddad 2012). *Drosophila* flies typically pupate at a specific height relative to the food source, and this height can be affected by various environmental cues such as humidity, food availability, temperature, and genetic constitution (Singh & Pandey, 1993).

Drosophila ananassae is a cosmopolitan and domestic species. Its occurrence in the Indian subcontinent is very prevalent (Singh *et al.,* 2014; Kumar & Singh, 2017). *D. ananassae* is known to be quite unique among hundreds of species of the genus *Drosophila* because it shows spontaneous crossing over in males which is usually absent in other species (Singh & Singh, 1988; Singh, 2010; Singh, 2024) and lack of genetic co-adaptation (Singh & Singh, 1987; Singh, 2010, Singh, 2024). In this study, we decided to see the impact of an elevated dose of protein on four different aspects, i.e., mating propensity, fecundity, pupation height, and biochemical parameters of *D. ananassae*. The altered food ecology may leave its noticeable effect on the reproductive as well as body homeostasis of flies and the results of this investigation unveil distinct consequences of elevated protein in the diet on the life of *D. ananassae.*

II. Materials and Methods

A) Isofemale lines of Drosophila ananassae

The isofemale line refers to the progeny of single impregnated female collected from nature. The experiments were conducted by utilizing five isofemale lines (Figure1) established from inseminated females collected from three geographical regions; 2 from Dehradun (Uttarakhand State), 2 from Delhi (Delhi State), and 1 from Varanasi (Uttar Pradesh State) of India. These five lines were fed the normal food ingredients (table1) commonly used to rear flies in the laboratory. Such lines have been considered as Control Isofemale Lines (CILs). From each CIL, five treated Isofemale Lines (TILs) were also rose which were fed on food containing a 10% higher dose of yeast extract, SRL (table1) than the control food. The increased amount of yeast in the treated group was calculated on the basis of the amount of water used in the food preparation (1000x10/100=100; 100+15=115gm). Therefore, the entire experiments were conducted by subjecting two groups of flies (control and treated) categorized as CILs and TILs.

Fig. 1: Five isofemale lines (each raised from a single naturally impregnated female) employed as control isofemale lines (CILs) and treated isofemale lines (TILs).

Table I. Food ingredients used to prepare one unit of food to culture CILs and TILs of *D. ananassae*.

Food ingredients	**Food for CILs**	**Experimental food for TILs**
Water	1000ml	1000ml
Agar agar	15gm	15gm
Maize powder	45gm	45gm
Yeast powder	15gm	115gm
Brown sugar	40gm	40gm
Nipagin	3.33gm	3.33gm
Propionic acid	3.33ml	3.33ml

B) Mating propensity

Virgin male and female flies were collected from the different lines of CIL and TIL and kept separately in their appropriate food mediums from each other for seven days. To observe mating in the individual line, 10 pairs of flies were introduced in the Elens-Wattiaux mating chamber (Figure2) in the morning hour, mainly commencing at 7 am. The mating was observed for the period of one hour and the number of pairs involved in mating was recorded. The experiments were repeated three times, for all the cases of CILs and TILs.

Fig. 2: Elens-Wattiaux mating chamber used to measure the number of mating/hour

C) Fecundity

Fecundity was observed in the mated females of CILs and TILs. This life history trait represents the number of eggs laid by an inseminated female in a definite period of its life. Seven days old virgin females and males were put individually in food vials to mate and produce the next generation. Thus the mated females of all the five CILs and their respective TILs were individually kept in the food vials containing 10ml of food and the number of eggs laid by them were counted daily for up to ten successive days (7-to-17-day age duration) of their life.

D) Pupation Height

For measuring the pupation heights, 100ml measuring cylinders containing food at their bottom (10ml) were used (Figure3). 7days old flies of CILs and TILs were cultured in the cylinders to get their next generation. Third instar larvae prefer to select a drier place and pupate at the inner walls of the cylinder. The height of the pupae from the food surface was noted down for all the five control and their respective treated lines.

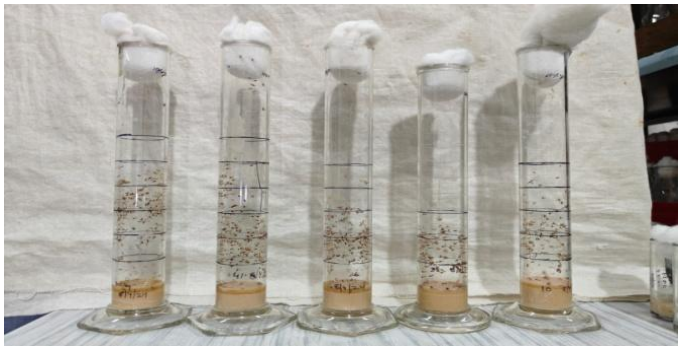

Fig. 3: Measuring cylinders (100ml) used to culture CILs and TILs to observing the pupation height.

E) Analysis of Biochemical Parameters (Metabolic Traits)

The three principal biochemical parameters, i.e., protein, glucose, and triglyceride were measured in CILs and TILs.

1) Estimation of Protein

Protein estimation was carried out in whole adult flies (n=5) using the Bradford method using bovine serum albumin as standard. Protein concentration (mg/dl/fly) was estimated in equal sex and sex-biased ratios for both the sexes using the standard curve of BSA (Bovine serum albumin). The estimation was done for three replicates of cohorts and repeated five times. The *D. anannase* flies were homogenized in 100µl PBS and centrifuged at 10,000 rpm at 4 ºC for 2 minutes. 10µl of supernatant was collected and mixed with 200 µl Bradford reagent and incubated at room temperature for 10 minutes in the dark. The absorbance for control and treated samples was taken at 595 nm.

2) Estimation of Glucose:

Quantification of glucose in whole adult flies of both the sexes was performed using glucose reagent, Beacon. Flies (n=5) were homogenized in 100 µl of cold PBS. The estimation was done for three replicates of cohorts and repeated five times and was done for equal sex and sex-biased groups for both the sexes of *D. ananassae*. Obtained supernatant after centrifugation at 12,000 rpm at 4 º C for 2 minutes was heated in the water bath for 10 min at 70 º C followed by another round of centrifugation. 200 µl of glucose reagent was added to 2 µl of supernatant in a 96-well plate and allowed to incubate at 37 º C for 10 min. The final reading was taken at 505 nm to measure the absorbance. Free glucose concentration (mg/dl/fly) was estimated in equal sex and sex-biased ratios for both the sexes using a glucose standard curve. The standard curve was obtained using glucose (1mg/ml).

3) Estimation of Triglyceride

Triglyceride estimation was done using a diagnostic kit, Beacon. Whole adult flies (n=5) were homogenized in 100 µl PBST (cold PBS + 0.05% Tween 20) and centrifuged at 10,000 rpm for 2 minutes. The estimation was done for three replicates of cohorts and repeated five times and was done for equal sex and sex-biased groups for both the sexes of *D. ananassae*. The supernatant obtained was heated in the water bath for 10 min at 70 ºC and to inactivate lipase and then used for triglyceride assay. 2µl of the homogenate was mixed with 200µl of triglyceride reagent and incubated for 10 min at 37 ºC. The final reading was taken at 490 nm using a microplate reader (BioTek ELx800 UV-Vis).

F) Statistical analysis and calculation:

Arithmetic mean ± SE were calculated to compute average mating, fecundity, pupation height, and biochemical parameters for each CIL and TILs. One-way ANOVA followed by post-hoc analysis (Tukey's multiple comparison) were performed to measure statistical differences among the different groups. The statistical significance level was decided at $p<0.05$. An unpaired t-test was also used to see a statistical difference between the different biochemical parameters of the two sexes. Narrow-sense heritability (h^2) was calculated by employing the formula, $h^2 = V_g/V_g+V_e$, where V_g+V_e depicted total phenotypic traits obtained by adding the variance value of different genetic lines with variance of lines fed on different food composition.

.

III. RESULTS

(a). Mating propensity: The mating propensity significantly varied among the control isofemale lines (CILs) as well as treated isofemale lines (TILs) with a more pronounced effect in CILs than their respective TILs. Figure4 depicts this aspect comparing the two groups (control and treated), where CIL 4 and 5 showed highly diminished frequency of mating than its other three lines. Increased dose of yeast-treated flies showed a significant difference compared to control in three lines, i.e., 1, 4, and 5. Based on the mating success data of the two groups, genetic variance (V_g) and variance due to diet (V_e) were computed to decipher narrow-sense heritability (h^2). The value of heritability remained 0.50 for four of the isofemale lines, a value that denotes 50 percent role of genetic variation that leads to variation in mating propensity. However, in one of the isofemale lines (3), only 25 percent of genetic variation causing trait differences could be observed.

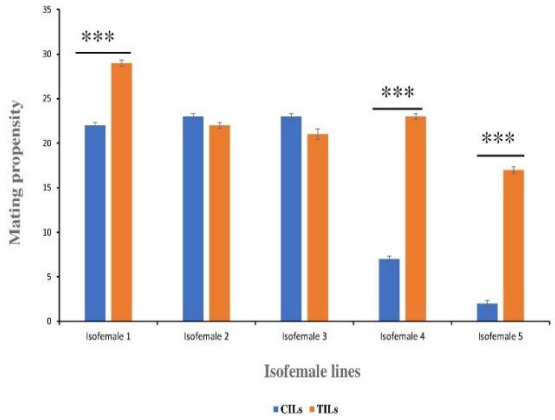

Fig. 4. Bar diagrams showing average number of mating propensity (mean ± SE) in CILs and TILs. ***p<0.001

(b). Fecundity: Fecundity in flies is one of the significant reproductive features that plays a role in the survival and sustainability of the species. This life history feature was also measured in all the five CILs and their respective TILs. Bar diagrams pertaining to this trait is presented in Figure5. The mean fecundity between CILs and their respective TILs were found to be statistically significant in all the five cases ($p<0.014$), however, when comparisons were made among the five CILs, no such distinct variation occurred. This indicates that all the genetically different lines lay a comparable number of eggs. Diet variation, particularly, due to an increase in protein dose leaves its significant impact on egg laying. To assess the trend of egg laying for the initial 10 days in CILs and TILs, the progressive average calculation was undertaken and the results fairly indicate an increasing trend of egg laying in all the lines, except that one of the CILs did not show a promising increasing trend (Figure6A & 6B). Increased dose of yeast causes a significant boost in the egg laying in their consecutive days of the treated lines. The role of genetic variation triggering this trait displayed less than 50% role of genetic variation causing fecundity variation for these lines (h^2 ranged between 0.29 to 0.48).

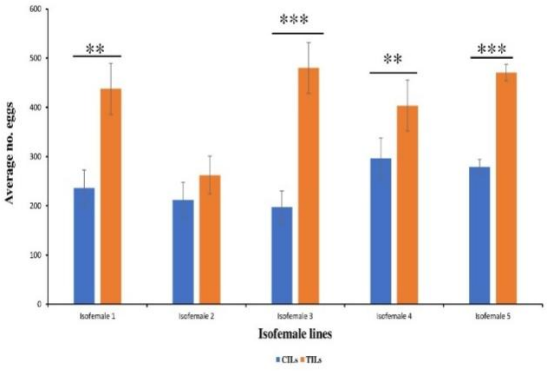

Fig.5. Bar diagram showing average number of eggs (mean ± SE) in CILs and TILs. **p<0.01; ***p<0.001

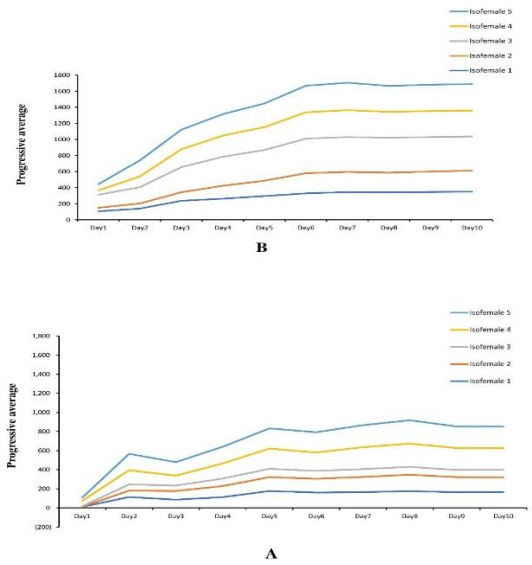

Fig. 6. Line diagram showing progressive average of fecundity in isofemale lines in CILs (A) & TILs (B).

(c). Pupation height: One of the pre-adult behaviors, i.e., pupation height variation in the five CILs and TILs showed interesting results. There occurred a significant difference in the pupation height among the control as well as treated groups ($p<0.00001$). Larvae reared on the normal food ingredients did not prefer to ascent high in the measuring cylinders and the majority of them even pupated on the food surface only. However, larvae reared on the increased dose of yeast moved upward and pupated significantly high in the culture cylinders (Figure7). The survival possibility of pupae does increase for the hatched flies eclosed in the drier area of the cylinder and therefore, high protein content leaves its impact on the pre-adult stage that indirectly enhances their survival possibility.

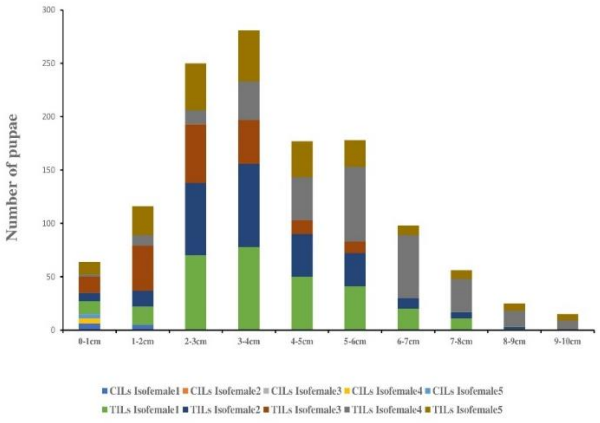

Fig. 7. Bar diagram showing distribution of pupae in CILs & TILs isofemale lines.

(d). Biochemical parameters: Three biochemical parameters were assayed in the two sexes separately in control and treated isofemale lines to envisage the impact of elevated protein on these parameters. In fact, it was hypothesized that an increased dose of protein (in TILs) may be responsible to boost protein along with their glucose and triglyceride content just because these biochemical molecules are inter-convertible through known pathways. The protein content measured in the two sexes of 5CILs and their TILs and their comparisons are presented in Figure8. Females in the control lines show a significantly higher content of protein than their respective males. The scenario was almost the same in treated lines where females possess higher protein content than their respective males (Figure8A & 8B). Comparison between control and treated females for their protein content clearly indicates the significant increase in the protein in all the five treated lines from their CILs. Similarly, between males of CILs and TILs, significant difference occurred, evidence that an extra amount of yeast causes an increase in the protein content of the two sexes.

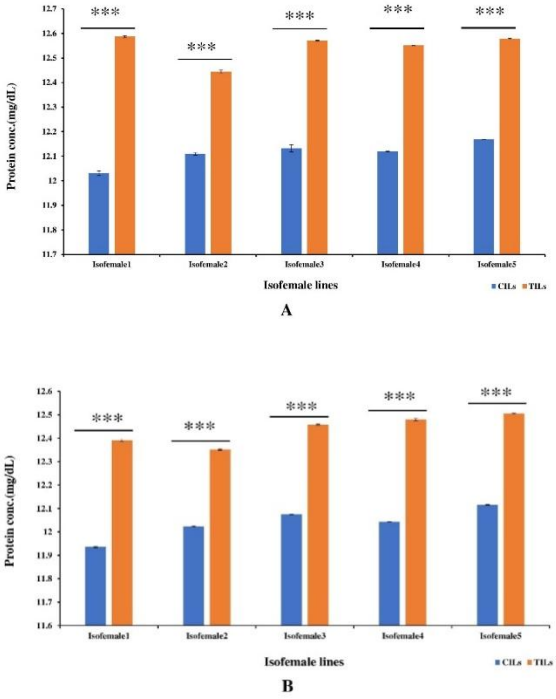

Fig. 8. Bar diagrams showing average protein concentration (mean ± SE) in CILs and TILs in males (A) & females (B). ***$p<0.001$

Glucose content was found to be always higher in females than males and among CILs, three lines showed highly significant difference between the two sexes. The average glucose content in CIL females ranged between 15.33 to 35.79 mg/dl and among males, it ranged between 11.64 to 14.47mg/dl. Further, the range of variation of glucose was evidently varying among females than males, which showed insignificant difference among the control lines (Figure9). Among treated lines also, females showed elevated glucose content than their respective males.

Here, in this group, all the five lines showed significant difference ($p<0.01$) between the two sexes, nevertheless, this difference was noted to be very high in TIL 3 and 5 (Figure9) than in other lines. A comparison between CILs and TILs females and males for their glucose content was also performed shown in Figure9A & 9B. The results of the female comparison indicate a significant increase in the glucose content of three lines (line 1, 2, and 4) of TIL whereas, similar comparisons done for males showed an increase in glucose content in four TILs from their respective control lines. Thus, increased yeast content as a source of protein does influence even glucose levels in *D. ananassae*.

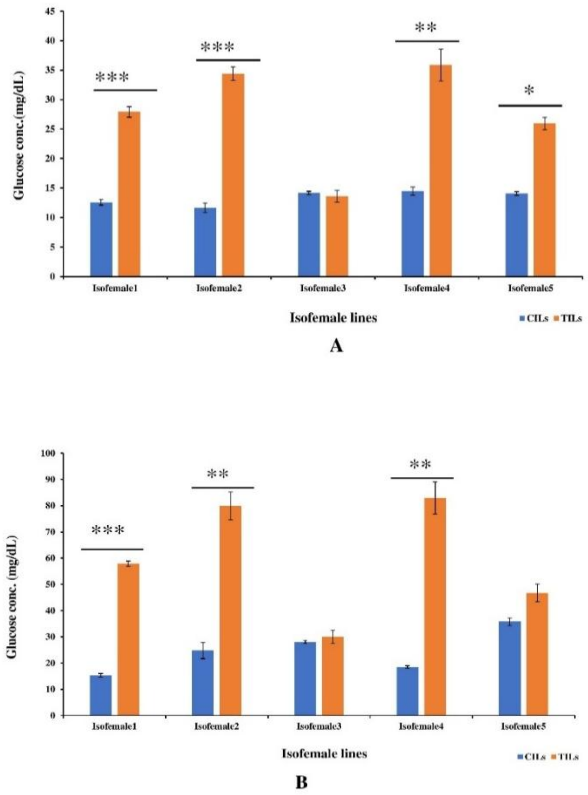

Fig. 9. Bar diagram showing average glucose concentration (mean ± SE) in CILs and TILs in males (A) & females (B). *p<0.05; **p<0.01; ***p<0.001

Average triglyceride content measured in the different lines of females of the two groups revealed a significant difference between CIL2, 3, and 5 and their respective TILs (Figure10 B), whereas, among males of the two groups, this variation could be observed in lines 2, 3 and 4 (Figure10 A). When males and females of CILs were compared, all the five lines showed significant difference in the triglyceride content with a uniform trend where females possessed higher content than their respective males. In some of the TIL males (e.g., 2, 3, and 4, figure10A) significantly high content of triglyceride was measured. However, no such excessive increase in triglyceride could be observed among males of CILs, except CIL5 which possessed its high content. A similar analysis performed with TILs females and males revealed that treated females possessed significantly higher triglyceride (except TIL3) than their respective males. The analysis also reveals that an extra dose of yeast boosts triglyceride content in males compared to the control males.

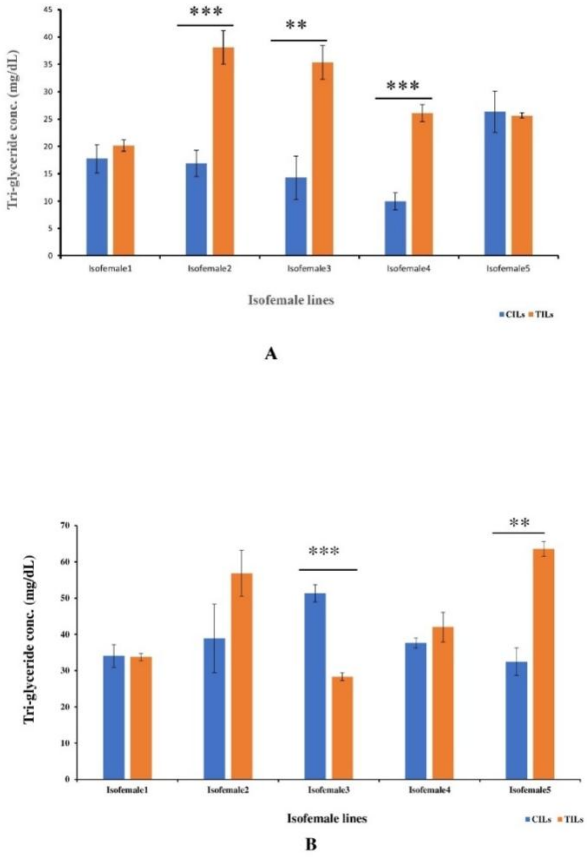

Fig. 10. Bar diagrams showing average tri-glyceride concentration (mean ± SE) in CILs and TILs in males (A) & females (B). **p<0.01; ***p<0.001

IV. DISCUSSION

The reproductive life of semelparous animals is prominently influenced by ecological factors especially the food availed during the development and adult survival as a whole. The increase in protein ingredients in the food may provide content towards the betterment of the reproductive ability. The results of this study clearly provide evidence that an increase in protein intake leaves its positive impact on the mating, egg laying, and pupation height, and a significant increase in all the three metabolites, i.e., glucose, triglycerides, and protein in itself in *D.*

ananassae. There may be a concern whether, the elevated protein in food can lead to some deleterious effect on the survival of the species and the answer for this could be, that insects like *Drosophila* flies, since the time of their maturity get actively involved in the reproduction and produce as many as they can and therefore, if extra protein in diet benefits their reproductive physiology, then it could be good for them. Mating propensity in the genus *Drosophila* is influenced by a number of genetic and environmental factors (Sisodia & Singh 2009; Krishnamoorti & Singh 2014). There are substantial reports that indicate the role of specific genes regulating sexual behavior in *Drosophila* (Demir & Dickson 2005; Rideout, *et al.,* 2010). Mutations in certain genes can alter mating propensity and several abiotic factors are responsible for influencing mating in different species of *Drosophila* (Singh & Singh 2014; David *et. al.,* 2005). Factors like temperature, light, population density, age, and nutritional status are the prime factors that affect mating success in this genus (Singh & Chatterjee 1987; Singh & Singh 2018).

Results of the present study clearly show that mating success increases in the flies of three treated lines compared to their respective control lines. Fecundity was found to significantly increase in the four lines, out of which, three had already shown increased mating propensity. Although fecundity in all the five treated lines was found to increase, however, line 2 did not depict a noteworthy difference from its control line. Progressive average calculation gives a very clear idea, whether a steady increase in the fecundity occurs with advancing age and, the results in this regard indicate that an increased dose of protein boosts egg production within ten days of the duration of observation. Even though, ageing would affect fecundity in the control as well as treated groups, the results of fecundity experiments give a clear sign that the initial phase of life in *Drosophila* becomes more productive for them particularly, when they are fed on a high dose of protein.

Pupation height in *Drosophila* is also known to be influenced by genetic as well as environmental factors (Zhang *et al.,* 2020; Bauer &Sokolowski 1985). Researchers have investigated the role of moisture and temperature on the selection of pupation sites which imparts the possibility of survival of the pupae (Du *et al.,* 2020; Rezende *et al.,* 2020). The variation in food composition triggering stimulus to pupate substantially away from the usual location has not been investigated so far. In this study, third instar larvae reared on high dose of protein moved significantly higher heights than those of control lines. Thus, there is a clear indication that food composition, particularly an increased dose of protein stimulates the third instar larvae to seek high heights for pupation, possibly it is a kind of adaptation to seek more drier areas for the enhanced chance of survival. Thus, the pupation behavior in *D. ananassae* is distinctly influenced by an increase in yeast content in the food medium. Treated larvae ascended significantly high in the upper strata of measuring cylinders used to rear flies and as a result, the scope of their survival gets increased than those flies who exit from the pupae settled at the food surface. The role of high protein may be as a triggering force for the change in pupation behavior that ultimately benefits the survival of the flies.

There are well-established routes in the biochemical pathways that help in the conversion of one bimolecular parameter into another to meet out energy demand in the living system (Judge & Dodd, 2020). Hence, it was decided to monitor the fate of increased protein content in genetically varied lines from the normal to treated conditions. The results clearly show that elevated content of food protein in the treated groups do increase the total protein content in them compared to the control lines. Besides this, an increase in the other two biochemical parameters (glucose and triglycerides) also takes place, which is an indication that the extra amount of protein finds its way to get modified into glucose and fatty acids. There might be biochemical pathways in insects including *Drosophila* where the increase in one major food constituent may lead to an increase in other body parameters to maintain biochemical homeostasis. Barragan-Fonseca *et al*., (2019) investigated the effects of dietary protein and carbohydrates on life-history traits and body protein and fat contents of the black soldier fly (*Hermetiaillucens*) and reported that heavier black soldier larvae had a higher crude fat content which they expected to be important for adult fitness because an increase in weight generally increases the fecundity of flies (Gobbi *et al.,* 2013). There are a few sporadic reports regarding the effect of diet on protein, glucose, and fat content in *D. melanogaster*. The protein content in *Drosophila* is influenced by dietary protein intake. High-protein diets increase overall protein content in the flies, affecting growth, reproduction, and longevity. Zanco *et al.,* (2021) explained that a high-protein diet can enhance growth and reproduction; it may reduce lifespan by compromising gut barrier function. High-sugar diets can lead to elevated glucose levels and are used to study diabetes-like conditions in flies (Musselman *et al.,* 2011). The fat content in *Drosophila* is significantly modulated by dietary fat and carbohydrate intake. High-fat diets increase lipid storage in flies, which is used to study obesity and related metabolic disorders (Heinrichsen & Haddad, 2012).

V. CONCLUSION

This study highlights the significant influence of dietary protein on key reproductive and developmental traits in *D. ananassae*. Flies reared on a high-protein diet demonstrated enhanced mating success, fecundity, and altered pupation behavior, likely contributing to better survival prospects. The increased biochemical reserves in protein-fed flies further suggest metabolic adaptations for meeting energy demands. These findings support the notion that high protein intake is

crucial for optimizing reproductive output in r-selected species like *Drosophila*.

ACKNOWLEDGMENT

AD and SU are thankful to University Grants Commission (UGC), New Delhi for financial support in the form of Senior Research Fellowship. Authors also wish to acknowledge the financial support from Institution of Eminence (IoE) grant, Banaras Hindu University.

AUTHOR'S CONTRIBUTION

Experiments were done by RB, AD, and SU. Statistical analyses were performed by AD, RB and AKS. Manuscript was written by AKS, AD, and SU.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Aguila, J.R., Hoshizaki, D.K., & Gibbs, A.G. (2013) Contribution of larval nutrition to adult reproduction in *Drosophila melanogaster*. *Journal of Experimental Biology*, 216: 399–406.

Alyokhin, A., Baker, M., Mota-Sanchez, D., Dively, G., & Grafius, E. (2013). Colorado potato beetle resistance to insecticides. *American Journal of Potato Research*, 90: 293-307.

Anagnostou, C., Dorsch, M., & Rohlfs, M. (2010). Influence of dietary yeasts on *Drosophila melanogaster* life-history traits. *EntomologiaExperimentalis et Applicata*, 136: 1-11.

Barragan-Fonseca, K. B., Gort, G., Dicke, M., & Van Loon, J. J. A. (2019). Effects of dietary protein and carbohydrate on life-history traits and body protein and fat contents of the black soldier fly *Hermetiaillucens*. *Physiological Entomology*, 44: 148–159.

Bauer, S. J., & Sokolowski, M. B. (1985). A genetic analysis of path length and pupation height in a natural population of *Drosophila melanogaster*. *Canadian Journal of Genetics and Cytology*, 27: 334–340.

Broderick, N. A., & Lemaitre, B. (2012). Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes*, 3: 307-321.

Canato, C. M., & Zucoloto, F. S. (1998). Feeding behavior of *Ceratitis capitata* (Diptera, Tephritidae): influence of carbohydrate ingestion. *Journal of Insect Physiology*, 44: 149–155.

David, J. R., Araripe, L. O., Chakir, M., Legout, H., Lemos, B., Pétavy, G., Rohmer, C., Joly, D., &Moreteau, B. (2005). Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. *Journal of Evolutionary Biology*, 18: 838–846.

Demir, E., & Dickson, B. J. (2005). Fruitless splicing specifies male courtship behavior in *Drosophila*. *Cell*,121: 785–794.

Du Plessis, H., Schlemmer, M., & Van Den Berg, J. (2020). The Effect of Temperature on the Development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Insects*, 11: 228-239.

Fuchs, S., Roussel, M., Cui, L., Moretti, R., & Briegel, H. (2014). Body size and reproductive traits of adult *Aedes aegypti* (Diptera: Culicidae) as affected by larval food quality and quantity. *European Journal of Entomology*, 111: 649-656.

Gobbi, P., Martínez-Sánchez, A. & Rojo, S. (2013) The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetiaillucens* (Diptera: Stratiomyidae). *EuropeanJournal of Entomology*, 110: 461–468.

Harrison, S. J., Raubenheimer, D., Simpson, S. J., Godin, J. J., & Bertram, S. M. (2014). Towards a synthesis of frameworks in nutritional ecology: interacting effects of protein, carbohydrate and phosphorus on field cricket fitness. *Proceedings of the Royal Society B Biological Sciences*, 281:20140539.

Heinrichsen, E. T., & Haddad, G. G. (2012). Role of high-fat diet in stress response in *Drosophila*. *Plos one*, 7: e42587.

Judge, A., & Dodd, M. S. (2020). Metabolism. *Essays in Biochemistry*, 64: 607–647.

Krishnamoorti, K., & Singh, A. K. (2014). Comparative behavioural analysis of mating between yellow and wild type *Drosophila of melanogaster* species group., *Journal of Scientific Research, Banaras Hindu University*, 58: 45–50.

Krittika, S., Lenka, A., & Yadav, P. (2019). Evidence of dietary protein restriction regulating pupation height, development time and lifespan in *Drosophila melanogaster*. *Biology Open*, 8: bio042952.

Kumar, S., & Singh, A. K., (2017) Population Genetics of *Drosophila*: Genetic variation and differentiationamong Indian natural populations of *Drosophila ananassae*. *Zoolical Studies*.56:1-10.

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., &Raubenheimer, D. (2008). Lifespan and reproduction in *Drosophila*: New insights from nutritional geometry. *Proceedings of the National Academy of Sciences*, 105: 2498-2503.

Musselman, L. P., Fink, J. L., Narzinski, K., Ramachandran, P. V., Hathiramani, S. S., Cagan, R. L., & Baranski, T. J. (2011). A high-sugar diet produces obesity and insulin resistance in wild-type *Drosophila*. *Disease Models & Mechanisms*, 4: 842–849.

Newell, P. D., & Douglas, A. E. (2014). Brewing Bugs: Yeast, Bacteria, and the Meaning of Symbiosis in a Cup of Coffee. *Microbial Ecology*, 67: 239-249.

Paoli, P. P., Donley, D., Stabler, D., Saseendranath, A., Nicolson, S. W., Simpson, S. J., & Wright, G. A. (2014). Nutritional balance of essential amino acids and carbohydrates of the adult worker honeybee depends on age. *Aging*, 6: 964-978.

Rezende, E. L., Bozinovic, F., Szilágyi, A., & Santos, M. (2020). Predicting temperature mortality and selection in natural *Drosophila* populations. *Science*, 369: 1242–1245.

Rideout, E. J., Dornan, A. J., Neville, M. C., Eadie, S., & Goodwin, S. F. (2010). Control of sexual differentiation and behavior by the doublesex gene in *Drosophila melanogaster*. *Nature Neuroscience*, 13: 458–466.

Schmidt, J. M., van Lenteren, J. C., Fransen, J. J., & Kanis, M. H. (1999). The effect of host diet on development and reproduction of Trichogrammapretiosum. *EntomologiaExperimentalis et Applicata*, 93: 215-223.

Singh, A., & Singh, B.N. (2014) Role of sexual selection in speciation in *Drosophila*. *Genetica*, 142: 23–41.

Singh, A. K., Kumar, S. & Ratnam,D. (2014) Genetic differentiation in natural populations and their mass culture stocks of *Drosophila ananassae*. *Genomics and Genetics*, 7: 123-132.

Singh, A. K. & Singh, B. N., (1988) Heterozygous inversions and spontaneous male crossing-over in *Drosophila ananassae*. *Genome*, 30: 445-450.

Singh, B. N., (2010) *Drosophila ananassae*: A good model species for genetical, behavioural and evolutionary studies. Indian Journal of Experimental Biology, 48: 333-345.

Singh, B.N. 2024. Hundred years of research on *Drosophila ananassae* has revealed a number of unusual genetic features of this species. Current Science 127: 14-16.

Singh, B. N. & Singh, A. K., (1987) The effects of heterozygous inversions on crossing-over in *Drosophila ananassae*. Genome, 29: 802-805.

Singh, B. N. & Chatterjee, S., (1987) Variation in mating propensity and fertility in isofemale strains of *Drosophila ananassae*. Genetica, 73: 237-242.

Singh, B. N. & Pandey, M. B., (1993) Evidence for additive polygenic control of pupation height in *Drosophila ananassae*. Hereditas, 119: 111-116.

Singh, R., & Singh, B. N., (2018). Influence of age on mating propensity in two sibling species, *Drosophila ananassae* and *D. pallidosa*. Indian Journal of Experimental Biology, 56: 7–13.

Sisodia, S. & Singh, B. N., (2009) Variations in morphological and life-history traits under extreme temperatures in *Drosophila ananassae*. Journal of Biosciences, 34: 263-274.

Zanco, B., Mirth, C. K., Sgrò, C. M., & Piper, M. D. (2021). A dietary sterol trade-off determines lifespan responses to dietary restriction in *Drosophila melanogaster* females. *eLife*, 10.

Zhang, W., Reeves, G. R., &Tautz, D. (2020). Identification of a genetic network for an ecologically relevant behavioural phenotype in *Drosophila melanogaster*. Molecular Ecology, 29: 502–518.

***