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Suitable culture media for *Drosophila Eugracilis*: Insights from fecundity and productivity

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Abstract
The present study was conducted to identify a suitable culture medium for the laboratory culture of *Drosophila eugracilis*. The study evaluated the effectiveness of three different culture media—wheat flour agar, corn flour agar, and banana agar—for the laboratory culture of *D. eugracilis* by analysing fecundity and productivity. Wild flies were collected from Karnatak University Campus, Dharwad, and cultured under laboratory conditions. The results showed that wheat flour agar medium recorded significantly higher numbers of eggs, pupae, and adult flies compared to corn flour agar and banana agar media. Although banana agar medium contained higher carbohydrate content and protein-to-carbohydrate ratio, it showed lower productivity and greater pupal mortality, likely due to excess sugar content and rapid fungal contamination. No significant difference was observed in developmental duration among the three media. Overall, wheat flour agar medium was found to be the most suitable medium for the successful culture and maintenance of *D. eugracilis* under laboratory conditions. The study provides preliminary information for establishing stable laboratory cultures of this species for future biological and genetic studies.

Keywords: *Drosophila eugracilis*, culture media, wheat flour agar, fecundity, productivity

Introduction

Drosophila is commonly known as the fruit fly. It is the most extensively used model organism in research because it is easy to culture, cheaper to maintain, high fecundity rate, short lifespan and adaptation time (Ranganath, 1999; Mitrovski and Hoffman, 2001; Markow and O'Grady, 2006; Kenney and Borisy, 2009) [6, 12, 13, 17]. The life cycle of fruit fly has four main stages: egg, larva, pupa, and adult, and duration of its development depend largely on different culturing conditions (Wayne *et al*., 2006; Parvathi *et al*., 2009; Kumar, 2014) [7, 15, 25]. A major success in using *Drosophila* as a research model is mainly because of the culture media, which is cheaper and easy to prepare in any research laboratory. About 300 species of *Drosophila* can be cultured successfully in the laboratory. Many culture media consisting of cereals like corn flour, oat, wheat, etc. are used as the most common ingredients for the solidification and nutritive supplements for the fruit fly culture media (Spencer, 1943; Flagg, 1998; Yee T. S., 2010 [3, 21, 24]). Widdowson and McCance (1935), and Keller (2007) [5, 26] have revealed that fruits consumed by *Drosophila* species are rich in glucose, sucrose and other carbohydrates; but they are generally not rich in proteins. The most common species cultured in the research laboratories is *Drosophila melanogaster* Meigen. The most common media used to culture these flies is standard corn meal agar medium. *D. melanogaster* can be cultured in different types of media, such as, wheat cream agar media (Shivanna *et al*., 1996) [20] and banana opuntia media (Markow and O'Grady, 2006) [12]. Studies have shown that different types of media comprise different nutritional value and sugar contents that can affect the rate of development of fruit flies. Several varieties of yeast and additives including fruits, sugar, flours, and molasses were used for the standardization of the *Drosophila* culture media (Robertson and Sang, 1944; Jaenike, 1986) [4, 18].

Drosophila eugracilis Bock and Wheeler is a member of genus *Drosophila* belonging to subgenus *Sophophora* and *melanogaster* species group, and *eugracilis* subgroup. Morphologically, *D. eugracilis* females possess larger body size and have swollen abdomen than males, posterior end of female is pointed due to the presence of their ovipositor and female genital organ; whereas males possess small hooked bristles on the mid-tibiae and metatarsi region on their first tarsal region (two teeth sex comb). This species is a native of the Indian subcontinent, South East Asia, and Australia. All these regions are having a humid tropical and subtropical climate (Bock and Wheeler, 1972) [1]. The presence of this species in Dharwad and surrounding areas is well established (Srinath and Shivanna, 2014; Srinath *et al*., 2023) [22, 23].

According to Markow and O' Grady (2006) [12], *D. eugracilis* can be bred on corn meal agar media. The lifespan of *D. eugracilis* is reported between 36 days for male and 53 in females (Yoon *et al*., 1990) [27]. Since *D. eugracilis* is not a popular research model, culturing techniques of this species in the laboratory is not well understood. This species is difficult to culture using standard laboratory wheat cream agar media, which is commonly used to culture *D. melanogaster*. At present, there are no reports of successful cultures of *D. eugracilis* in the laboratory. Hence, an attempt is made to identify suitable culture media using easily available ingredients which can result in adequate development of the species. The present study will analyse corn flour agar media, banana agar media and wheat flour agar media and determine which of the above-mentioned media is more suitable for the culture of *D. eugracilis* in terms of fecundity, productivity, and viability.

Materials and Methods

Collection of Flies

D. eugracilis flies were collected from Karnatak University Campus, Dharwad by using net sweeping method. Generally, females possess larger body size and have swollen abdomen than males, posterior end of female is pointed due to the presence of their ovipositor and female genital organ; whereas males possess sex comb on their first tarsal region. Based on these morphological characters, collected flies were etherized using diethyl ether, were separated based on their sex. Males were directly identified to their respective species whereas; female flies were transferred individually into each culture vial and plugged with cotton and were cultured in laboratory using different culture media at 20-25^{0}C with relative humidity of 70-80%. The culture of *D. eugracilis* was maintained from November to April month.

Identification of *D. eugracilis*

After 10-11 days, *D. eugracilis* flies were identified based on the males emerging from F1 culture vials. The male genital organs are species-specific and differ from species to species. The male flies were identified based on the sex comb pattern and genital plate (Bock and Wheeler, 1972; Markow and O' Grady, 2006) [1, 12]. The genital plates and sex combs were boiled in 10% KOH, approximately for 15-20 minutes and were dissected under stereomicroscope, and later mounted using DPX (Figure 1).

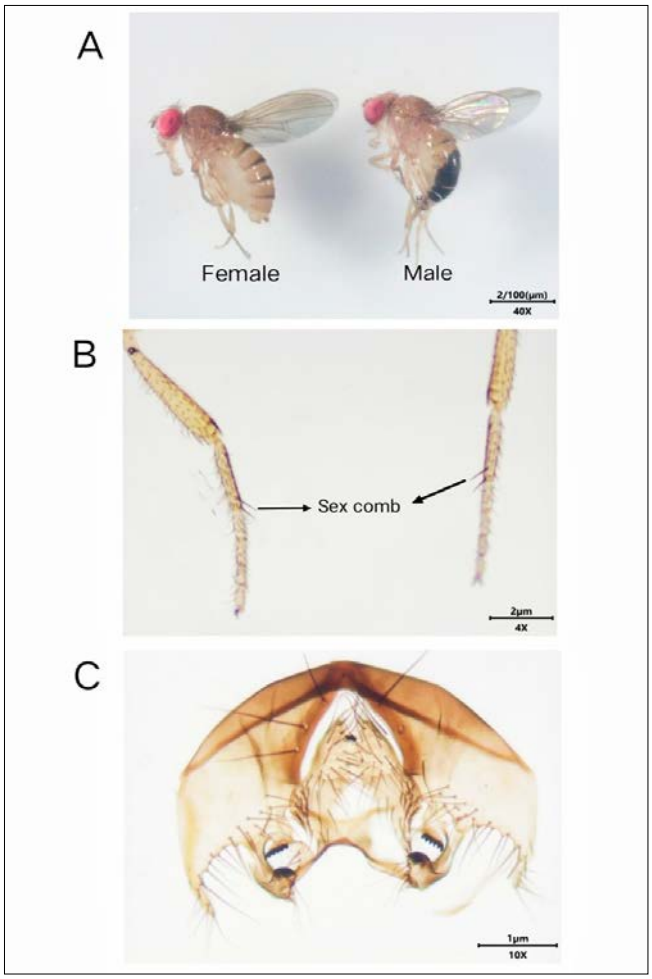


Fig 1: A. Morphology of male and female flies, B. Fore leg of male showing sex comb, C. Genital plate

Different Culture Media to Culture *D. eugracilis*

Wheat Flour Agar Media:

Wheat flour agar medium was prepared as per the procedure described by Mohapatra and Pandey (2018) [14]. For 500 ml media, 250 ml of distilled water was boiled in a vessel and 50 grams of wheat flour was mixed with another 250 ml of distilled water. 50 grams of jaggery was dissolved in boiling water. Then mixture (wheat flour and water) was added and boiled thoroughly with constant stirring. 3 grams of agar agar and 3 ml of propionic acid was added and boiled for 10-15 minutes. The hot media was poured into the sterilized cultured bottles, after cooling the water vapour on the side of culture bottles was blotted off with the help of blotting paper/tissue paper. A yeast solution (2-3 drops) was added into the bottles and it was tightly plugged with sterilized cotton.

Banana Agar Media:

Banana agar medium was prepared as per the procedure described by Pearl (1926) [16]. 500 ml of distilled water was boiled in a vessel. Then 5 grams of agar agar was added and allowed to boil for 5 minutes. Then bananas were mashed and added to boiling water (ripened bananas). 5 ml of propionic acid was added then allowed to boil for 3 minutes. The hot media was poured into the sterilized cultured bottles, after cooling the water vapours on the side of culture bottles was blotted off with the help of blotting paper/tissue paper. Yeast solution (2-3 drops) was added into the bottles and it was tightly plugged with sterilized cotton.

Corn Flour Agar Media

Corn flour agar medium was prepared as per the procedure described by Lakovaara (1969) [8]. 500 ml of distilled water was boiled in a vessel, and 22 grams of glucose was dissolved in it. 36 grams of maize flour with 10 grams of sugar was added and boiled thoroughly with constant stirring. 8 grams of yeast and 3 ml of orthophosphoric acid were added, allowed to boil for 5-10 minutes. Then 10 grams of agar agar and 2 ml of propionic acid were added, allowed to boil for 5 minutes. The hot media was poured into the sterilized cultured bottles, after cooling the water vapours on the side of culture bottles was blotted off with the help of blotting paper/tissue paper. Yeast solution (2-3 drops) was added into the bottles and it was tightly plugged with sterilized cotton.

Experimental Procedure

Fecundity and Productivity

In order to analyse the best media for culture of *D. eugracilis*, fecundity (number of eggs laid) and productivity (number of flies emerged) were studied separately using the experimental culture media i.e., wheat flour agar media, corn flour agar media, and banana agar media (Figure 2). Virgin male and female flies were transferred into 15 culture vials which were plugged immediately with sterilized cotton in each culture media. For the ease of analysis of data, only 3 vials with virgin male and female were considered and the data was recorded. These vials were subsequently transferred into fresh vials for 15 days (equivalent to 15 vials). The number of eggs, pupa and flies were counted in respective culture media. The parental flies from each media vials were discarded on 16th day.

Fig 2: Different culture media bottles. A. Corn flour agar media B. Banana agar media C. Wheat flour agar media

Statistical Analysis

In order to check the distribution of the data, it was subjected to normality test (Shapiro-Wilk test of normality). The data showed normal distribution for number of eggs (p 4.191≥0.05), number of pupa (p 2.340≥0.05), number of flies (p 3.350≥0.05) and pupal mortality (p 1.117≥0.05). The data showed normal distribution for developmental time from egg to pupa (p 1.690≥0.05) and pupa to flies (p 5.833≥0.05). To study the effect of different media on fecundity and productivity, a one-way ANOVA test was conducted to compare the means of fecundity and productivity between different media for any significant difference. To test the difference between the different media within a single vial, a non-parametric Kruskal-Wallis's test was conducted. The statistical analysis was done using SPSS software version 21.0.

Results

Table 1: Mean ± SE of fecundity and productivity in different culture media of *D. eugracilis.*

Sl.no	Media	Eggs	Pupa	Flies
1	Wheat	31.977 ± 2.886^{A}	28.044 ± 2.582^{A}	25.911 ± 2.379^{A}
2	Corn	13.488 ± 2.611^{B}	8.711 ± 1.802^{B}	7.022 ± 1.460^{B}
3	Banana	15.00 ± 2.232^{B}	8.888 ± 1.549^{B}	6.088 ± 1.186^{B}

^{A} indicates significant difference at $p\leq0.05$, ^{B} indicates no significant difference.

Table 1 shows the mean of fecundity and productivity of *D. eugracilis* cultured in different culture media. It revealed that the number of eggs (fecundity), pupa and number of flies (productivity) on an average were more in wheat flour media. Among the banana agar and corn flour media, banana media recorded more egg laying and pupa, whereas corn flour recorded a greater number of emerging flies. One-way ANOVA revealed an overall significant ($p\leq0.05$) difference between fecundity, pupa and number of flies among different culture media. Post-hoc tests in particular reported that there is a significant difference for number of eggs, pupa and flies between wheat flour agar media and corn flour agar media and banana agar media. But no significant difference was found between corn flour agar and banana agar media for any of the above-mentioned variables.

Table 2: Mean ± SE of developmental time (in days) in different culture media of *D. eugracilis*.

Sl. no	Media	Egg to pupa	Pupa to flies	Pupal mortality
1	Wheat	4.48 ± 0.089^{A}	5.00 ± 0.123^{A}	2.133 ± 0.340^{A}
2	Corn	4.29 ± 0.081^{A}	4.85 ± 0.139^{A}	1.688 ± 0.374^{A}
3	Banana	4.19 ± 0.059^{A}	5.21 ± 0.109^{A}	2.8 ± 0.435^{A}

^{A} indicates significant difference at $p\leq0.05$, ^{B} indicates no significant difference.

The mean of developmental time of *D. eugracilis* in different culture media reported that the developmental time from egg to pupa and pupa to flies in all media requires 9-10 days. One-way ANOVA revealed no significant ($p\leq0.05$) difference for the developmental time among all the three-culture media. The maximum pupal mortality was observed in banana agar media followed by wheat flour agar media and least in corn flour agar media. One-way ANOVA showed no significant difference for any of the variables among all the culture media (Table 2).

Table 3: Chemical composition of different culture media (500 ml)

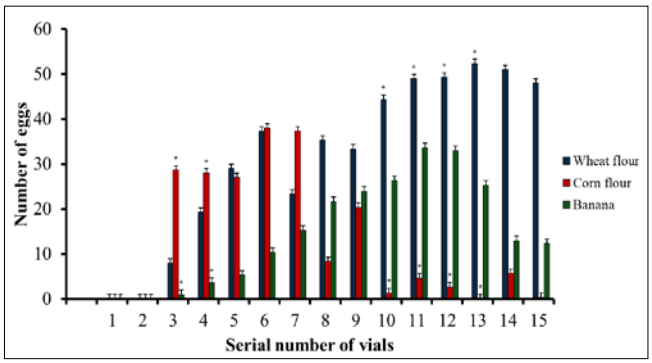
Sl. no	Media	Total carbohydrate content (gm)	Dietary fiber (gm)	Fat (gm)	Protein (gm)	Minerals (gm)	Ratio (P:C)	Calories (kcal)
1	Wheat	91	10.5	1.3	6.6	325.8	1:13	358
2	Corn	70.2	14.5	1.8	7.3	380.8	1:10	289
3	Banana	120	19	1.7	5.5	1816.3	1:24	445

Data obtained from United States Department of Agriculture (USDA) and Nutritional facts.

Table 3 comprises the chemical composition of different culture media (500 ml). Banana agar media consist of highest quantity of carbohydrate, dietary fibre, minerals and calories compared to wheat flour agar and corn flour agar media, whereas corn flour agar media comprises highest quantity of protein and fat among different culture media. The above information was obtained from the source "Nutritional facts" and United States Department of Agriculture (USDA). The protein-to-carbohydrate (P:C) ratio value was maximum in banana agar media followed by wheat flour agar media and minimum in corn flour agar media.

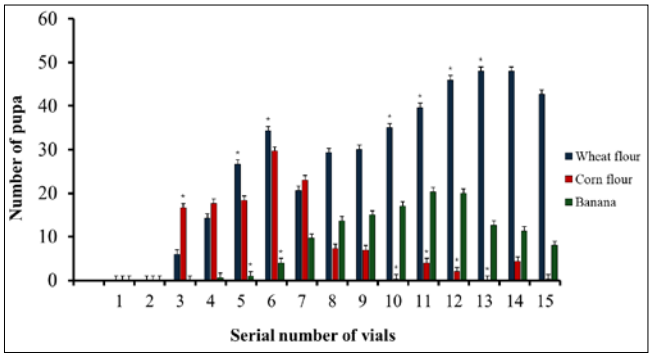
Figure 3 represents the number of eggs laid by *D. eugracilis* for 15 days in 15 vials in different culture media. Maximum number of eggs was laid on day 13 in wheat flour agar media, day 6 in corn flour agar media and day 11 in banana

agar media. The number of pupae of *D. eugracilis* for 15 days in different culture media was also observed. It revealed maximum number of pupae on day 13 and 14 in wheat flour agar media, day 6 in corn flour agar media and day 11 in banana agar media, respectively (Figure 4). Figure 5 represents the number of flies emerged of *D. eugracilis* for 15 days in different culture media. Maximum number of flies emerged were observed on day 14 in wheat flour agar media, day 6 in corn flour agar media and day 11 and 12 in banana agar media. Non-parametric Kruskal-Wallis test revealed that there is significant difference between the following media with respect to different vials (number of eggs-corn agar vs. banana for vial 3 and 4, wheat vs. corn for vials 10-13), (number of pupa: banana vs. corn for vial 3, wheat vs. banana for vials 5 and 6, wheat vs. corn for vials 10-13), (number of flies: banana vs. corn for vial 3, wheat vs. banana for vials 5 and 6, corn vs. wheat for vials 10-13 and 15).



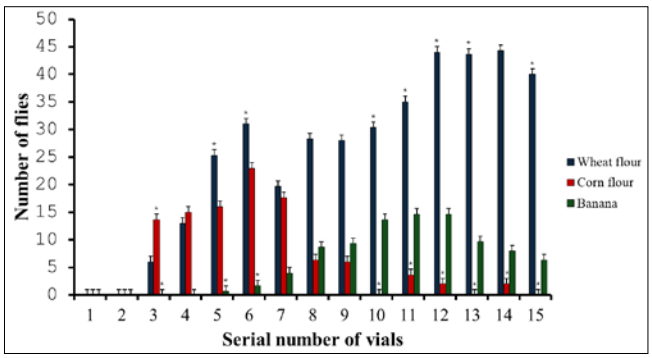
*Significant at $p \leq 0.05$

Fig 3: Number of eggs laid in different culture media of *D. eugracilis*



*Significant at $p \leq 0.05$

Fig 4: Number of pupae in different culture media of *D. eugracilis*



*Significant at $p \leq 0.05$

Fig 5: Number of flies emerged in different culture media of *D. eugracilis*

Discussion

The pursuit to identify a suitable media for *D. eugracilis* is challenging. In the present study, *D. eugracilis* species can be easily cultured in wheat flour agar media compared to corn flour agar and banana agar media. Fecundity, number of pupa and productivity were significantly more compared to other media. Mohapatra and Pandey (2018) [14] studied the effect of different solid culture media on *D. melanogaster*. They reported that *D. melanogaster* can be easily cultured in wheat meal solid media. Similarly, in the present study *D. eugracilis* could be easily propagated in wheat flour agar media.

According to Lee *et al.* (2008) [10], P:C ratio also plays a vital role in determining the effectiveness of media. In the present study, among the selected media, banana agar medium has highest P:C ratio. But the overall fecundity and productivity is more for wheat flour agar media (Table 1), which indicates that P:C ratio is not the only factor responsible for *Drosophila* culture. Even though proteins and carbohydrates are required as main energy source for growth and development, their requirement is determined by the feeding choice of the flies (Rodrigues *et al*., 2015) [19]. The present study favours wheat flour agar media which has moderate levels of P:C ratio, where carbohydrate and protein values are between the values of corn flour and banana agar media. This also reveals that even though the corn media with all its artificial ingredients was considered as a promising media, but the overall fecundity and productivity was lower. Banana agar media tends to disintegrate quickly and formation of fungal moulds was more. Lushchak *et al.* (2014) [11] reported that the media containing high carbohydrate content, low protein content may lower metamorphosis of pupa. Since banana is a natural source of high sugar and low protein, it could influence the development of flies. Hence, reasonably, pupal and fly mortality was more in this media. When considering the desiccation factor, banana agar media was less influenced compared to wheat and corn flour media. According to Catchpoole (2005) [2], the desiccation stress is one of the major reasons for the mortality of fruit flies, reduced reproductive ability and reduced metamorphosis of the hatched larva in the culture media. Adding live yeast to has enhanced the culture of *D. eugracilis* in all the media. Live yeast is a source of proteins and free amino-acid, polyols and sugars: adding live yeast to a food media containing killed yeast increases body mass of females and their protein or fat contents (Le Bourg *et al*., 2015) [9].

From a temporal perspective, there was no statistical significance between the duration from egg to pupa and pupa to flies. This observation provides an insight that the media has no effect on developmental time of *D. eugracilis* (Table 2). The only difference that can be discussed in this context is that in case of wheat flour agar media, 39/45 vials recorded for egg to pupa, whereas in case of corn flour agar media it was 24/45 vials and for banana it was 26/45 vials. Similarly, for pupa to flies it was 39/39 vials for wheat flour agar media, 20/24 and 23/26 vials for banana agar media. This indicates that the wheat flour agar media had maximum vials for fecundity (86%) and corn flour agar had minimum percentage (53.3%). Similarly, 100% viability was observed for corn flour agar media and minimum (83.8%) for corn

flour agar media. From this perspective, we can infer that wheat flour agar media is better than the other two media. The effect of all the three media on pupal mortality is not significant. Hence, pupal mortality is not influenced by different media.

Another interesting pattern of egg laying capacity was observed between corn flour agar and banana agar media (Figures 3-5). The corn flour agar media initially showed more conducive environment for fecundity, number of pupa and flies from vial 3 to vial 7, whereas there was a rise in fecundity, number of pupa and flies for banana agar media from vial 8 to vial 15. This reveals that corn flour agar supports the more egg laying process initially compared to banana agar media. The wheat flour agar media appears to be robust because throughout the experimental duration of 15 vials/days, significant number of eggs, pupa and flies were found from day 3-15. Mohapatra and Pandey (2018) [14] studied fecundity and productivity in different culture media (wheat meal solid, cornmeal and barley media), they reported that wheat meal solid culture media is more preferable media than cornmeal and barley media. Similarly, from these experimental observations and recordings, the usage of wheat flour agar media for the culture of *D. eugracilis* species is more effective than the other two culture media.

Conclusion

On the basis of experimental evidences, the number of eggs, pupa and flies observed in wheat flour agar media was significantly higher than the results for all other culture media. Hence, it can be inferred that the wheat flour agar media is the most preferable culture media for breeding *D. eugracilis*.

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