Further evidences of cannibalism and partial carnivorism in *Drosophila* species larvae.

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In our earlier communiqué (Bhattacharyya, 2014), evidences had been provided in the favor of cannibalism and 'partial carnivorism' in *D. melanogaster* (Oregon R) larvae and other *Drosophila* species. As a student of 2nd year M.S., an attempt was taken to answer more questions before we could conclude on any proper reason for cannibalism in this group of Dipterans being known for decades as the "fruit flies". Whether it is the resultant of culturing them on an artificial diet and environment for several decades? Or whether such attribute is genetically pre-disposed and has remained to be conserved in the process of evolution?

Materials and Method

All the wild type *Drosophila* stocks and different mutant strains of *D. melanogaster* were maintained, unless otherwise mentioned, in standard (maize-yeast-agar-jaggery) culture medium at 22+1°C temperature. The wild type species used in the study were *D. melanogaster*, *D. virilis*, and local species collected from three distinctly different demographical regions. While the first one was collected from the Dooars at the Himalayan foothills (Ethelbari Tea Estate; Jalpaiguri), the second one was collected from the north-eastern fringe of this metropolis (Ultadanga, Kolkata), and the third one, from the Gangetic delta region, the Sunderbans (Canning, South 24 Parganas).

Live specimens other than *Drosophila* were either collected personally or purchased, as were the live yeast and the food colors. Each experiment was conducted at least in five vials and repeated for at least three times. Observations were made under a Magnus stereoscopic binocular (model -MS24) using 2× and 4× objectives. Photographs, both still and video, were captured initially by a cell phone (Lumia520), and later by digital cameras (Canon A1100s or Powershot A800 and Sony).

Experiments

Previous reporters, (Vijendravarma *et al*., 2013) have emphasized on the artificial culture media with lower content of protein to be the driving cause of cannibalism in this group of Dipterans. Hence, to ascertain, we had to answer the question: Does the depleted quantum of protein in the artificial culture media compel the growing larvae to practice cannibalism and/or carnivorism in order to compensate the deficient nutrition?

To answer this question, three different culture media were prepared by just varying the amount of yeast in the media, (i) a normal media with the prescribed amount of yeast, (ii) media with doubled amount of yeast, and (iii) media completely devoid of yeast.

Yeast being the major source of protein in the culture media- more cannibalism was expected to occur in the media without yeast if it had been due to compensation for deficiency of proteins in the culture media. However, the observations that followed were quite contradictory to this- where cannibalism remained unaltered in all the three, in fact somewhat increased in the vials containing doubled amount of yeast but remained at a basal level only in the vials with culture media devoid of any yeast- that was comparable to (if not exactly) to the control vials (although the development rate in these vials were slower compared to the other two). This observation further supported our previous experiments reported in our previous communiqué enabling us to reach the most possible conclusion that neither supplementation *of excess proteins to an otherwise normal culture medium, nor the relative number of larvae (intraspecific competition) can prevent larval cannibalism.*

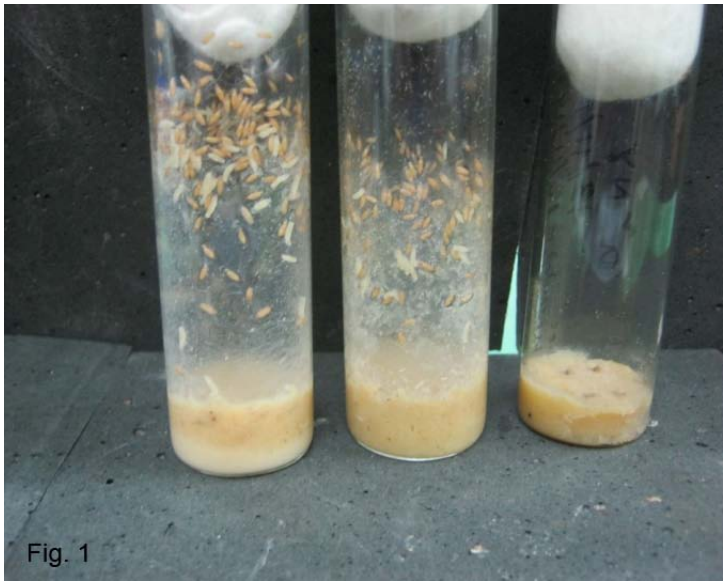

Figure 1. *D. melanogaster* larvae cannibalising on adult bodies irrespective of the gender in a culture vial that had been supplemented with three etherized white ebony males and three wild type females, belonging to the same species.

However, mention must be made that there is a direct correlation between the amount of yeast supplemented in the culture media with the size and developmental rate of the larvae (Figure 1). Especially intriguing were the larvae growing in the yeast deprived media- where the larvae were arrested in a particular developmental stage and did not undergo moulting into the next stage. However, the addition of exogenous protein into these protein-deprived culture media helps compensate in the delayed/retarded developmental rate. The larvae developing into the adults in the yeast deprived vials, were exclusively cannibals- as the only possible source of the protein metabolised in the process of metamorphosis had to come from the conspecific larvae only, as no other protein source was present in the rearing culture medium. However, an intriguing observation that needs mention about these flies is that the adults emerging from these strictly cannibalistic diet culture media showed an altered sex ratio (from the general 1:1 to a 2:1 ratio for male: female).

The above experiment however, clearly ruled out any direct correlation with the incidences of cannibalism to the quantum of protein (yeast) in the culture media. Nonetheless, the affinity towards an exogenous protein source was also clearly demonstrated. Hence these observations put forth yet another question- as to what is the cue -rather the driving force to this cannibalistic behaviour of these otherwise non-carnivorous flies? What type of cue attracts the larvae to an exogenous protein source, even in a culture media with high protein content?

In our previous communiqué, we provided evidence in which the larvae fed indifferently on flies from the same lineage as well as other members from the same species. Furthermore, the cannibalistic behavior was not only restricted to members of the same genus, but also to the evolutionarily distant species when supplemented to culture media ("partial carnivorism") –which allowed us to logically conclude that even if there is any chemical attractant that acts as the cue, it is not specific for either the species or the genus. Hence the next most potent candidate for being an attractant could be specific to a particular sex of the victim.

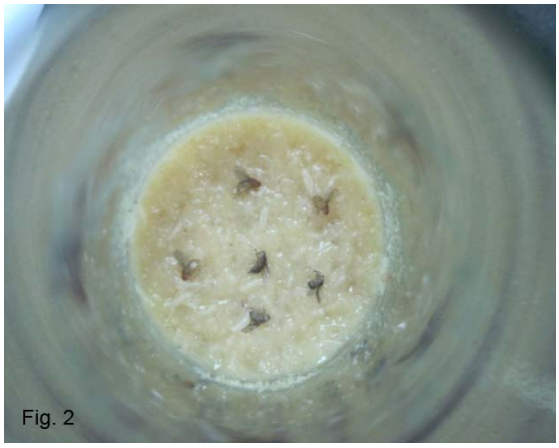

Figure 2. Different rates of development in double-yeast medium, normal yeast medium, and yeast deprived medium (from left to right) cultured at the same time.

To find out the validity of such cues in serving as an attractant for cannibalistic larvae towards a potent victim, an experiment was designed in such a manner, such that it was possible to identify the gender of the victimised adult bodies upon which the larvae fed. In fresh cultures containing exclusively larvae in the growing stages, etherized white ebony males (three) and wild type females (three) were

supplemented.

No gender biasness was noticed with respect to larval feeding upon the dead adults (Figure 2). Similar observations were recorded in reciprocal event where white ebony females and wild type males were taken in same numbers.

Thus with such diverse victims of the feeding larvae, it was necessary to see is there any preference towards a particular exogenous protein- specifically to a cannibalistic source, when evidences were available on the preference towards a cannibalised conspecific even when the quantum of protein available in the immediate environment was sufficing? Moreover, is there any relation with cannibalism (and/or carnivorism) with the rate of development?

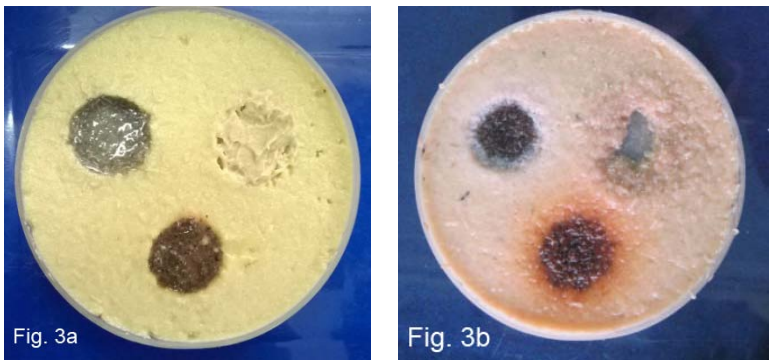

Figure 3. (a) The Three Way Assay (clockwise from bottom: killed adults, injured larvae, and live yeast containing wells). (b) The same after the first day of feeding by the developing first instar larvae in the petridish.

Two separate experiments were conducted to answer the addressed questions. In the first experiment, (*three way assay system*), three wells were produced within the petridish (Figure 3a) which was filled with normal culture medium in which flies were cultured for few days before discarding them. After the emergence of the first instar larvae, three different protein sources were added in the prepared wells- live yeast, freshly killed larvae, and freshly killed adults. The most significant observation from this experiment was that the first instar larvae were initially found to be attracted towards the well containing live yeast (Figure 3b). However, a gradual shift in preference from live yeast towards live protein source, better to say, a cannibalistic source of protein supplement was observed with each successive moult.

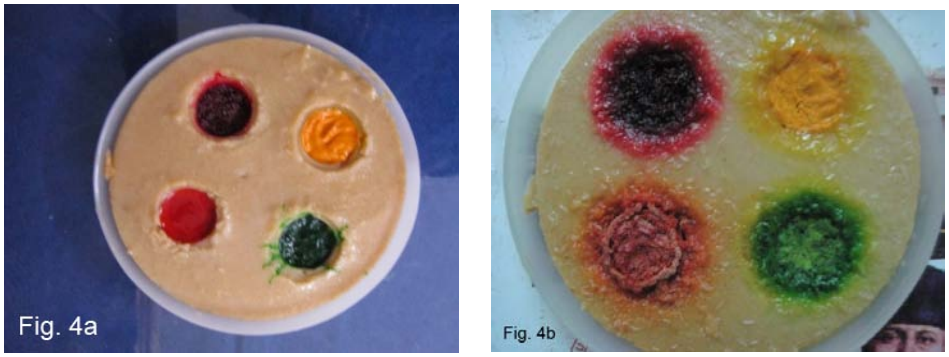

Figure 4. (a) Four Way Assay wells with the supplemented proteins: clockwise from bottom-left: live yeast (orange), freshly killed adults (crimson), amino acids in the form of *Amino-Fit* (yellow) and injured larvae (green)- added after the appearance of first instar larvae in the surrounding protein deprived media. (b) The same after two days of larval feeding- showing a shift in the affinity to the well containing killed larvae.

For a better resolution of this observed picture of cannibalism, a second experiment was designed based on the same logic, but was slightly modified. In this set of experiment (four way assay system), four wells were prepared within the petridish which was filled with a protein deprived, (*i.e*., completely yeast free) culture medium. After the emergence of the first instar larvae, the four wells were filled with different exogenous source of protein (nearly similar to the previous experiment) along with a synthetic amino acid source (*Amino-Fit*). These different sources of protein were mixed with different food colors (Figures 4-a to

b) in order to determine which particular protein source was preferred by the growing larvae, simply by examining the color of the gut from the otherwise colorless translucent exterior (Figure 5-a to c). Indeed, as expected, it was found that the first and second instar larvae mostly selected live yeast as their most preferred protein source, followed by a shift in the preference to the killed larvae in subsequent stages. Dead adults were found to be mainly preferred by the later instars. However, though in separate experiments- *Amino-Fit*, when added to otherwise protein deprived vials helped resume development in the arrested larvae, the same when added in the wells did not attract a greater number of wandering larvae to it. This observation further helped to emphasize on the specific chemical "cue" offered by the protein from living sources that attracts wandering larvae (arrested in a particular moulting stage). Hence the artificial amino acids in the form of *Amino-Fit*, being devoid of any cue- did not attract.

Figure 5. (a) Developing larva feeding on yeast which is clear from the food color added (orange- to the yeast in the well) being visible from the translucent exterior of the larva. (b) Developing larva feeding on killed conspecific larvae- which is clear from the food color (green- added to the well)- being visible from the translucent exterior of the larva. (c) Matured larva feeding on killed conspecific adults which is clear from the food color (crimson, added to the well) being visible from the translucent exterior of the larva.

Thus, logically, some specific nutritional requirement is being met by a cannibalistic source for the successive moults to occur. Although in separate experiments conducted by us as well as previously by Vijendravarma *et al*, have shown that metamorphosis of the larvae is completed even without cannibalism- hence we cannot conclude on the obligatory pre-requisite in this group of Dipterans. Even though we cannot deny on a possible evolutionary advantage in such modified preference of these fruit flies- the same cannot be considered as plasticity in response to a poor nutritional condition. Hence, our experimental observations logically demand the behavior of cannibalism to be an innate behavioral attribute, pre-disposed genetically- and the *Drosophila sp*. larvae as facultative cannibals.

Now, the question that naturally arise is, why cannibalism?

- Does the incidence of predatory cannibalism result from excessive inbreeding, rearing in relatively crowded conditions on artificial culture medium and environment for several decades?
- Or has this behavioral characteristic evolved even before culturing the flies in the laboratory conditions?

If the former alternative is more probable, then when did this transition, at least partial, from frugivory to predatory cannibalism take place? (Do populations harbour genetic variations for the propensity to cannibalism, allowing it to change with the changing environmental conditions?) Is it restricted to this particular species of fruit fly (*Drosophila melanogaster*), which has been in the cultures for over 90 years? (Lindsley and Grell, 1968).

Comparable experiments were also conducted in ancestral *D. virilis* (Bhattacharyya, 2014). Identical feeding behavior observed in this ancestral species belonging to the same genus suggests its possible existence of cannibalism from the time of evolution of the genus.

Figure 6. A possible cannibalistic aggregation observed in nature- growing on a banana in the wild.

Alternately, depriving both the species from normal diet and environment, as well as space limitation for considerable period (*D. virilis* has also been cultured in the laboratory for some decades from now) might compel them to adapt cannibalistic approach, which thus might have originated as the product of parallel evolution in recent time after speciation. The alternative approach logically demands absence or lower rate of conspecific consumption in larvae living in the wild or introduced very recently in the laboratory. Interestingly, larval cannibalism has been noticed in all culture vials where flies collected from the wild- from three distinct geographical regions were introduced in the lab just a couple of weeks prior to the experimental observations. Moreover, aggregation behavior has also been observed in larvae growing in bananas in nature as well (Figure 6).

Thus it will be wise to speculate that artificial food and environment are not the exclusive cause of predatory cannibalism and such behavior is genetically predisposed in the genome of most, if not all, species of *Drosophila* and might have possibly evolved to compensate inadequate protein required for their metabolism allowing us to conclude on the facultative nature of the cannibalistic behavior.

Acknowledgement: I am grateful to Dr. Debasis Mutsuddi, for his guidance, constant encouragement and valuable suggestions during the preparation of this work. Also, Professor Subir Chandra Dasgupta, Head of the Department of Zoology, and Professor Mamata Roy, Principal in office at Maulana Azad College, Kolkata, for their great help and laboratory facilities provided.

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Drosophila suzukii (Matsumura) found in Uruguay.

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Abstract

Drosophila species collected from two southern localities in Uruguay are reported. The spotted wing *Drosophila suzukii* was the most abundant species (96%) of the total *Drosophila* flies (n = 46) emerged from ripened, decayed, or damaged blueberries collected at rural Canelones Department, while it represented 0.50% among the *Drosophila* samples (n = 5007) collected from banana-baited traps in urban Montevideo city. Data suggest that *D. suzukii* has successfully invaded anthropic environments in urban and agrosystem ecosystems in southern localities in Uruguay.