

traps at low, though comparable, frequencies to those calculated for the other *Drosophila* species. Excepting for *D. suzukii* all other *Drosophila* species were previously reported in urban and suburban Montevideo city (Goñi *et al.*, 1997, 1998).

The new data discussed above expand the geographic/climatic range of the invasive *D. suzukii* in the Americas, and place an alert to the Uruguayan and regional agricultural sanitary control authorities about the potential economic effects this propagule on fruit production.

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**“Pack Hunting” or “Social Digestion” as a possible cause of larval clustering associated with the evolution of cannibalistic behavior in *Drosophila* species larvae.**

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The seminal observation of cannibalism in *Drosophila melanogaster* cultured in laboratory culture media for more than nine decades (Lindsley and Grell, 1968) has led us to a few of the major questions - as to

why cannibalism as such - what is the driving force that compels these otherwise non-carnivore species to become cannibals?

Previous reports (Vijendravarma *et al.*, 2013) have emphasized on the propensity to a more cannibalistic diet in the flies grown in protein compromised culture media for successive generations, giving them a direct fitness to combat with the protein deficiency. However, the fact that larvae show a basal level of cannibalism even when grown in optimum (conventional culture media used in the laboratory for generations) nutrient conditions (Bhattacharyya, 2014) and that comparable observations of cannibalistic behavior were made in the ancestral species, *i.e.*, *Drosophila virilis*, hint at the existence of this behavior from the time of evolution of the genus. Moreover, experiments conducted on locally collected species (possibly derived species, as the local species prevalent in this part of the country being *D. ananassae*, *i.e.*, a derived species characterised by a pericentric inversion of the X chromosome) that had been introduced in our laboratory for only 2 weeks just prior to the experimental observations that led to comparable observation, further emphasized on this evolutionary concept. Thus we preferred to consider these Dipterans as Facultative cannibals with the behavior of cannibalism being an innate behavioral character rather than being considered as mere plasticity in response to poor nutrient conditions.



Figure 1. Cannibalistic aggregations in *D. melanogaster* larvae.

This hypothetical view was further supported by the observation of cannibalistic behavior in the wild (nature, in a rotting banana), which would be expected considering cannibalism as an innate behavioral attribute not influenced by the artificial culture medium. Earlier workers have proposed on the aggregational

properties of the larvae (Figure 1) seen even in the wild as a manifestation of the feeding behavior of the larvae and hence have proposed a possible role in the development of cannibalistic behavior in the otherwise non-carnivorous flies (Gregg *et al.*, 1990; and later by Vijendravarma *et al.*, 2013).

Gregg *et al.* (1990) considered “external digestion” as the possible cause of such cannibalistic aggregation. They even applied the term, “social digestion”, to explain such clustering behavior and correlated this attribute to the evolution of exceptionally large size of the larval salivary glands.



Figure 2 (left). ‘Large predator- small victim’ cannibalism observed in a culture of *D. melanogaster* larvae.

Figure 3 (right). ‘Equivalent size predator-prey’ cannibalism in *D. melanogaster* larvae.

Vijendravarma *et al.* (2013), in their communication, highlighted cannibalism in the light of such aggregations where they solely emphasized the advantages of smaller larvae to overpower a large victim in such aggregations, which they termed “pack hunting”, it being a manifestation of cannibalism in the *Drosophila* sp. larvae. They observed, unlike most other cases of cannibalism, ‘size-reversed’ cannibalism (large pre-pupal larvae were cannibalized by younger and smaller ones). Such over-sized victims were found to be attacked by multiple smaller sized larvae. Their observations allowed them to infer that this ‘pack hunting’ presumably helped the attackers to overpower a large victim being attracted by chemical cues to form such aggregations.

We, apart from “size reversed cannibalism”, also observed both ‘large predator- small victim’ (Figure 2) as well as ‘equivalent size predator-prey’ cannibalism (Figure 3). In our experiments “multiple-larvae-single victim” encounters were found to be mostly prevalent in crowded and overcrowded situations, whereas incidences of “one-to-one encounter” between predator and prey were not uncommon (Figure 4), and found mostly in uncrowded, protein deprived and adverse conditions. Our studies so far have shown that apart from conspecific larvae, these cannibalistic larvae also fed on conspecific pupae and dead adults. Comparable observations were reported earlier from *D. hydei*, where larvae were found to consume the puparium (Gregg *et al.*, 1990). As there is no question to overpower the stationary puparium and dead adults, cannibalistic aggressiveness is less likely to be associated with overpowering the large victim, and more likely that “clustering behavior has evolved to facilitate external digestion” (Gregg *et al.*, 1990).



Figure 4. “One-to-one encounter” observed between predator and prey (here a conspecific adult) in a culture vial of *D. melanogaster*.

It has been demonstrated that besides conspecifics, *D. melanogaster* larvae fed on a wide variety of exogenous protein sources when supplemented from outside (Table 1). It includes immediately killed adults, both within and outside the genus, and most surprisingly even on cooked fish (washed thoroughly off any spice or cooked flavor, before being added

to the culture vials) and raw sliced chicken meat. Aggregations in such vials were observed, which again hinted at an evolutionary advantage to the practice of social digestion, rather than pack hunting, where the proponents emphasized completely on the advantage of overpowering an oversized victim.

Hence, from our studies and the earlier evidence and reports, it would be justified enough to conclude that the *feeding behavior, as such, of the Drosophila sp. larvae seems to have originated from “social digestion” rather than “pack hunting”*. Hence the evolution of cannibalism must have originated from the same practice of social digestion. (Figure 5).

Table 1. List of exogenous specimens that were fed on by the *Drosophila melanogaster* larvae.

| Common name               | Scientific name                   | Family                            |
|---------------------------|-----------------------------------|-----------------------------------|
| Housefly                  | <i>Musca domestica</i>            | Muscidae                          |
| Flesh fly                 | <i>Parasarcophagus ruficornis</i> | Sarcophagidae                     |
| Grasshopper               | <i>Gesonula punctifrons</i>       | Acridae                           |
| Honey bee                 | <i>Apis</i> sp.                   | Apidae                            |
| Black ant                 | <i>Camponotus compressus</i>      | Formicidae                        |
| Rohu fish (cooked)        | <i>Labeo rohita</i>               | Cyprinidae (Class-Actinopterygii) |
| Chicken (sliced raw meat) | <i>Gallus</i> sp.                 | Phasianidae (Class-Aves)          |



Figure 5. Social digestion as a possible manifestation of cannibalistic behavior in *Drosophila* species.

It is noteworthy to mention here that clustering behavior was very prominent in the natural environment of the larvae. Clustering of larvae in a natural culture of banana was also observed in the wild (Figure 6). Such aggregations could be related to a cannibalistic

behavior being at its play, hence emphasizing at the innateness of this attribute, being present even in the natural environment.

However, Gregg *et al.* (1990) as an extension of their work- argued that the unexplained massiveness of both the larval salivary glands and giant chromosomes are the resultant of producing digestive enzymes in huge quantities required for external digestion, relative to the digestive tract enzymes, to counteract the effects of dispersion in the external environment. They even provided strong evidence for the external digestion of amylose, cellulose, and chitin by *Drosophila* larvae. The work of Gregg *et al.* (1990), therefore, contradicted the popular belief (Ashburner and Berendes, 1978) that salivary glands do not produce digestive enzymes.



Figure 6. Aggregation and scraping behavior of larvae observed in the wild on a rotting banana, hinting at a possible presence of cannibalism in nature.

Hence, logically larvae growing in a strictly cannibalistic diet should have massive glands to meet with the nutrient deficiency by socially digesting conspecifics. However, when larvae growing in an otherwise protein deprived culture media, but flourishing entirely by cannibalizing conspecifics, were dissected for the preparation of salivary gland chromosome showed

abnormally small sized glands compared to normal larvae. Moreover, the giant chromosomes prepared from the same, which normally undergo  $2^8$  times endoreplication, were also very thin in appearance, almost comparable to the Malpighian tubules polytene chromosomes in wild type larvae (Figure 7). Hence again, what effect does the cannibalistic diet, or protein deprivation have on the larval development and metamorphosis remains a big question that stands, yet to be answered by further studies.

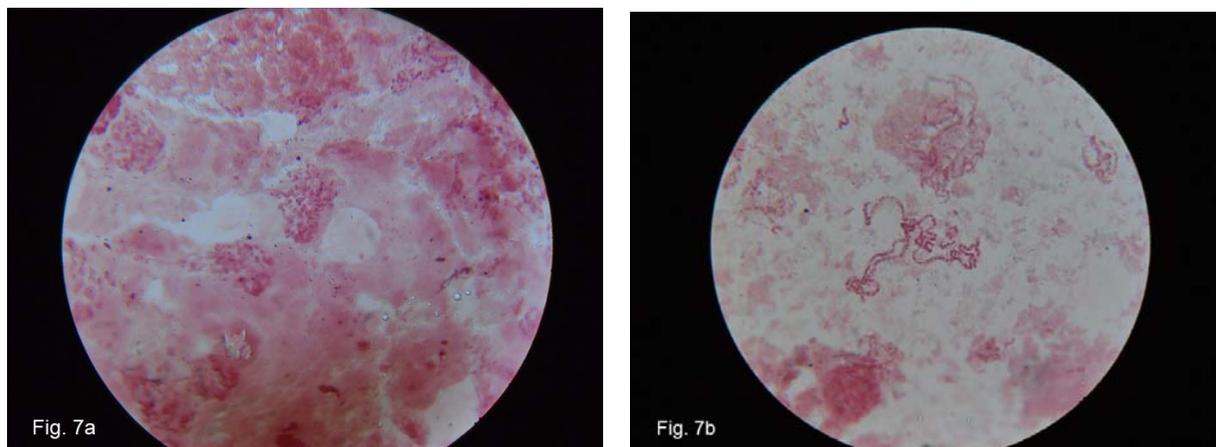


Figure 7. a, Salivary gland chromosome prepared from larvae growing on a strictly cannibalistic diet; b, Salivary gland chromosome prepared from larvae reared in a normal culture medium with only a basal level of cannibalism at play.

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### Comparison of the genotoxic and antigenotoxic activity of three *Ipomoea* species with medicinal properties in *Drosophila melanogaster*.

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#### Abstract

Some plants are recognized for their therapeutical properties, which could be associated to the presence of one or more metabolites. The resins from the root from *Ipomoea* species are commonly used for their purgative activity, being *I. purga* the most effective. Nevertheless their low activity as purgative, *I. orizabensis* and *I. jalapa* are used to adulterate the officinal preparation. In spite of the medicinal activity, and the wide use of traditional resources, little is known about collateral effects of plants derivatives. The genotoxicity of three species of the *Ipomoea* (Convolvulaceae) was compared using the Somatic Mutation and Mitotic Recombination Test (SMART) of *Drosophila*. Larvae were chronically exposed to different concentrations of resins from *I. purga*, *I. jalapa*, or *I. orizabensis* before (Pretreatment) or after (Post treatment) acute exposure to sucrose 5% or N-