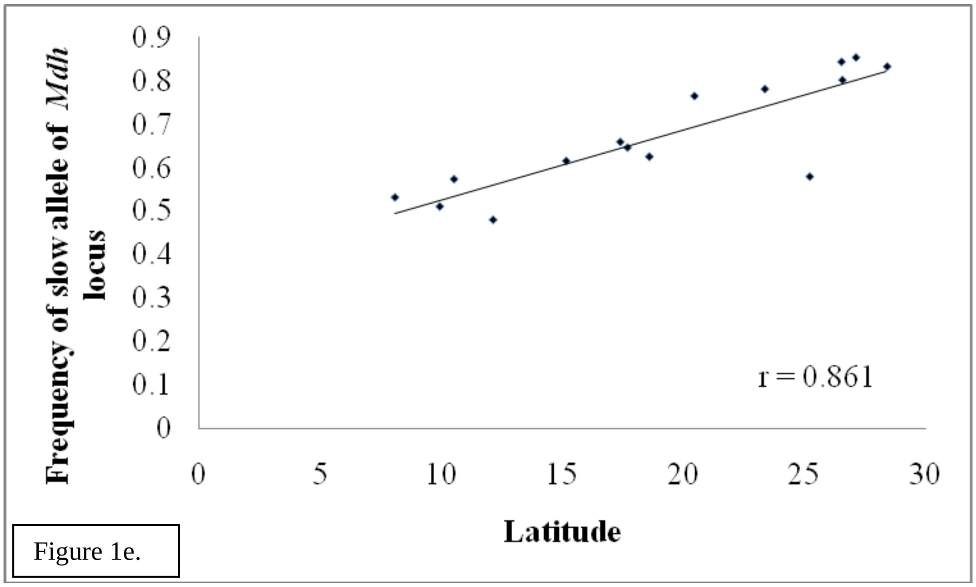

Figure 1e.

Acknowledgment: We are thankful to UGC (University Grants Commission), New Delhi, for providing financial support in the form of major research project to AKS and research fellowship to SK.

References: Arthur, A.L., A.R. Weeks, and C.M. Sgro 2008, J. Evol. Biol. 21: 1470–1479; Ayala, F.J., J.R. Powell, M.L. Tracey, C.A. Mourao, and S. Perez-Salas 1972, Genetics 70: 113-139; Bock, I.R., and M.R. Wheeler 1972, In: *The* Drosophila melanogaster *species group*. Univ. Texas Pub. 7213: 1-102; Bubliy, O.A., A.G. Imasheva, and O.E. Lazebnyi 1994, Genetika 30: 467-477; Bubliy, O.A., B.A. Kalabushkin, and A.G. Imasheva 1999, Hereditas 130: 25–32; Cavener, D.R., and M.T. Clegg 1981, Genetics 98: 613–623; Hoffmann, A.A., and A. Weeks 2007, Genetica 129: 133–147; Krishnamoorti, K., and A.K. Singh 2013, Dros. Inf. Serv. 96: 54-55; Kumar, S., and A.K. Singh 2012, Dros. Inf. Serv. 95: 18-20; Kumar, S., and A.K. Singh 2013, Dros. Inf. Serv. 96: 52-54; Kumar, S., and A.K. Singh 2014, Genetika 46: 227-234; Lakovaara, S., and A. Saura 1971, Genetics 69: 377-384; Land, V.T., J. Van Putten, W.F.H. Villarroel, A. Kamping, and W.V. Delden 2000, Evolution 54: 201–209; Moraes, E.M., and F.M. Sene 2002, J. Zool. Syst. Evol. Res. 40: 123–128; Mulley, J.C., J.W. James, and J.S.F. Barker 1979, Biochemical Genetics 17: 105–126; Oakeshott, J.G., J.B. Gibson, P.R. Anderson, W.R. Knibb, D.G. Anderson, and G.K. Chambers 1982, Evolution 36: 86-96; Parkash, R., S. Shamina, and Neena 1994, Gen. Sel. Evol. 26: 485-494; Prout, T., and J.S.F. Barker 1993, Genetics 134: 369–375; Santos, M., A. Ruiz, and A. Fontdevila 1989, Amer. Nat. 133: 183–197; Shaw, C.R., and R. Prasad 1970, Biochem. Genet. 4: 297-320; Singh, A.K., S. Kumar, and Bhumika 2013, J. Sci. Res. 57: 104-108; Singh, B.N., 2010, Ind. J. Exp. Bio. 48: 333-345; Umina, P.A., A.R. Weeks, M.R. Kearney, S.W. McKechnie, and A.A. Hoffmann 2005, Science 308: 691-693.

Cannibalism and "partial carnivorism" in *Drosophila* sp. larvae.

Bhattacharyya, Dipita. Cytogenetics Laboratory, P.G. Department of Zoology, Maulana Azad College. 8, Rafi Ahmed Kidwai Road, Kolkata-700013, India.

The seminal observation of cannibalism in *Drosophila* sp. larvae in our laboratory is purely accidental rather than being the resultant of meticulous execution of a well-designed research plan. Initially, as post graduate students, we were in the process of conducting an experiment to separate the two mutants - one, X-linked (white), and the other, an autosomal (ebony) from a double mutant strain, (*i.e.*, white ebony). During this time, due to some unavoidable circumstances, severe mite infection compelled us to discard some of the severely infected vials and regularly monitor the remaining vials under a dissection microscope (magnification 20× and 40×) in order to establish the cultures completely free of mites.

At this time of rigorous monitoring, it was discovered that a few of the larvae seemed to somehow associate with some of the dead bodies of the adult flies in the vial. It had been observed earlier as well. However, unaware of the fact that comparable observation was reported in Canton-S flies in the previous year, (Vijendraverma *et al.*, 2013), the thought of a possible cannibalistic behavior was of course discarded. However this time, having kept the vials under frequent observation under the dissection microscope, we had direct evidence that some of the larvae were feeding on the dead adults (parental flies). In fact, further studies revealed that larvae, especially first and second instar, fed on other larvae and pupae as well. Such repeated observations, coupled with the aforesaid publication, tempted us to extend our observations in different experimental situations.

Observations

Some of the notable observations that are worth mentioning are as follows:

- Cannibalistic behavior initiated from the first instar larvae itself and continued till the early third instar stage.
- There is a gradual decrease in the cannibalistic behavior as the mid-third instar larvae start crawling out from the culture medium, up along the wall of the glass vial.
- Finally, in the late third instar stage, there is complete absence of cannibalism as the larvae gradually stops feeding, turns motionless and enters into the pupal stage. These pupae in turn might serve as an easy target for the cannibalistic larvae, if they form near the surface of the culture medium.

The Exhibition of Cannibalism Was Characterized as Follows

The larvae were found to approach the victimized counterpart, followed by continuous scrapping of the cuticle of the victim with their jaws (Figure 1, a-b), until they were successful to make a lesion. They then entered into the body and started eating the soft internal body parts, leaving only the empty exoskeleton of the victim either on the food surface or deep in the culture medium (Figure 1, c-d). They were also found to have consumed the wings of the dead parental flies.

Fig. 1a

Figure 1. (a) White ebony larvae approaching the victimized counterpart (adult parental fly) before feeding on the same, (b) White ebony second instar larva scraping against the cuticle of the victimized counterpart (a first instar larva), (c) White ebony larva feeding on the soft internal body part of a dead adult, (d) Remnant of the victim on the culture medium.

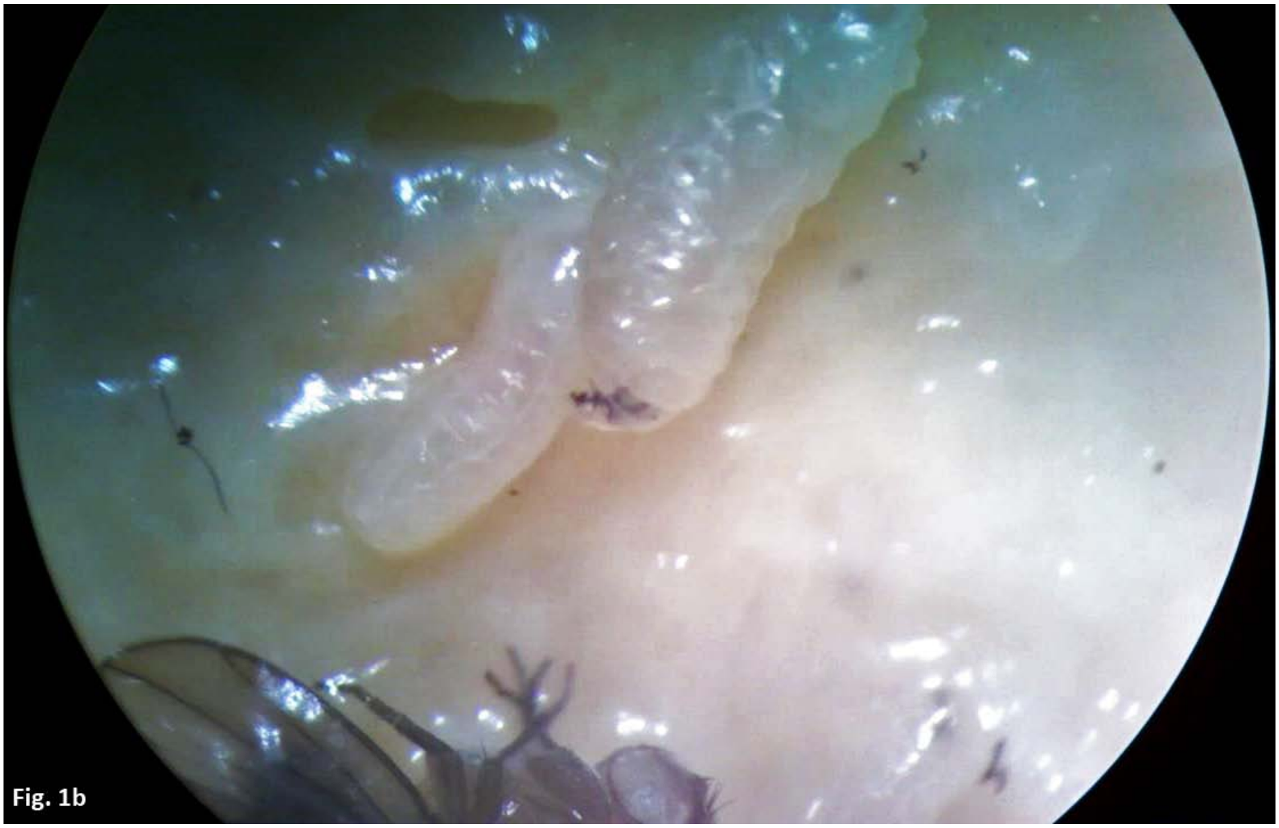

Fig. 1b

Fig. 1c


Fig. 1d

Experiments

As the larval cannibalism was first observed in a double mutant strain, extension of observations to wild type (Oregon R) culture became necessary to determine whether these two mutants somehow influenced the cannibalistic behavior of the larvae, especially when the mutant, white, has been known to have pleiotropic effects, influencing both adult and larval characteristics (Lindsley and Grell, 1968). Interestingly, identical results with respect to cannibalism were also observed in the wild-type stock (Figure 2, a-b). These observations exclude the possibility of influence of these two markers on the cannibalistic behavior.


Fig. 2a

Figure 2. (a) Cannibalism as observed in the wild-type (Oregon R) stock, (b) Having made a successful lesion, the larva enters into the body of the victimized counterpart and feeds on the soft internal body parts.

Fig. 2b

Next, a series of simple experiments were designed to answer the following questions:

- *In cannibalism, among the larvae, is the cannibalism the resultant of specific competition for a limited resource?*
- *Whether the cannibalistic behavior of the larvae is limited to their own parents?*
- *Whether such behavior is species specific? If the answer is a negative one, is there any preference?*
- *Is (Are) the victim(s) of cannibal larvae only restricted to the members belonging to the same genus?*
- *Whether such a phenomenon is limited only to one species under the genus* Drosophila*?*

Experiments conducted to answer the above questions

- *In cannibalism, among the larvae, is the cannibalism the resultant of specific competition for a limited resource?*

When *D. melanogaster* larvae were cultured in normal maize-jaggery-yeast-agar medium, in the following vials, at 22±1°C,

1. Uncrowded (5 males and 10 females, kept for 2 days),
2. Crowded (10 males and 20 females, kept together for 3 days), and
3. Overcrowded (10 males and 20 females, kept together for 5 days),

cannibalistic behavior was found to remain unaltered significantly in all three situations.

The experimental observations cited above, point to the general conclusion that cannibalistic behavior in the larvae might not be the resultant of competition for limited food resources. But at the same time, further experimental verifications are required by altering the parameters to reach any definite satisfactory conclusion, as food limitation was considered to be one of the major cause of killing conspecifics in spiders (Wise, 2006) and *Drosophila* sp. (Vijendraverma *et al.*, 2013).

- *Whether the cannibalistic behavior of the larvae is limited to their own parents?*

All the adult flies from the vials were discarded after allowing them to mate for three days. After the emergence of first instar larvae, etherized adult flies from separate vials were introduced into the vials. The first instar larvae were found to attack the etherized flies within 10-15 minutes and on the next day, only exoskeletons were found, either on the food surface or in some cases inside the culture medium (observed from the sides of the vials).

- *Whether such behavior is species specific? If the answer is a negative one, is there any preference?*

The same experiment was repeated with the locally collected species. When etherized locally collected adult flies were introduced into the vials containing only *D. melanogaster* larvae, the larvae were found to feed on them as well. Next, when equal number of both *D. melanogaster* and locally collected *Drosophila* sp adults were added together in vials culturing only *D. melanogaster* larvae, they fed equally upon all the flies, irrespective of the species.

- *Is (Are) the victim(s) of cannibal larvae only restricted to the members belonging to the same genus?*

In an extension of this series of experiments, adult houseflies (*Musca domestica*; family Muscidae), adult flesh flies (*Parasarcophaga ruficornis*; family Sarcophagidae), and sliced raw chicken meat were supplemented separately to vials containing only *D. melanogaster* first instar larvae. The larvae fed on these equally, as was seen in the earlier cases (Figure 3, a-c).

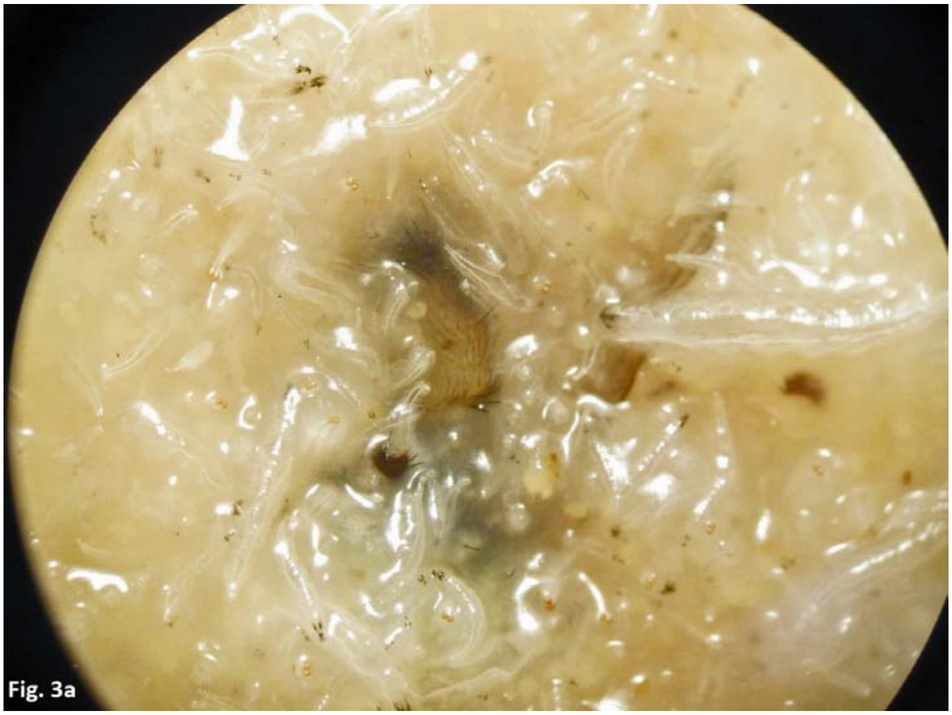

Figure 3. (a) *D. melanogaster* larvae feeding on *Musca domestica* added to vial, (b) *D. melanogaster* larvae feeding on the body of adult flesh fly added to vial (eye pigment having been consumed already), (c) Wild type *D. melanogaster* larvae feeding on sliced raw chicken meat.

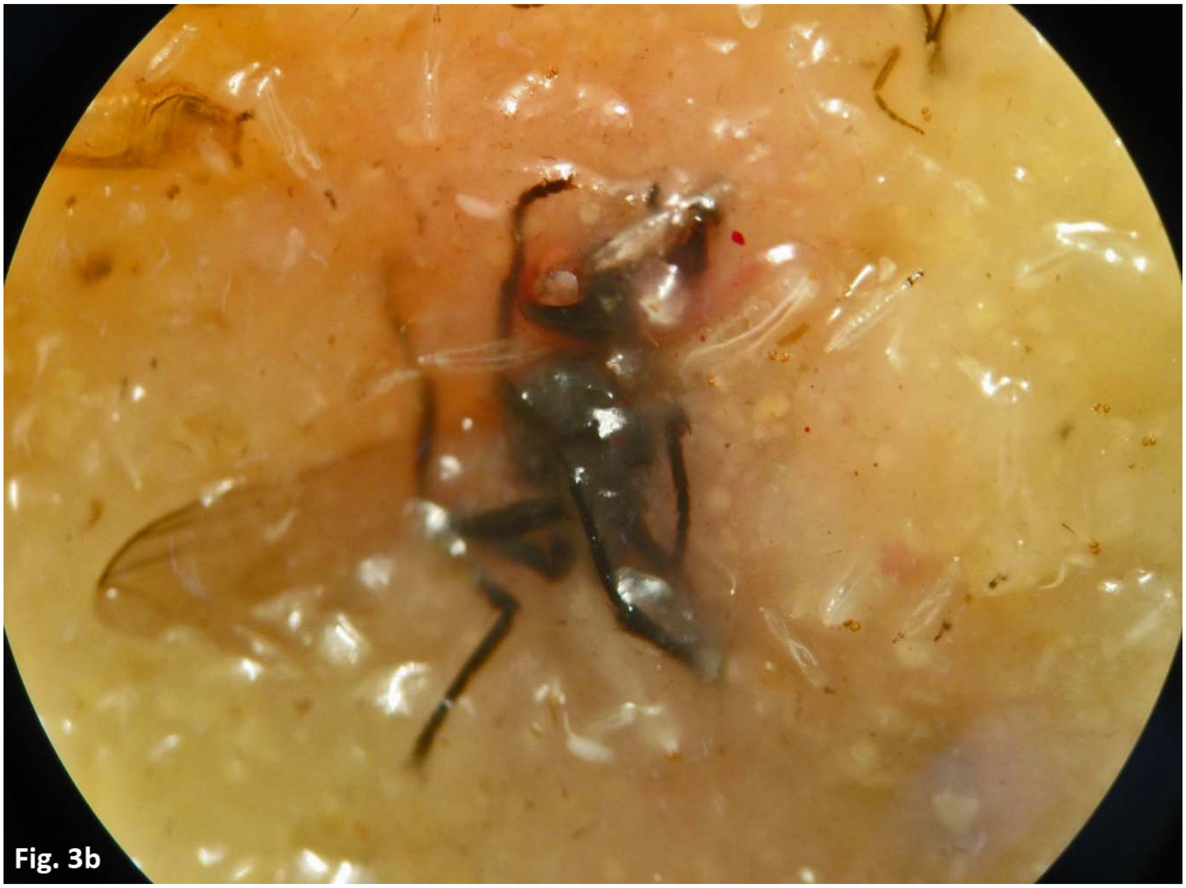

Fig. 3b

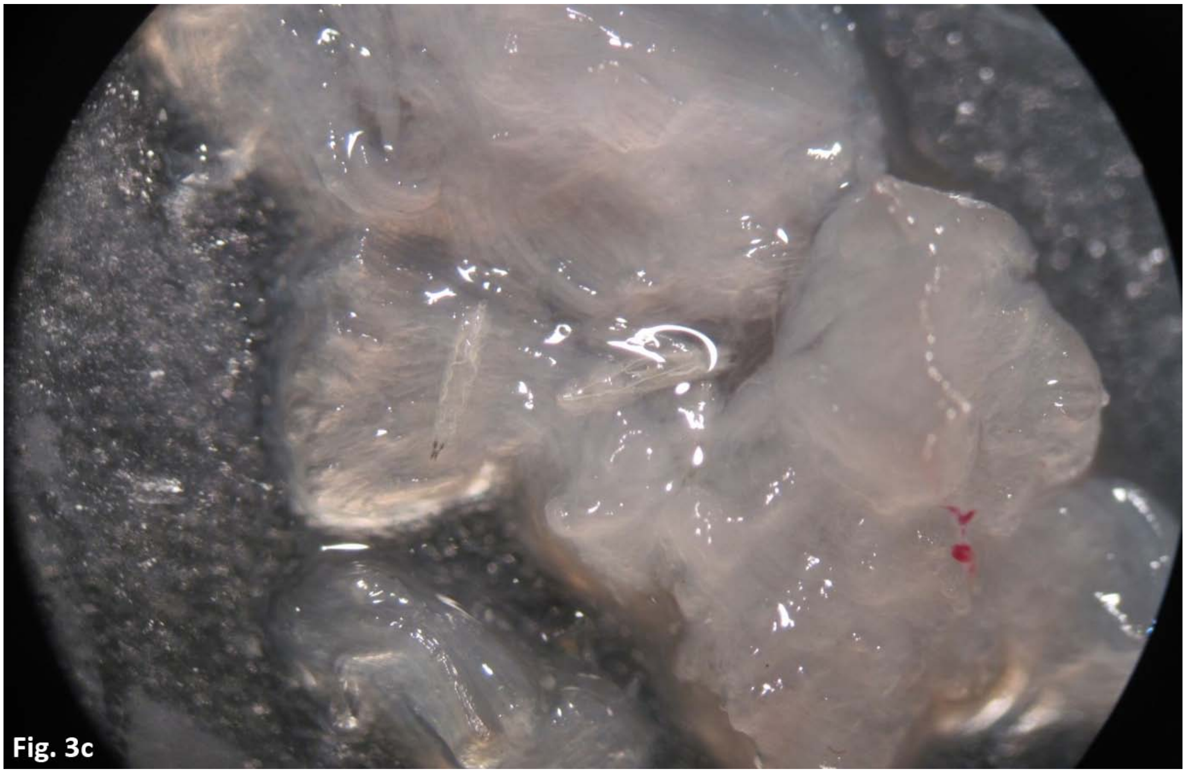

Fig. 3c

- *Whether such a phenomenon is limited only to one species under the genus* Drosophila*?*

Comparable cannibalistic behavior was also observed in locally collected *Drosophila* species, in all the experimental situations performed earlier using *D. melanogaster* larvae (Figure 4, a-b).

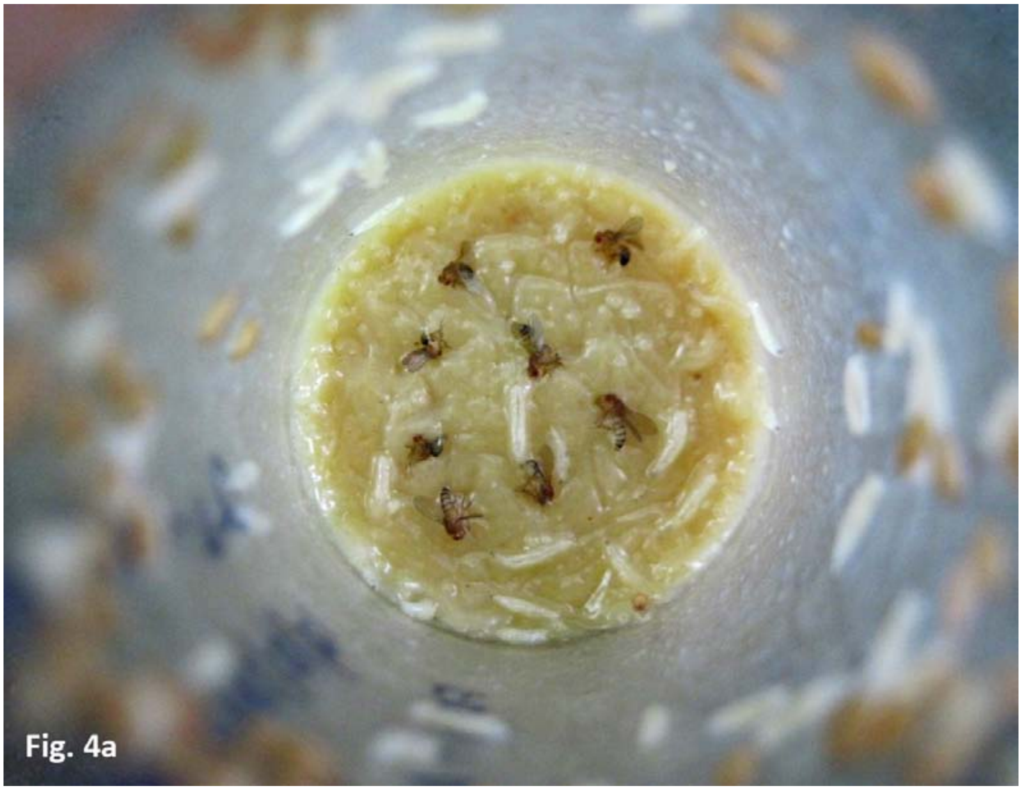

Fig. 4a

Fig. 4b

Figure 4. (a) Cannibalistic behavior observed in locally collected *Drosophila sp*. larvae, as seen after supplementing dead bodies of adult flies to the vial, (b) A closer view depicting larvae of the locally collected *Drosophila* sp., feeding on the adult of the same species.

All the experimental observations so far tempted us to speculate that cannibalistic behavior of the larvae is not restricted to *D. melanogaster* only, it is not the resultant of intraspecific competition among larvae for limited amount of food resources, and there is no species and genus specificity with respect to larval cannibalism. The last point we would like to add is that the feeding on *Musca* sp., *Parasarcophaga* sp., and raw chicken meat although allowed us to use the term, "partial carnivorism" in *Drosophila* larvae; we thus far failed to culture the flies in vials containing only raw chicken meat.

Acknowledgments: This work is financially supported by Department of Biotechnology, Star College scheme (BT/HRD/11/03/2011 dt 25.5.11) that has been granted to Maulana Azad College, Kolkata. I would like to express my gratitude towards Dr. D. Mutsuddi for his guidance and Professor Dr. S.C. Dasgupta, Head of the Department, for providing the necessary laboratory facilities.

References: Wise, David H., 2006, Annu. Rev. Entomol. 51: 441-465; Lindsley, Dan L., and E.M. Grell 1968, *Genetic Variations of* Drosophila melanogaster, Carnegie Institution of Washington publication no. 627; Vijendraverma, R.K, S. Narasimha, and T.J. Kawecki 2013, Nature Communications 4: 1789, DOI:10.1038/ncomms2744.

The natural breeding sites of *Drosophila funebris* in Chile.

Jara, Claudia[1], Sergio Flores[2], Luis Pino[1], María Cristina Medina[3], Eduardo Alvarez[1], Francisco Del Pino[1], and Raúl Godoy-Herrera[1]. [1]Programa de Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile, Santiago; [2]Departamento de Antropología, Facultad de Ciencias Sociales, Universidad de Chile; [3]Departamento de Biología y Ciencias Naturales, Facultad de Ciencias, Universidad de Playa Ancha de Ciencias de la Educación, Valparaíso.

Drosophila funebris, an Holoartic, cosmopolitan species, can be collected in Chile to South of the latitude 43°- 44°. There, natural populations of the species live in extreme climatic conditions near the Strait of Magellan (53° 10´S) and in Caleta Josefina in Tierra del Fuego (53° 40´S). *D. funebris* is one of the Southernmost *Drosophilidae* (Brncic and Dobzhansky, 1957; Brncic and Sanchez, 1958). On the other hand, *D. funebris* adult flies can emerge from fungi, walnut husks, decaying fruits, potatoes, and onions (Carson, 1965).

In Central Valley of Chile, latitude 29°- 37° S, *D. funebris* larvae and adults exploit a variety of decaying materials in a diversity of habitats very different to those of Tierra del Fuego, attesting to the versatility and flexibility of *D. funebris* genome. For example, populations of *D. funebris* utilize decaying pumpkin (*Cucurbita maxima*), prickly pear decaying tissue (*Opuntia ficus-indica*), and decaying tissue of a Chilean cactus (*Echinopsis chilensis*). Depending on the locality, the three types of breeding sites may be separated for a few meters as in Pelequén (34° 28´S) and Melipilla (33° 31' S) or scattered over a surface of about 50 km^2 as in Til-Til (33° 06' S).

The Pelequén, Melipilla, and Til-Til localities also differ in climate. For example, in annual rain: (i) Pelequén, 563.4 mm; (ii) Melipilla, 397.7 mm; (iii) Til-Til, 318.7 mm. Annual mean temperature in these three localities is: (i) Pelequén, 13.5°C, (ii) Melipilla, 14°C, and (iii) Til-Til, 17°C.

On the other hand, depending on the type of decaying fruit, larvae of D. *funebris* coexist with larvae of other *Drosophila* species. In pumpkin, *D. funebris* shares the fruit with *D. immigrans*. In prickly pear tissues, *D. funebris* lives together with the Chilean endemic *D. pavani* and the cosmopolitan *D. buzzatii*. From decaying cactus tissue *E. chilensis*, adults of *D. funebris* emerge together with *D. busckii* and *D. buzzatii*.

The ecology of Chilean natural populations of *D. funebris* offers an opportunity to investigate the role of ecological factors in the origin of new species. Likewise, these populations are a good biological material to study behavioral barriers that restrict gene flow. Recent results (unpublished data) suggest that there are gene flow restrictions between natural populations of *D. funebris* that live in sympatry on different substrates (pumpkin, cactus, and prickly pear). These restrictions also exist between allopatric populations of *D. funebris* reared on the same type of fruit. That is, sexes prefer to copulate with individuals emerged from the same type