

A WONDERFUL ANIMAL MODEL, *Drosophila* THAT MAY FULFILL THE ACADEMIC DEMAND OF BIOLOGICAL STUDIES OF 21ST CENTURY

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ABSTRACT

Drosophila is an important animal model which has extensively been used in various disciplines of life sciences. Several branches of zoology involve use of this animal to study various life processes. Its several unique genetical traits make it ideal to be used in scientific research. *Drosophila* flies are still in tremendous use to explore several intricacies pertaining to development, disease problems, expression and silencing of genes, role of genes on behavior and several other aspects. One of the well known species of this genus i.e. *D. melanogaster*, has been used as a model organism for understanding molecular mechanisms of human diseases. The genome wide analysis of this organism and humans have revealed that a number of genes have been well conserved in the two groups during the long course of evolution. A number of basic biological, physiological, behavioral and neurological properties are conserved between *Drosophila* and the vertebrates including humans. Approximately 75% of human disease-causing genes are known to have a functional homolog in the *Drosophila*. The *Drosophila* flies can be effectively used for drug screening and in drug discovery. Hundreds of zoologists employ this organism in their teaching and research work from all over the world. Our country forms abode of hundreds of species of this genus and therefore Indian subcontinent is quite rich in *Drosophila* biodiversity. If procuring wild animals for class room teaching has become a serious issue and if that results removal of certain curricula from the syllabi then it is our responsibility to seek an alternative animal model which can be largely reared and used for teaching and research. It seems that it is *Drosophila* that can only serve this purpose and will be the boon for biological studies.

KEYWORDS : Sexual dimorphism, Biological Studies, 21st Century, *Drosophila*

Dissection of animals was introduced as a part of our curriculum in the 1920's in life science education. During the last decade it has seriously been felt that the population of a number of animal species is declining due to their habitat loss. Thus the practice of animal dissection has come under criticism all over the world including India for its relevance and value for teaching purposes in Zoology. According to an estimate, if the undergraduate students of our country studying zoology, dissect the conventionally prescribed animals in their practical classes, they will sacrifice 18 million animals per academic session. A committee constituted by the human resource development ministry and the UGC in January 2010 decided to completely stop the animal dissection. During these days every educational institution is receiving letters from the concerned agencies to restrict the use of animals in dissection and not to use wild animals in class room dissection. As an alternative for this, it is being suggested to adopt computer animation programmes to display such exercises. Although, it is really need of the present time as the populations of several animals which were conventionally involved in dissection are dwindling but

people having experience in this matter feel that the computer assisted dissection would not justify the teaching as well as research purposes. In the field of biological studies, dissection of animals, in fact forms one small part of this subject. There are a number of other areas e.g. genetics, developmental biology, behaviour, taxonomy, morphology etc. which are addressed with equal importance to enrich our knowledge towards the life. If procuring animals for studies become an issue to remove certain curricula from the syllabi then we must think for alternative animal models which can be largely reared and employed for teaching and research. It seems that *Drosophila* is one of the animal models that can serve the purpose of teaching in a number of areas of biological studies.

Drosophila means "dew-loving". These flies are popularly known as fruit flies. *D. melanogaster* has been one of the most extensively used species in genetical research. It became one of the popular organisms in the field of genetics because of its several unique features. Some of them are: distinct sexual dimorphism, rapid life cycle, easy to cultured on simple food medium, availability of a number of mutants, produce large number of progeny in a single

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generation, low chromosome number, small genome size, presence of polytene chromosomes, do not act as vector of any known disease, etc.

This insect has extensively been utilized in genetical research since very long. This organism gained further significance when the developmental biologists and ethologists were able to decipher several intricacies of developmental and behavioural aspects by using it as a model organism (Lints, and Soliman, 1988). One can study embryonic and larval developmental stages in this animal as efficiently as we observe such aspects in the study of development in other vertebrate animals. Study of behavior, form another area of interest in the present era. Effect of genetic strains and mutant forms and their role in behavior have now been studied in a number of *Drosophila* species. Thus besides conventional use of this animal in genetical studies, a number of other areas of teaching and research involve this animal model. Some of the experiments of evolutionary biology can be skillfully planned and demonstrated to undergraduate and post graduate students by utilizing only this unique fly. Several experiments pertaining to the field of evolution can be efficiently done by utilizing *Drosophila* flies. Experiments related to natural selection, hybridization, sexual isolation (reproductive isolation) and inheritance of quantitative traits can be demonstrated to biology students and thus such endeavor substantiate their knowledge regarding organism diversity and their evolution. *Drosophila* is extensively used as a model organism in genetics (including population genetics), cell-biology, biochemistry, and developmental biology. The entire DNA sequence of *Drosophila melanogaster* has now been sequenced. The genomes of some other species have been fully or partially sequenced. The data obtained from such studies have helped us to compare genome similarity or dissimilarity between those species and therefore one can establish evolutionary genome comparisons. The *Drosophila* genome is also compared with the genomes of other species of insects such as honey bees and mosquito.

Significance as an Animal Model

There are many salient features of this fly that make it an attractive animal model for scientific study. It is being used in cytogenetics study for the last 100 years. The

total genome sequencing of *D. melanogaster* and some other species have now been accomplished. It is esteemed in genetical research due to its features like: it is cosmopolitan in distribution, can be collected from the nature by putting banana bait or fermenting fruits. Hand sweeping with the help of net or picking individual flies with an aspirator are the common methods to collect them. One can clearly observe them under stereo binocular/dissecting microscope for their morphological features. The two sexes are easily identifiable. Virgin males and females can be collected and employed in making required crosses and utilizing them in mating behavior studies. Larval behavior makes another important field of study to observe their behavior towards food preference and pupation site preference. The larvae could also be used for the non sexual behavior studies like geotaxis, phototaxis, locomotion behavior etc. Sperm mobility can be observed and demonstrated in the genital tract of mated females. Polytene chromosomes help in the study of chromosomal aberrations like deletions, duplications, inversions and translocations. Experiments pertaining to sexual selection can also be demonstrated.

It's Life Cycle and Culture

This organism is preferred for genetical and other studies for one of its features that it completes its life cycle in just 10 to 12 days. The fertilized egg undergoes embryogenesis that is completed within 24 hours after fertilization. The first instar larva hatches from the egg in 1 day. It grows for 24 hours and then moults into a larger second instar larva. The process is repeated to yield a third instar larva that feeds and grows for 2 to 3 days. The third instar larva settles on a dry place and undergoes the process of pupation. It metamorphoses into an adult form from pupal stage in about 3 to 4 days. Thus the life cycle of *Drosophila* fly shows the dipteran pattern of development. The eggs are about 0.5 mm long and a pair of filaments extends from their antero-dorsal surface. The third instar larvae are very active and voracious feeder that the culture medium in which they are crawling becomes heavily channeled and furrowed. During the third instar stage one can sacrifice the larva for its salivary glands for the preparation of polytene chromosomes. (Griffiths, et al. 1996, Snustad and Simmons, 2010). The adult fly averages in its size 2mm. The adult emerges out from pupal case by

forcing its way through the anterior end. At the time of hatching it is comparatively longer with constricted wings but after a short while it gains normal size and the wings get expanded.

These flies being small in their size occupy quite less space in the culture vial or bottles and thus hundreds of flies can be reared in the single culture bottle. Males and females become sexually mature in about 8-10 hours. The flies can be anesthetized using anesthetic ether. Ether is inflammable, has a strong odor and may kill flies if they are over-etherized. Etherized flies are transferred on a glass plate and are then observed under the stereo-binocular. A fine hair brush is used to handle the flies on the plate or at the time when they are being transferred in the food vials or bottles.

Drosophila flies are cultured on the simple food medium. The different ingredients required to rear them can be easily managed (Brooker, 1999 Demerec and Kaufmann, 1965). The food ingredients are: Agar agar, sugar (molasses), Yeast, Maize powder, Propionic acid, Nepazin (as an antifungal agent) and Water. These contents are boiled and poured in the glass vials or bottles which solidify after getting cooled. Flies collected from natural habitat can be cultured in the food bottles for further generations. Students can also be familiarized with these organisms by rearing them on banana food kept in the glass bottles. In this case flies which are trapped in the bottle are plugged with cotton and left in a cool and dry place. One can observe the larval stages crawling on the food within three to four days. When enough numbers of larvae are there in the bottle, flies can be set free, and within a week other stage like pupae are seen on the wall of the glass bottle. The practice of culturing flies on banana food can be done in a college that can thus help students to have idea about the developmental stages of an insect.

Setting a Cross to Understand the Inheritance Pattern of Genetically Determined Traits

Presently a number of mutant stocks are available for several species of *Drosophila*. These mutants either appear spontaneously in the fly population or can be created by exposing them to mutagens like radiations or chemicals. The location of such mutants on the chromosomes can be

ascertained by doing crosses and analyzing the further generations. In *D. melanogaster*, hundreds of mutants are known and their position on different chromosomes is well documented. These mutants could be used in several genetical experiments and their inheritance pattern can also be studied (Graf et al 1991, Roberts, 1986). To set a cross between mutant type and wild type flies, one has to collect mutant virgin females and males from the culture bottles. The separated males and females are aged for 4-5 days and then they are allowed into a fresh food bottle to produce progeny. Females begin laying fertile eggs soon after mating. The parental flies are removed after four or five days stay in the bottle because by this time mated females have already laid enough number of eggs. The F1 flies obtained from the cross can be subjected to either self-cross or testcross to study genetic phenomena. The pattern of sex-linked inheritance was well documented by T. H. Morgan in *D. melanogaster* as he involved a white (eyed) mutant male in the cross. Based on the inheritance pattern of the white eye gene he could explain that it is a sex-linked gene and it shows criss-cross pattern of inheritance. His work on *Drosophila* earned him the 1933 Nobel prize in Medicine. Some of the mutant flies of *D. melanogaster* can be procured from the known laboratories and can be maintained in the laboratory conditions for practical classes. We can reconstruct our teaching syllabi in such a way that the practical exercises include those aspects in which *Drosophila* related experiments form substantial portion.

Drosophila Species Found In Indian Subcontinent

Indian subcontinent is quit rich in *Drosophila* fauna due to its varied ecological diversity. It is represented by number of Dipteran insect species. More than 200 species of *Drosophila* have been reported from India and its neighbouring regions (Gupta, 1993). *Drosophila* flies collected from nature can be identified for their species by examining their morphological traits like sex-comb and other features (Gupta, 2005). Several species of *Drosophila* reported from other parts of the world also occur in this region. Indian *Drosophila* workers have chosen more than a dozen species to study the genetical, cytological, behavioural, evolutionary and molecular aspects in different

species. *D. melanogaster*, *D. ananassae*, *D. malerkotliana*, *D. bipectinata*, *D. nasuta*, *D. parabipectinata* are some of the species which have been involved for the study of their chromosomal polymorphism, allozyme polymorphism, taxonomic features, quantitative traits, and behavioural characteristics. *D. ananassae* is one of the cosmopolitan and domestic species and it is widely distributed in our country. It is considered as an important species among the whole of genus *Drosophila* because of its several genetic features e.g. spontaneous male meiotic recombination, mutability, parthenogenesis, Y-4 linkage of nucleolus organizer, lack of genetic coadaptation etc (Singh, 2010, Singh and Singh 1990, Singh, 2012). This species harbours several distinct structural chromosomal aberrations like inversions and translocations. One can use this animal model to study several aspects of evolutionary events like natural selection, random genetic drift, sexual isolation etc. by setting simple experiments. Several species of *Drosophila* are genetically close to each other and therefore can be hybridized in laboratory conditions. In most of the cases, the hybrid individuals formed show sterility. Thus this organism is of immense importance to study reproductive isolation and the mechanism of speciation.

New Directions in *Drosophila* Research

One may undermine that *Drosophila* being an insect, may not be of substantial use for applied aspects of studies. The development of recombinant DNA technology and the results of genome sequencing of a vast number of organisms have confirmed that all life forms have a common origin. Due to this, genes with similar functions in different organisms tend to be similar or identical in structure and nucleotide sequence. By studying genetics of *Drosophila* and a mammal, scientists have been able to learn the causes and treatment of some of the genetically determined human diseases. The genome sequencing of *Drosophila* has revealed that it shows genome homology with a number of invertebrate animals and vertebrates including humans. We find that certain genes which are in single or few copies in *Drosophila* are represented by several copies of it in mammals. This helps us to elucidate that how evolutionary changes might have occurred during the long course of evolution. Due to genome similarity

between this fly and humans for some of the genes people used it in deciphering several gene expression/ gene regulation aspects. *D. melanogaster* has now become an important animal model in the study of a number of human diseases like cancer, parkinson's, sleep, Alzheimer's, neurodegeneration, and visual defects such as retinal degeneration and also in finding valid alternative in the drug discovery process. Actually fly models of human diseases are generated by creating targeted mutations, in the homolog of a human disease-related gene or by expression of the human form of the gene, that produce a distinct countable phenotype. Stem cell research has helped the cytogeneticists to derive useful concepts of cellular activities. It is therefore concluded that this organism be given due weightage for the teaching of biological phenomenon to our young generations.

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