WONDER ANIMAL MODEL FOR GENETIC STUDIES - *Drosophila Melanogaster* –ITS LIFE CYCLE AND BREEDING METHODS – A REVIEW

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ABSTRACT:

Drosophila *is a genus of small flies, belonging to the family Drosophilidae, whose members are often called "fruit flies". The entire genus, however, contains about 1,500 species and is very diverse in appearance, behavior, and breeding habitat. One species of Drosophila in particular D. melanogaster, has been heavily used in research in genetics and is a common model organism in developmental biology. Basic genetic mechanisms are shared by most organisms. Therefore, it is only necessary to study the genetic mechanisms of a few organisms in order to understand how the mechanisms work in many organisms, including humans. Drosophila melanogaster, the fruit fly a little insect about 3mm long, is an excellent organism to study genetic mechanisms. The general principles of gene transmission, linkage, sex determination, genetic interactions; molecular, biochemical and developmental genetics, chromosomal aberrations, penetrance and expressivity, and evolutionary change may all be admirably demonstrated by using the fruit fly Drosophila melanogaster. The life cycle of Drosophila is short and completes in about three weeks. Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membrane. The egg produces larva, which eats and grows and at length becomes pupa. The pupa, in turn develops into an imago or adult. The duration of these stages varies with the temperature. Drosophila cultures ought to be kept in room temperature where the temperature does not range below 20°C or above 25°C. They are bred on fermenting medium which contains corn, dextrose, sugar and yeast extract. Their breeding ratio is 1:3 (male:female). The common culture contaminants include fungi, mites and bacteria. The male and the female are differentiated (under the microscope) based on their size, markings on their abdomen and presence of sex combs following anesthetization with ether.*

Key Words: Drosophila melanogaster, Genetics, Breeding

INTRODUCTION:

Drosophila is a genus of small flies, belonging to the family Drosophilidae, whose members are often called "fruit flies". One species of *Drosophila* in particular *D.melanogaster*, has been heavily used in research in genetics and is a common model organism in developmental biology. The entire genus, however, contains about 1,500 species and is very diverse in appearance, behavior, and breeding habitat. Scientists who study Drosophila attribute the species' diversity to its ability to be competitive in almost every habitat, including deserts.

Why Drosophila?

Drosophila melanogaster is a fruit fly, of the kind that accumulates around spoiled fruit. It is also one of the most valuable organisms in biological research, particularly in genetics and developmental biology. Basic genetic mechanisms are shared by most organisms. Therefore, it is only necessary to study the genetic mechanisms of a few organisms in order to understand how the mechanisms work in many organisms, including humans. *Drosophila melanogaster*, a little insect about 3mm long, is an excellent organism to study genetic mechanisms. The general principles of gene transmission, linkage, sex determination, genetic interactions; molecular, biochemical and developmental genetics, chromosomal aberrations, penetrance and expressivity, and evolutionary change may all be admirably demonstrated by using the fruit fly. *D. melanogaster* and its hundreds of related species have been extensively studied for decades, and there is extensive literature available (1).

The extensive knowledge of the genetics of *D. melanogaster* and the long term experimental experience with this organism together with extensive genetic homology to mammals has made it of unique usefulness in mutation research and genetic toxicology. Many *Drosophila* genes are homologous to human genes and are studied to gain a better understanding of what role these proteins have in human beings. Much research about the genetics of *Drosophila* over the last 50 years has resulted in a wealth of reference literature and knowledge about hundreds of its genes.

It is an ideal organism for several reasons:

- Fruit flies are hardy with simple food requirements and occupy little space.
- The reproductive cycle is complete in about 12 days at room temperature, allowing quick analysis of test crosses.
- Fruit flies produce large numbers of offspring to allow sufficient data to be collected. Examination and data collection is easy because the flies can be quickly and easily immobilized for examination.
- Many types of hereditary variations can be recognized with low-power magnification.

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- Drosophila has a small number of chromosomes (four pairs), a genome size smaller than the human complement of 23 pairs of chromosomes. The giant ("polytene") chromosomes in the salivary (and other) glands of the mature larvae.
    - Show far more structural detail than do normal chromosomes, and
    - They are present during interphase when chromosomes are normally invisible
- A large number of genetically defined mutants are available which define most aspects of the fly's biology.
- Many Drosophila genes are homologous to human genes and are studied to gain a better understanding of what role these proteins have in human beings. Much research about the genetics of Drosophila over the last 50 years has resulted in a wealth of reference literature and knowledge about hundreds of its genes.
- The genome is relatively small for an animal (less than a tenth that of humans and mice).
- Mutations can be targeted to specific genes (1,2).

Life cycle of Drosophila

Stages and duration:

Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membrane. The egg produces larva, which eats and grows and at length becomes pupa. The pupa, in turn develops into an imago or adult. *(Fig. 1)* The duration of these stages varies with the temperature. At 20ºC, the average length of the egg-larval period is 8 days; at 25ºC it is reduced to 5 days. The pupal life at 20ºC is about 6.3 days, whereas at 25º C is about 4.2 days. Thus at 25º c the life cycle may be completed in about 10 days, but at 20ºC about 15 days are required. Drosophila cultures ought to be kept in room temperature where the temperature does not range below 20ºC or above 25ºC. Continued exposure to temperatures above 30˚ C may result in sterilization or death and at low temperatures the viability of flies is impaired and life cycle prolonged.(2)

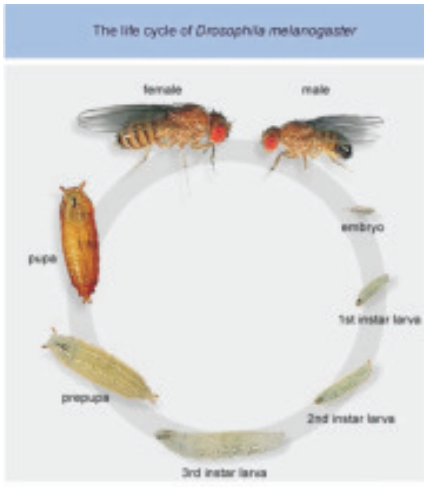

Figure 1: Lifecycle of *D. melanogaster*

Source : Dept. of Anatomy & Cell Biology University of Melbourne

The egg:

The egg of *Drosophila melanogaster* is about 0.5 of a millimeter long. An outer investing membrane, the chorion, is opaque and shows a pattern of hexagonal markings. A pair of filaments, extending from the anterodorsal surface, keeps the egg from sinking into soft food on which it may be laid. Penetration of spermatozoa into egg occurs through a small opening or micropyle, in the conical protrusion at the anterior end, as the egg passes through the uterus. Many sperms may enter an egg, through normally only one functions in fertilization. The spermatozoa have been stored by the female since the time of mating. Immediately after the entrance of the sperm, the reduction (meiotic) divisions are completed and the egg nucleus (female pronucleus) is formed. The sperm nucleus and the egg nucleus then come into position side by side to produce the zygote nucleus, which divides to form the first two cleavage nuclei, the initial stage of development of the embryo. Eggs may be laid by the mother shortly after they are penetrated by the sperm, or they may be retained in the uterus during the early stages of embryonic development. (2)

The Larval Stages:

The larva , after hatching from the egg , undergoes two molts, so that the larval period consist of three stages (instars).The final stage , or third instar may attain a length of about 4.5millimeters. The larvae are such intensely active and voracious feeders that the culture medium in which they are crawling becomes heavily channeled and furrowed (2).

The larva has 12 segments: the 3 head segments, 3 thoracic segments, and 8 abdominal segments. The body wall is soft and flexible and consists of the outer noncellular cuticula and the inner cellular epidermis. A great number of sense organs are spread regularly over the whole body. (Fig. 2) (2,3)

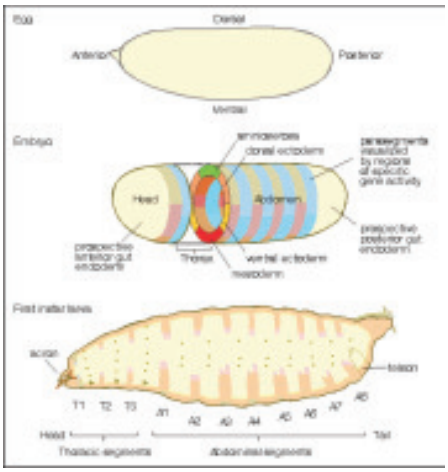

Fig. 2: Anatomy of egg, embryo and larva of *D. melanogaster*

Source : Bloomington Stock Center, Indiana University

The larvae are quite transparent. Their fat bodies, in the form of long whitish sheets, the coiled intestine, and the yellowish malpighian tubules , as well as the gonads embedded in the fat body can easily be distinguished in the living larva when observed in transmitted light. The dorsal blood vessel is the circulatory organ of the larva. The larval muscles, segmentally arranged, are transparent but can be made visible when the larva is fixed in hot water. The larva contains a number of primitive cell complexes called imaginal discs, which are the primordia for later imaginal structures(2,3).

The primary mechanism by which the larva grows is molting. At each molt the entire cuticle of the insect, including many specialized cuticular structures, as well as the mouth armature and the spiracles, is shed and has to be rebuilt again. During each molt, therefore many reconstruction processes occur, leading to the formation of structures characteristic of the ensuing instar. The growth of the internal organs proceeds gradually and seems to be rather independent of the molting process, which mainly affects the body wall. Organs such as Malpighian tubes, muscles, fat body, and intestine grow by an increase in cell size; the number of cells in the organ remains constant. The organ discs, on the other hand, grow chiefly by cell multiplication; the size of the individual cells remains about the same. In general, one might say that purely larval organs grow by an increase in cell size, whereas the presumptive imaginal organs grow by cell multiplication(2,3).

The Pupa:

A series of developmental steps by means of which the insect passes from the larval into the adult organism is called "metamorphosis". The most drastic changes in this transformation process take place during the pupal stage.

Shortly before pupation the larva leaves the food and usually crawls onto the sides of the culture bottles, seeking a suitable place for pupation, and finally comes to rest. The larva is now very sluggish, everts its anterior spiracles, and becomes motionless. Soon the larva shortens and appears to be somewhat broader, thus gradually acquiring its pupal shape. The shortening of the larval cuticle, which forms the case of the puparium, is caused by muscular action. The puparium, which is the outer pupil case, is thus identical with the cuticle of the last larval instar. When the shaping of the puparium is completed, the larval segmentation is obliterated, but the cuticle is still white. This stage lasts only a few minutes and is thus an accurate time mark from which to date the age of the pupa. Immediately after the cuticle reaches the white prepupal stage, the hardening and the darkening of the cuticle begin and proceed very quickly. About three and a half hours later the puparium is fully coloured. Pigmentation apparently starts in the external surface of the cuticle and proceeds inward (2,3).

Four hours after the formation of the puparium , the animal within it has separated its epidermis from the puparium and has become a headless individual having no external wings or legs and known as the "prepupa". A very fine prepupal cuticle has been secreted and surrounds the prepupa. (2,3).

Pupation takes place about 12 hours after puparium formation. By muscular contraction the prepupa draws back from the anterior end of the puparim and everts its head structures. This movement also ejects the larval mouth armature, which until now was attached to the anterior end of the prepupa. The wings, halteres and legs are also everted. A typical pupa with head, thorax, and abdomen is thus shaped. In section it is seen that the pupa now lies within three membranes: an outer membrane, the puparium: an intermediate membrane, the prepupal cuticle; and an inner membrane, the newly secreted pupal cuticle(2,3).

Now metamorphosis involves the destruction of certain larval tissues and organs (histolysis) and the organization of adult structures from primitive cell complexes, the imaginal discs. It must, however, be realized that some larval organs are transformed into their imaginal state without any very drastic change in their structure. The duration and extent of these transformation processes vary greatly for the different organs involved. Larval organs which are completely histolyzed during metamorphosis are the salivary glands, the fat bodies, the intestine and apparently the muscles. All these organs are formed anew, either from imaginal disc cells already present in the larva or from cells which come visibly into being in the course of pupal reorganization. The Malpighian tubules are relatively little altered during metamorphosis but nevertheless undergo some change in their structural composition. The same situation seems to prevail in the brain, which is not completely histolyzed. The extremities, eyes, mouthparts, antennae, and genital apparatus differentiate from their appropriate imaginal discs, which were already present in the larval stage and which undergo histogenesis during pupal development. The body wall of the imaginal head, thorax, and abdomen is also formed from imaginal disc cells. The body wall of head and thorax is formed by the combined effort of all the imaginal discs in this region, each of which contributes its part. The hypoderm of the abdomen is formed by segmentally arranged imaginal cells which first become visible in young prepupae(2,3).

Adult stage

When metamorphosis is complete, the adult flies emerge from the pupa case. They are fragile and light in color and their wings are not fully expanded. These flies darken in a few hours and take on the normal appearance of the adult fly(4). Upon emergence, flies are relatively light in color but they darken during the first few hours. It is possible by this criterion to distinguish recently emerged flies from older ones present in the same culture

bottle.(2).They live a month or more and then die. A female does not mate for about 10 to 12 hours after emerging from the pupa. Once she has mated, she stores a considerable quantity of sperm in receptacles and fertilizes her eggs as she lays them. Hence, to ensure a controlled mating, it is necessary to use females that have not mated before. These flies are referred to as virgin females (3,4).

Female Male

Figure 3: *Male and female adult Drosophila*

Features to determine the sex of adult fly *(Fig. 3)*:

1. ***Size of adult***

   The female is generally larger than the male.

2. ***Shape of abdomen***

   The tip of the abdomen is elongated in the female, and somewhat more rounded in the male(2).

3. ***Markings on the abdomen***

   Alternating dark and light bands can be seen on the entire rear portion of the female; the last few segments of the male are fused(5). The abdomen of the female has seven segments that are readily visible with low power magnifiers, whereas that of the male has five(2).

4. ***Appearance of sex comb:***

   The males have so called sex combs, a fringe of about ten stout black bristles on the distal surface of the basal (uppermost) tarsal segment of the fore leg *(fig. 4)*. Such bristles are lacking in the female (2). Sex identification via the sex comb can also be done successfully in the pupal stage.

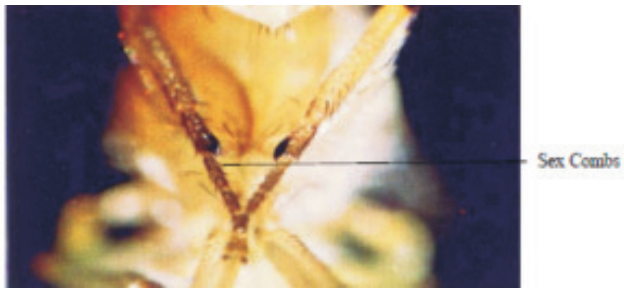

Figure 4: Sex combs in a male fly

5. ***External genitalia on abdomen***

   Located at the tip of the abdomen, the ovipositor of the female is pointed. The claspers of the male are darkly pigmented, arranged in circular form, and located just ventral to the tip (5).

6. ***Sex organs during larval stage***

   During the late larval stage males can be distinguished by the presence of a large, white mass of testicular tissue. This tissue is located at the beginning of the posterior third of the larva in the lateral fat bodies and can be seen through the integument. The corresponding ovarian tissue of the female constitutes a much smaller mass (5).

Tools for culturing Drosophila

Basic fly handling equipment includes a binocular microscope with a good light source, an etherizer or a CO_2 plate for anesthetization, a fly pusher, an aspirator, a pounding pad, and a morgue. For most purposes flies can be kept at room temperature , but one or two constant temperature rooms, preferably humidified, or incubators are generally useful and are necessary for some techniques(6).

Microscope and light source:

A binocular or trinocular microscope with good quality optics, easy access to the magnification changer and a smooth accessible focusing mechanism is ideal (6).

Anesthetizing flies:

Ether and CO_2 are the fly anesthetics of choice .CO_2 requires more setup and maintenance than ether. If ether is chosen an etherizer and a sorting plate is required , on the other hand a CO_2 pad serves as both an anesthetizer and a sorting plate(6).

Ether is flammable, has a strong odor and will kill flies if they are over-etherized. Carbon dioxide works very well, keeping flies immobile for long periods of time with no side effects, however CO_2 mats (blocks) are expensive and a CO_2 source (usually a bottle) and delivery system (vials and clamps) are necessary, increasing the costs.

The least harmful to the flies is either carbon dioxide or cooling anesthetizing. Of these two choices, cooling is the simplest, requiring only a freezer, ice and petridishes. In addition, it is the only method which will not affect fly neurology, therefore behavior studies may begin after the flies have warmed up sufficiently.

Anesthetizing flies by cooling

In order to incapacitate the flies, place the culture vial in the freezer until the flies are not moving, generally 8-12 minutes. Flies are dumped onto a chilled surface. This can be constructed by using the top of a petridish, adding crushed ice, then placing the bottom of the petridish on top. Adding flies to this system will keep them chilled long enough to do each experiment. Simply place the flies back into the culture vial when finished. There are no long-lasting side effects to this method, although flies left in the refrigerator too long may not recover (7).

Stock Keeping:

Most stocks can be successfully cultured by periodic

mass transfer of adults to fresh food. Bottles or vials are tapped on the pounding pad to shake flies away from the plug, the plug is rapidly removed, and the old culture is inverted over a fresh bottle or vial. Flies are tapped into the new vessel, or some are shaken back into the old one, as necessary, and the two are rapidly separated and replugged. Good tossing technique combined with plugs that are easily removed and replaced results in very few escapes. The frequency with which new subcultures need to be established depends on health and fecundity of the genotype, the temperature at which it is raised, and the density of the cultures (6).

It is very good practice to keep the old cultures for 2 weeks (at 18ºC) after transfer, so that they can be used as a backup should the new stocks fail for any reason(8).

Culture contaminants:

Drosophila is largely pestilence-free, but mites, fungi, and bacteria can be problems in laboratory cultures. Benchtop and fly pushing equipments must be regularly cleaned. Benchtop and all equipments that come into contact into potentially contaminated stocks should be cleaned with 70% ethanol or soap and water after use. Sharing pounding pads, CO_2 pads, fly pushers, and sorting plates can aid in the spread of contaminants (6).

Mites are egg predators and are the most dangerous contaminating species. Even those that simply feed on the medium can outcompete weak genotypes and compromise experimental observations. Frequent stock transfer, tight plugs and zero mite tolerance by all fly workers in a building are best defenses. Cultures that are grown at 24 – 25º C must never be kept for more than 30 days. If mites are known to be a problem cultures should be checked and discarded after 18 – 20 days. To prevent the import of mites from outside sources, all stocks new to the lab should be quarantined for atleast two generations. Any culture found to contain mites should be autoclaved immediately and replaced with a mite free source (6).

Fungi and bacteria can also contaminate the culture. If mould is the problem in isolated cultures, it can usually be eliminated by daily transfer of adults for 7 – 10 days. Visually inspect cultures from the later transfers for hyphae (look around the pupal cases) and use one that appears to be free of fungal growth for further subculture. If fungal contamination is a widespread problem be sure that fungal inhibitor (p-hydroxy-benzoic acid methyl ether) is being added to the medium after it is cooked (boiling destroys the inhibitor) (6).

A variety of bacterial contaminants can occur in fly cultures. Most common problems are caused by mucous - producing bacteria. Although not directly toxic, larva, and to some extent adults become trapped in the heavy layer of mucous that coats the surface of the food. Large numbers of larvae overcome the effect of the bacteria in a healthy stock, but weak stocks or pair matings can be seriously compromised. A wide spread bacterial problem may indicate that the pH of the medium is too high; try lowering the pH (6,7).

Culture conditions:

Timing & Lighting

Fruit flies are "cold-blooded" so rate of growth and development varies with temperature. The duration of the different stages varies with the temperature. At 20˚C the average length of the egg-larval period is 8 days; at 25˚C it is reduced to 5 days. The flies are attracted to lights. Part of fly courtship behavior is visual, so it is probably a good idea to keep them in an area with good lighting most of the time(9).

Methods of breeding drosophila:

Drosophila melanogaster is found in abundance on soft fruits like grapes, bananas, and plums, especially if they are overripe and have begun to ferment. Adult flies as well as larvae feed on fruit juices: and since yeast is present wherever fermentation is in progress, it is believed that yeast constitutes an important part of their diet .Therefore Drosophila may be raised on any fermenting medium. The different types of medium routinely used for breeding Drosophila include cornmeal medium, banana jaggery medium, sucrose dextrose medium and maltose corn medium. The composition of the food predominantly includes sugar, yeast extract, dextrose and corn flour. They can be bred in glass bottles to obtain large numbers of the progeny *(Fig. 5)*. And most often crosses and experiments are set up in glass vials.

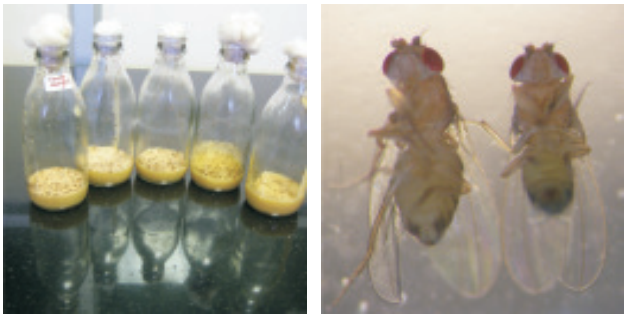

Figure 5: *Breeding Drosophila*

Scientists who study Drosophila attribute the species' diversity to its ability to be competitive in almost every habitat, including deserts. The extensive knowledge of the genetics of *D. melanogaster* and the long term experimental experience with this organism together with extensive genetic homology to mammals has made it of unique usefulness in mutation research and genetic toxicology. Many *Drosophila* genes are homologous to human genes and are studied to gain a better understanding of what role these proteins have in human beings. Much research about the genetics of *Drosophila* over the last 50 years has resulted in a wealth of reference literature and knowledge about hundreds of its genes. Specific mutations can be targeted and analyzed. Its

ease of handling, short reproductive cycle allows scientists to analyze test crosses. Also, the offspring are produced in large numbers which provides statistically significant data and phenotypic mutant changes are easily recognizable under the microscope. This review details on the lifecycle *of D. melanogaster*, its importance in genetic studies and also basic tools required for culturing flies in laboratory.

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