Drosophila Melanogaster

Drosophila melanogaster is a species of Fly (the taxonomic order Diptera) in the family Drosophilidae. The species is known generally as the common fruit fly or vinegar fly. Starting with Charles W. Woodworth's proposal of the use of this species as a model organism, *D. melanogaster* continues to be widely used for biological research in studies of genetics, physiology, microbial pathogenesis and life history evolution. It is typically used because it is an animal species that is easy to care for, breeds quickly, and lays many eggs.

Drosophila melanogaster lives in a wide range of habitats. Native habitats include those in the tropical regions of the Old World, but the common fruit fly has been introduced to almost all temperate regions of the world. The only aspects that limit the habitats Drosopila melangaster can live in is temperature and availability of water. The scientific name Drosophila actually means "lover of dew", implying that this species requires moist environments.

The development of this species' offspring is extremely dependent on temperature, and the adults cannot withstand the colder temperatures of high elevations or high latitudes. Food supplies are also limited in these locations. Therefore, in colder climates Drosophila melanogaster cannot survive.

In temperate regions where human activities have introduced Drosophila melanogaster, these flies seek shelter in colder winter months. Many times Drosophila can be found in fruit cellars, or other available manmade structures with a large supply of food.

Physical description

Drosophila mature through complete metamorphosis, as do all members of the order Diptera. Similar to all insects Drosophila is covered in a chitinous exoskeleton; has three main body segments; and has three pairs of segmented legs. In Adult stage the common fruit fly is normally a yellow brown color, and is only about 3 mm in length and 2 mm in width. The shape of the common fruit fly's body is what one would normally imagine for a species of the order *Diptera*. It has a rounded head with large, red, compound eyes; three smaller simple eyes, and short antennae. Its mouth has developed for sopping up liquids. The female is slightly larger than the mal. There are black stripes on the dorsal surface of its abdomen, which can be used to determine the sex of an individual. Males have a greater amount of black pigmentation concentrated at the posterior end of the abdomen.

Larvae are minute white maggots lacking legs and a defined head.

Reproduction

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. 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.

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 function 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.

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. 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. 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.

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.

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. Four hours after the formation of the puparium , the animal within it has separated its epidermis from thepuparium 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. 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. 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 a new, 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.

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. 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.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. Features to determine the sex of adult fly:

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.

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. The abdomen of the female has seven segments that are readily visible with low power magnifiers, whereas that of the male has five.

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. Sex identification via the sex comb can also be done successfully in the pupal stage.

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.

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.

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. And most often crosses and experiments are set up in glass vials.
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.

Model organism in genetics

Drosophila melanogaster is one of the most studied organisms in biological research, particularly in genetics and developmental biology. There are several reasons:

- Its care and culture requires little equipment and uses little space even when using large cultures, and the overall cost is low.
- It is small and easy to grow in the laboratory and their morphology is easy to identify once they are anesthetized (usually with ether, carbon dioxide gas, by cooling them, or with products like FlyNap)
- It has a short generation time (about 10 days at room temperature) so several generations can be studied within a few weeks.
- It has a high fecundity (females lay up to 100 eggs per day, and perhaps 2000 in a lifetime).[2]
- Males and females are readily distinguished and virgin females are easily isolated, facilitating genetic crossing.
- The mature larvae show giant chromosomes in the salivary glands called polytene chromosomes—"puffs" indicate regions of transcription and hence gene activity.
- It has only four pairs of chromosomes: three autosomes, and one sex chromosome.
- Males do not show meiotic recombination, facilitating genetic studies.
- Recessive lethal "balancer chromosomes" carrying visible genetic markers can be used to keep stocks of lethal alleles in a heterozygous state without recombination due to multiple inversions in the balancer.
- Genetic transformation techniques have been available since 1987.
- Its complete genome was sequenced and first published in 2000

Anesthetizing flies:
Ether and CO2 are the fly anesthetics of choice. CO2 require more setup and maintenance than ether. If ether is chosen an etherizer and a sorting plate is required, on the other hand a CO2 pad serves as both an anesthetizer and a sorting plate. 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 CO2 mats (blocks) are expensive and a CO2 source and delivery system 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.