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Comprehensive study on the life cycle, morphology, and culturing techniques of drosophila melanogaster in laboratory conditions

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Abstract
This research was carried out in the laboratory of the Department of Zoology, Kokrajhar Govt. College, Kokrajhar, and it delves into the life cycle, morphology, and culturing techniques of Drosophila melanogaster under laboratory conditions. D. melanogaster, often referred to as the fruit fly, is a member of the Drosophilidae family. This diminutive fly is known for its yellow-brown body and distinctive brick-red, rounded eyes, measuring roughly 3 mm in length and 2 mm in width, with females being slightly larger than males. The fruit fly's body is segmented into the head, thorax, and abdomen. The life cycle consists of four stages: embryogenesis, larval stage, pupal stage, and adult stage, with sexual dimorphism evident in adults, facilitating sex differentiation. Widely used in genetic research, fruit flies serve as a model organism in developmental biology. Typically bred in fermenting mediums derived from decaying vegetables and fruits, they exhibit a breeding ratio of one male to three females. The Drosophila genus encompasses around 1,500 species, each with unique appearances, behaviors, and breeding environments. Temperature significantly impacts their development, as adults cannot tolerate the colder conditions of high latitudes or areas with insufficient food sources. Consequently, cooler environments are generally unsuitable for D. melanogaster, often found in locations rich in food supply, such as fruit cellars.

Keywords: Drosophila melanogaster, laboratory, fruit fly, zoology, temperature

Introduction

Drosophila, commonly known as the "fruit fly," encompasses about 1,500 species, each displaying a diverse range of appearances, behaviors, and breeding habitats [1]. They belong to the phylum Arthropoda, class Insecta, order Diptera, and family Drosophilidae and are also known as "vinegar flies" or "pomace flies" [2]. These flies are easily identifiable by their brick-red eyes, brownish body, and distinctive black rings around the abdomen. Exhibiting sexual dimorphism, females are roughly 2.5 mm long, while males are slightly smaller with darker backs and a distinct black patch on the abdomen. Typically attracted to spoiled and rotten fruits and vegetables, Drosophila plays a pivotal role in biological research, especially in genetics and developmental biology [1]. For over a century, Drosophila has been utilized as an *in vivo* model organism for genetic and developmental studies, and more recently for toxicology, particularly nanotoxicology [3].

Drosophila is characterized by a chitinous exoskeleton and a body segmented into the head, thorax, and abdomen. It has three pairs of segmented legs and a single pair of wings developing from the middle thoracic segment, along with rudimentary wings called halteres, serving as balancing organs [4]. Fruit flies reproduce rapidly, with a single pair capable of producing hundreds of offspring within a few weeks, reaching sexual maturity within one week [5]. Male fruit flies have sex combs on their front legs, believed to aid in mating, though their removal has little impact on mating success [6].

Fruit flies exhibit simple behaviors, such as being attracted to the smell of food and light. When cultured in a tube, they tend to migrate toward the brightest light source [5]. In 1910, Thomas H. Morgan utilized Drosophila to provide the first evidence supporting the chromosomal theory of inheritance, asserting that chromosomes carry genetic information. Morgan pioneered the use of Drosophila in genetic research. The fruit fly genome is 60% homologous to humans, with about 75% of genes associated with human diseases having homologs in flies. These characteristics, combined with a short generation time, low maintenance costs, and powerful genetic tools, make fruit flies invaluable for studying complex pathways relevant to biomedical research.

Fruit flies coexist with numerous microbes and other arthropods in their decaying host resources, interacting in various ways. Some microbes serve as food for the flies, while others decompose fruit and plant matter into attractants or substances consumed by the flies [7]. Researchers using Drosophila have been awarded five Nobel Prizes [8]. Originally an African species, non-African lineages share a common origin [9]. Under optimal conditions at 25°C, D. melanogaster's lifespan is about 50 days from egg to death [10]. Development time varies with temperature, with the shortest time (egg to adult) of seven days occurring at around 28°C, increasing at higher temperatures due to heat stress [11].

Aims and Objectives

The aims and objectives of our study on Drosophila culture are as follows

The study aimed to

- Understand the life cycle of Drosophila melanogaster, including its metamorphosis, fertilization process, and instar differentiation.
- Gain knowledge about the fruit fly’s appearance, characteristics, sexual dimorphism, optimal growth conditions, such as suitable temperature and lifespan.
- Learn culturing techniques for Drosophila, mastering methods for effective cultivation in a controlled environment.

Methodology

The study took place in the Zoology Department at Kokrajhar Govt. College, Kokrajhar, during November 2023. The steps included

1. **Preparation of Culture Medium:** Various wastes, including vegetable and banana peels, and lemons, were stored in a transparent jar to create a suitable medium for culturing Drosophila melanogaster. The jar was left open to allow fruit flies to enter.

2. **Capturing Fruit Flies:** Once flies entered the jar, it was covered with a net to prevent escape. It took approximately 10-12 days to observe all stages of the fruit fly's life cycle, from egg to adult.

3. **Collection and Observation of Different Stages:** Eggs, larvae, and pupae were carefully removed with a brush and placed in a clean petri dish for microscopic examination.

4. **Anesthetizing Adult Fruit Flies:** Capturing adults was challenging due to their quick movements. To facilitate observation, an anesthetizing method was used. Adult flies were tapped into an empty jar, chloroform was added, and the jar was sealed to render the flies unconscious.

5. **Microscopic Examination:** Anesthetized flies were observed under a dissecting or stereo microscope, with detailed observations recorded and photographs taken for documentation.

Results and Discussions

The life cycle of Drosophila melanogaster spans approximately 10-12 days from egg fertilization to adult emergence, undergoing significant morphological changes between its larval and adult stages. The stages include:

- **Embryogenesis:** Females lay small, white eggs about 0.5 mm long. This phase lasts 24 hours post-fertilization. The egg is covered by the chorion, displaying a hexagonal pattern, with filaments preventing it from sinking into the food medium.

- **Larval Stage:** This stage spans about four days, with three instars. Larvae are transparent, undergoing two molts, resulting in three instars. The third instar can reach about 4.5 mm in length. The larva has 12 segments and tracheal breathing via spiracles at both ends.

- **Pupal Phase:** The third instar larva encapsulates, marking the start of the pupal phase, lasting about four days. New structures form from imaginal discs, growing from undifferentiated cells of the larva.

- **Adult Stage:** Adults emerge from the pupal case, initially fragile and light in color. Within hours, they darken, taking on the typical adult appearance. Newly emerged flies are lighter in color compared to older flies in the same medium. Adult flies live for about a month, with females becoming sexually mature after 8-10 hours post-emergence.

Traits to differentiate the sex of adult fruit flies include size, shape, and markings on the abdomen, presence of sex combs, and external genitalia.

Traits to Determine the Sex of Adult Fruit Flies

1. **Size of Adult:** Females are generally larger than males.

2. **Shape of the Abdomen:** Females have elongated tips, while males have rounded, circular tips [13].

3. **Markings on the Abdomen:** Females have alternating light and dark bands on their abdomen, while the last few segments of males are fused [12]. The abdomen of females has seven visible segments under low-power magnification, whereas males have five segments [13].

4. **Appearance of Sex Combs:** Males have sex combs, a fringe of stout black bristles on the distal surface of the basal tarsal segment of the foreleg, which are absent in females. Sex identification via the comb can also be done in the pupal stage [13].

5. **External Genitalia on Abdomen:** Females have a pointed ovipositor at the tip of the abdomen, while males have darkly pigmented claspers arranged in a circular form ventral to the tip [14].

6. **Sex Organs During Larval Stage:** During the late larval stage, males can be distinguished by the presence of large white masses of testicular tissue located in the lateral fat body near the posterior third of the larva. The corresponding ovarian tissue in females is much smaller [1].



Fig(s): Images captured during the culture process

Conclusion

The study of Drosophila melanogaster highlights its rapid and well-defined life cycle, spanning approximately 10-12 days from egg fertilization to adult emergence. The complete metamorphosis and distinct stages make it an ideal model organism for genetic and developmental research. Its short generation time, high reproductive rate, and well-characterized genetic makeup underscore its value in studying fundamental biological processes and genetic inheritance.

The cultivation and observation techniques employed, such as preparing the culture medium, capturing and anesthetizing flies, and microscopic examination, have proven effective in revealing the intricate details of Drosophila development. These methods contribute to a deeper understanding of its biology and support its continued use as a model organism in research.

Overall, the insights gained reinforce the significance of Drosophila melanogaster in scientific research, providing a solid foundation for future studies in genetics, developmental biology, and toxicology.

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