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Drosophila Development and Reproduction

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Abstract

One of the many reasons that make *Drosophila* an extremely valuable organism is that the molecular, cellular, and genetic foundations of development are highly conserved between flies and higher eukaryotes such as humans. *Drosophila* progress through several developmental stages in a process known as the life cycle and each stage provides a unique platform for developmental research. This video introduces each stage of the *Drosophila* life cycle and details the physical characteristics and major developmental events that occur during each stage. Next, the video discusses the genetic regulation of pattern formation, which is important for establishing the body plan of the organism and specifying individual tissues and organs. In addition, this video gives an overview of *Drosophila* reproduction, and how to use the reproductive characteristics of *Drosophila* to set up a genetic cross. Finally, we discuss examples of how the principles of *Drosophila* development and reproduction can be applied to research. These applications include RNA interference, behavioral assays of mating behaviors, and live imaging techniques that allow us to visualize development as a dynamic process. Overall, this video highlights the importance of understanding development and reproduction in *Drosophila*, and how this knowledge can be used to understand development in other organisms.

Transcript

Drosophila melanogaster, are widely used as a model organism in the study development and reproduction. *Drosophila* progress through several developmental stages in a process known as the life cycle and each stage provides a unique platform for developmental research. In this video, we will present the basics of *Drosophila* development and reproduction, including how to set up a genetic cross and discuss how this research can be applied to understand processes ranging from wound healing to behavior.

First, let's discuss the *Drosophila* life cycle. *Drosophila* progress through 4 main stages of development: embryo, larva, pupa, and adult.

The embryo is a fertilized egg that is about 0.5 mm long and oval shaped. Immediately after fertilization, the embryo undergoes rapid mitotic division without growth. The zygotic nucleus undergoes nine rounds of nuclear division, but does not undergo cytokinesis, forming a multi-nucleate cell called a syncytial blastoderm. Since all the nuclei in the syncytial blastoderm share a common cytoplasm, proteins can diffuse freely, forming morphogen gradients, which are important for establishing the body plan and patterning of individual organs and tissues in the fly. After the 10th nuclear division, the nuclei migrate to the periphery of the syncytial blastoderm . Following the 13th round of nuclear division, which occurs approximately 3 hours after fertilization, the 6000 nuclei in the syncytial blastoderm become individualized forming the cellular blastoderm . The cellular blastoderm contains a monolayer of cells and is transformed into a complex multi-layered structure, in a process known as gastrulation. During gastrulation, cell shape changes drive invaginations of the monolayer, ultimately creating the endoderm, mesoderm, and ectoderm germ layers. The endoderm gives rise to the gut, the mesoderm gives rise to the muscles and heart, and the ectoderm gives rise to the epidermis and central nervous system. After 24 hours, embryos hatch as larvae.

Larvae are white with worm-like segmented bodies. They crawl around in wet food eating constantly, leading to rapid growth. Larvae progress through three stages: the first instar for 24 hours, second instar for another 24 hours, and third instar for 48 hours. Molting occurs between each stage. When ready for pupation, third instar larvae leave their food source and attach to a firm surface, such as the side of a vial.

Pupa are immobile and are initially soft and white but eventually harden and turn brown. Over a period of four days, larval tissues degenerate and adult tissues form. Eclosion marks the end of the pupal stage and the flies emerge as adults.

8 hours after eclosion, the adults become sexually receptive and begin to mate, starting the life cycle all over again.

The complete life cycle takes about 10 days at 25 °C, but it can be affected by temperature. For example, at 18 °C the life cycle is about 19 days and at 29 °C, the life cycle is only 7 days.

Throughout development, careful genetic regulation of pattern formation establishes the body plan and specifies individual tissues and organs. Importantly, the establishment of the anterior-posterior axis defines the head to tail orientation of the organism, and is regulated by several groups of genes.

First, maternal effect genes are supplied in the oocyte and inherited from the female. They are important in the syncytial blastoderm for initially establishing the anterior and posterior of the embryo. In particular, the bicoid gene defines the anterior of the embryo including the head and thorax, while the nanos gene defines the posterior, including the abdomen.

Second, the segmentation genes, which are regulated by maternal effect genes, include the gap genes and pair rule genes. Gap genes establish a segmented body plan along the anterior-posterior axis by broadly subdividing the embryo. Pair rule genes are expressed in a striped pattern perpendicular to anterior-posterior axis, further dividing the embryo into smaller segments. Then the segment polarity genes, such as engrailed begin to establish cell fates within each segment.

Lastly, homeotic genes are responsible for defining particular anatomical structures, such as wings and legs. Interestingly, the order of the genes on the chromosome reflect how they are expressed along the anterior-posterior axis.

Drosophila are extremely fertile organisms that can produce thousands of progeny in a lifetime. Females lay hundreds of eggs per day and continue to fertilize eggs well after mating has occurred.

Drosophila are also sexually dimorphic organisms meaning that the females are phenotypically distinct from males. In particular, males are smaller than females and have darkly colored external genitalia, as well as more black pigment on their lower abdomens. Males also have a patch of bristles on their forelegs called sex combs used to latch onto the female during copulation. These distinct phenotypic differences make it very easy to distinguish males from females, which is particularly useful when setting up a genetic cross.

Setting up a cross with *Drosophila* is a useful technique for studying genetics. So let's get started!

The first step to setting up a cross is to collect virgin females of the desired genotype, so that you can control exactly which male with whom she will mate. *Drosophila* are unable to mate during the first 8 hours after eclosion, so collecting very young adults guarantees virginity. To collect newly eclosed females, clear the vial into the morgue to get rid of all adults. Every 3-4 hours, check the vial for newly eclosed adults, and collect the females in a new vial without any males until ready for use. Virgin females are identified by their very light body color and a dark spot on their abdomen, known as the meconium.

When ready to begin the cross, combine 4-6 males with 4-6 virgin females of your desired genotypes in a dated food vial, and store at 25° C and 60% humidity. After 3-4 days, larvae will be present and the parents should be transferred to a new vial, preventing the parents from mating with the progeny. After approximately 10 days, new offspring will emerge and their phenotypes can be examined.

One tool that *Drosophila* researchers use are balancer chromosomes that prevent genetic recombination and contain genetic markers such as curly wings, which are useful in determining the correct genotype of a fly. If you wanted flies that are heterozygous for two different mutations, you can cross a stock with mutation #1 over the balancer chromosome CyO, to a second stock with mutation #2 also balanced over CyO. Any progeny that emerge without curly wings are heterozygous for both mutations.

Another commonly used tool in *Drosophila* research is the UAS-GAL4 system, which allows researchers to express or knockdown a gene in a specific tissue. GAL4 is a yeast transcription factor that is driven by a tissue specific promoter and UAS is the Upstream Activating sequence, which controls the expression of the gene of interest . When you cross a fly with a tissue specific GAL4 transgene to a fly with a UAS transgene with your gene of interest directly downstream, the GAL4 protein binds the UAS and drives expression of your desired gene. For example, UAS-GFP crossed to apterous-GAL4, which is specific for the wing discs in pupa, expresses GFP specifically in those cells.

There are many applications that can be used to study *Drosophila* development and reproduction. One application is behavioral analyses - specifically courtship behavior. During courtship, the male orients himself towards the female and follows her while tapping her with his forelegs. If the female is receptive, she allows the male to mount her. The male curls his abdomen and transfers seminal fluid into the female, a process known as copulation. The analyses of these behaviors of courtship in various mutants gives insight into the genetic control of behavior

Drosophila development is an extremely dynamic process that includes many cell movements and shape changes, which can be studied via live imaging. For example, dorsal closure during embryogenesis is when a gap in the epithelium is closed in a zipper-like manner involving the coordination of many cell types. Dorsal closure during development is often used as a model to study wound closure, which may have clinical implications.

A third application used to understand processes during *Drosophila* development is RNA interference, which knocks down the activity of individual genes and can be used in large scale reverse genetic screens. For example, dsRNA can be injected into embryos, and the impact of the gene knockdown on organ development, for example, can be assessed. Here, RNA interference revealed a gene important for fusion during tracheal development.

You've just watched JoVE's introduction to *Drosophila melanogaster* reproduction and development . In this video we reviewed: the *Drosophila* life cycle, including details about each stage of development. We also learned how to use the reproductive capabilities of *Drosophila* to study genetics and set up a cross. Finally, we learned how *Drosophila* development and reproduction are useful for understanding complex processes such as behavior, wound closure, and organ development.