

Fly Cycle²

The Lives of a Fly

Drosophila melanogaster

Booklet by
Mary S. Tyler

CD-ROM & DVD Created by
Mary S. Tyler
and
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University of Maine

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An Adaptation of the Film
Fly Cycle: The Lives of a Fly
Drosophila melanogaster
by
Mary S. Tyler, Jamie W. Schnetzer,
and David Tartaglia

This film is dedicated to Mary Roelofs Stott, poet and observer of nature. Her joy and fascination in observing nature, measured out always with reverence for its power, inspired and educated all who knew her. With a writer's eye and philosopher's wit, she reminded us of the poetry in the grandest and smallest of things.

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Sequence 8 © 1991 by Rachel Fink and Eric Wieschaus
Sequence 9 © 1996 by Daniel Kiehart, Shinya Inoué, and Paul Young
All other sequences © 1996 by Mary S. Tyler

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PREFACE

About the CD and DVD

The common fruit fly *Drosophila melanogaster* has become one of the most studied organisms in biology and for this reason is important for students at all levels to examine. The CD & DVD show the many stages of the living animal, demonstrating the intricacies and fascination of the *Drosophila* life cycle, and incorporating details such as dissections, histological sections and a gallery of mutants used in research. The CD&DVD are an adaptation of the film, *Fly Cycle: The lives of a Fly*, *Drosophila melanogaster*, by Mary S. Tyler, Jamie W. Schnetzer, and David Tartaglia, published by Sinauer Associates (1996). Its purpose is to make the material on the film accessible to students in a more flexible format.

The CD&DVD should serve as an educational tool at many levels. As an introduction to the life cycle and to mutants, it can be used at the high-school and introductory undergraduate levels to acquaint students with an organism they can use in the classroom. At more advanced undergraduate levels in courses in developmental biology and genetics, it can help students in the techniques used to examine the organism in detail. For example, students will see how to sex larvae and adults, to dissect larval tissues such as salivary glands and imaginal discs, and to dissect pupae from their pupal cases. For graduate students beginning their research on *Drosophila*, it will serve as a guide to many of the aspects of development, growth, and metamorphosis they will need to know.

CD/DVD Production: The CD&DVD were created and produced by Mary S. Tyler and Ronald N. Kozlowski at the Department of Biological Sciences, University of Maine, using Final Cut Pro, Macromedia Director, Adobe Photoshop, Peak DV and Media Cleaner. Film sequences were digitized into QuickTimes by Ronald Kozlowski at the Department of Biological Sciences, University of Maine, using Media Cleaner and QuickTime 6.0, on an Apple G4 Dual Processor Power Mac. The DVD was assembled in DVD Studio Pro.

Filming: Most of the filming was done by Mary S. Tyler, with Jamie W. Schnetzer, using an NEC high-resolution color CCD video camera and a Cohu high-sensitivity, high-resolution, black-and-white video camera mounted on an Olympus dissecting microscope with Fiber-lite il-

lumination and a Leitz compound microscope with bright-field and phase contrast optics. Recordings were made with a Sony SV0-2000 recorder. Speeded up sequences were prepared from real-time sequences, compressed during production after digitization. The two sections on embryonic development were filmed by others: Rachel Fink and Eric Wieschaus recorded with a Dage-MTI Newvicon camera on a Nikon Diaphot inverted microscope, and Daniel Kiehart, Shinya Inoué, and Paul Young used a Dage Newvicon camera on a transilluminating, universal polarizing microscope (Inoué, 1986, pp. 495–496) with Nomarski DIC optics. In both embryonic sequences, an Image-1 image-processing system was used, and frames were recorded on a Panasonic optical memory disc recorder.

Histological sections: In several sequences, histological sections prepared by Mary S. Tyler are shown. These are 8- μ m-thick paraffin sections stained with hematoxylin, eosin, and alcian blue.

Film Production: The film sequences were produced by David Tartaglia at ASAP Media Services, and Mary S. Tyler, University of Maine. Film was assembled using Media 100, Adobe After Effects, Adobe Illustrator, Adobe Photoshop, Sound Edit, Peak DV and Final Cut Pro.

About This Booklet

This booklet should be helpful to anyone using the CD or DVD in teaching and any viewer who needs further details. It presents, in modified form, the spoken text from the film sequences and additional information where useful. For those who are inspired to begin their own observations of this organism, there is a section on “getting started” which includes limited instructions as well as a list of suppliers. At the end of the booklet, you will find a selected bibliography and a glossary.

Accompanying Materials Available from Sinauer

Tyler, M.S. and R.N.Kozlowski, 2003. *Fly Cycle 2: The DVD & CD of The Lives of a Fly*, *Drosophila melanogaster*. Sinauer Associates, Sunderland, MA.

Tyler, M. S. 2003. *Developmental Biology: A Guide for Experimental Study*. 3rd Edition. Sinauer Associates, Sunderland, MA.

Gilbert, S. F. 2003. *Developmental Biology*, 7th Ed. Sinauer Associates, Sunderland, MA.

ACKNOWLEDGMENTS

There are many to be thanked.

We thank the expert “Drosophilists” who provided information, advice and data from their own work. The CD&DVD, and film from which they were adapted, couldn’t have happened without them: John M. Ringo, Erik C. Johnson, Becky Talyn, and Harold B. Dowse.

We thank those who contributed their high-quality footage for the embryogenesis portions of the film: Rachel Fink, Eric Wieschaus, Daniel Kiehart, Shinya Inoué, and Paul E. Young. And Rachel and Dan in particular, thanks for your encouragement and help.

Thanks also to the Society for Developmental Biology for allowing us to rescue the footage from Rachel Fink and Eric Wieschaus that was previously published in the film, “A Dozen Eggs,” edited by Rachel Fink.

We thank all our friends and in particular Matthew Tyler, at Swarthmore College, for his expert advice and help with artwork throughout this project. We extend our special thanks to Matthew Snow, who prepared the picture files for this booklet. We are deeply indebted to Michael Scott, the organizer and overseer of ASAP Media Services, who was generous with his time and invaluable to the original project.

We thank the great people at Sinauer Associates, whose enthusiasm and support for this project never flagged. We are indebted in particular to Andy Sinauer and Dean Scudder for believing in the project, to Kathaleen Emerson and Jason Dirks for guiding us with calm and skill, to Mara Silver for her careful copyediting, and to Marie Scavotto for her skilled promotion of the product.

A sincere thank you to Rachel Fink, Scott Gilbert, Harold Dowse, Jeffrey Hall, and Seth Tyler for their careful reviews that gave polish to the product. And my thanks to the Society for Developmental Biology’s Education Committee for their time in previewing the film sequences.

We owe a special thanks to Lilian Morgan Scherp, whose kind correspondence, giving permission to use pictures of her father, Thomas Hunt Morgan, are now filed under treasures.

The words of gratitude we owe the collaborators of the original film, Jamie W. Schnetzer and David Tartaglia, go beyond the bounds of this booklet. They gave heart and soul to their work.

And we thank those who kept things going with their loving support: Amy Kozlowski, Anna Tyler, Matthew Tyler, and Seth Tyler.

The work was supported in part by a Bird and Bird Instructional Grant and a Regular Faculty Research Fund Award from the University of Maine, and by NSF-DUE-CCLI Grant # 0087657.

Mary S. Tyler

Orono, ME 04469

April 2003

Most of the royalties from this CD-ROM will go to supporting research in developmental biology at the University of Maine.

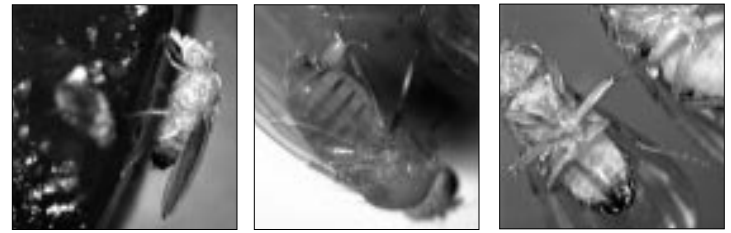


1. LIFE CYCLE (SUMMARY)

Sequence built as a moving collage by David Tartaglia from footage filmed by Mary S. Tyler and Jamie W. Schnetzer at the University of Maine.

This sequence summarizes the life cycle of the fruit fly, *Drosophila melanogaster*. Adults start the cycle with courtship and mating. During courtship, a male sings a **love song** to the female by vibrating his wings one at a time. After mating, the female lays fertilized eggs into the food source. After about a day of development, a small, wormlike larva hatches from the egg shell. The larva eats its way through the food, **molting** twice as it grows. Molting separates the larval stage into three **instars**. During the last instar, the larva crawls out of the food source to begin **pupariation**, during which the outer larval covering transforms into a hard, protective case. Becoming first a **prepupa** and then a **pupa**, the organism is transformed through the process of **metamorphosis** into an adult fly as most of the larval organs are destroyed. This adult is built primarily from sets of imaginal cells set aside in the larva for this purpose. After several days, a young fly **ecloses**, flipping open an anterior door in the pupal case and pushing itself free, usually into the moisture of the early morning dew.

The name of this movie comes partly from the realization that in its life cycle, *Drosophila* goes through what appears to be two distinct lives: the wormlike larva and the appendaged adult. This exquisite adult is constructed, not primarily from modified larval structures as might be expected, but mostly anew from undifferentiated cells that were carried around in the larva like excess baggage. It is an astonishing way to travel through life. Welcome to the lives of a fly, *Drosophila melanogaster*.



2. THE ADULT OR IMAGO

Sequence filmed by Mary S. Tyler at the University of Maine.

The adult stage of *Drosophila* is beautifully constructed for its tasks. The body, made up of three major regions—the head, **thorax**, and **abdomen**—is a feeding and reproductive machine whose success we've all experienced on our overripe bananas.

Because the adult male and female are sexually dimorphic, they can be easily distinguished from one another. The male is smaller than the female, with a shorter abdomen that is solid black at its posterior dorsal surface. The female's posterior end is striped dorsally.

On the ventral surface of the abdomen, males have heavily bristled **anal plates** that are darker than the female's. Also, the female's posterior end is pointed, while the male's is rounded. The female shown in this sequence is extruding an egg, which often occurs while recovering from anesthesia.

Males have a pair of black **sex combs** on their forelegs. These fringes of tiny black stout bristles, shown in the film at low- and higher-power magnifications, help the male pull himself up onto the female's back during mating.

The thoracic region of the fly bears the locomotory appendages. Ventrally, there are three sets of walking legs; dorsally, there is a pair of wings and a pair of fleshy balancing organs, called **halteres**. The halteres, sitting behind the wings, represent a modified second pair of wings. They vibrate at the same rate as the wings during flight and are needed for equilibrium during flight.

The thoracic region is also noteworthy for its extensive musculature. This is demonstrated in histological sections through the fly. These 8- μ m-thick sagittal sections, stained to increase contrast, are lateral to the midline.

The head region is a complex of sensory organs with a pair of large **compound eyes**, three light-sensitive **ocelli** on top of the head, and ante-

riorly, a pair of **antennae** with feathery extensions called **aristae**. It is through the aristae that vibrations, such as those produced by the male's love song, are picked up. These are transmitted down the antenna to the **antennal organs**, called Johnston's organs, near the base of the antenna, and relayed to the antennal lobes of the brain by way of the antennal nerve. A histological section through the head shows the antennal organs and the antennal lobes of the brain.

In the histological section through the head, shown both at low- and higher-power magnifications, you will also see the complexity of the large compound eyes, with their numerous light-sensitive **ommata**, each with its own **corneal lens**. To service the eyes, the brain has large **optic lobes**. A histological section taken from a more ventral region of the head shows a peculiar feature, the passage of the esophagus right through the middle of the brain.

A high-power magnification of the living animal shows a prominent feature of the head, the **proboscis**, which is the feeding organ of the adult. It is shown being extended and retracted. The soft **labial palps** at the distal end of the proboscis contact the surface of the food, and a strong **sucking pump** at the base of the proboscis sucks it into the esophagus.

A histological section through the head shows the hard **sclerotized plates** and extensive musculature of the sucking pump.

Adults feed in the moist crevices of their food supply. Females in particular need to feed on a protein-rich diet to reproduce successfully. When sexually mature, flies will court and mate.

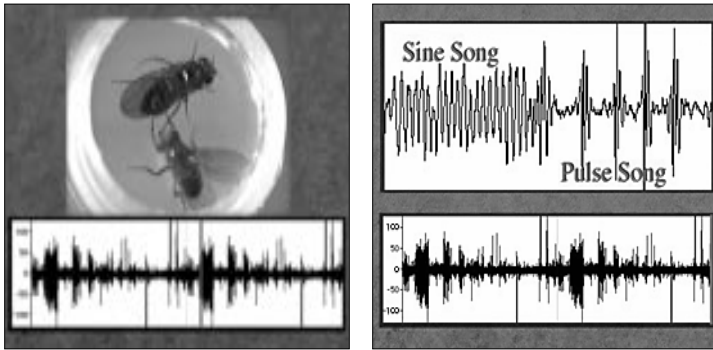


3. COURTSHIP

Sequence filmed by Mary S. Tyler and analyzed by John M. Ringo at the University of Maine.

Courtship precedes mating. Typically, the male first orients towards the female. You may see the male tap the female's **abdomen** with his forelegs, most likely "tasting" her to make sure she is the right species. He then follows her and, vibrating his wings one at a time, produces his **love song**. When he gets close to the posterior end of the female, he extends his **proboscis** and attempts to lick the female's **vaginal plates**. At this point, he often curls his abdomen and tries to intromit and crawl onto the female's back. Note that the male often interrupts his courtship to preen.

The female in these sequences is unreceptive. This is typical in females that have already mated or that are newly eclosed (for up to about 10 hours). The female exhibits a series of behaviors that represent rejection of the male. She rejects the male by fending him off with her middle leg, walking away (**decamping**), **flicking** her wings, and kicking the male with her hind legs. She also extends her **ovipositor** in rejection. Sometimes a female also moves both wings rapidly in a scissoring motion as a rejection signal.



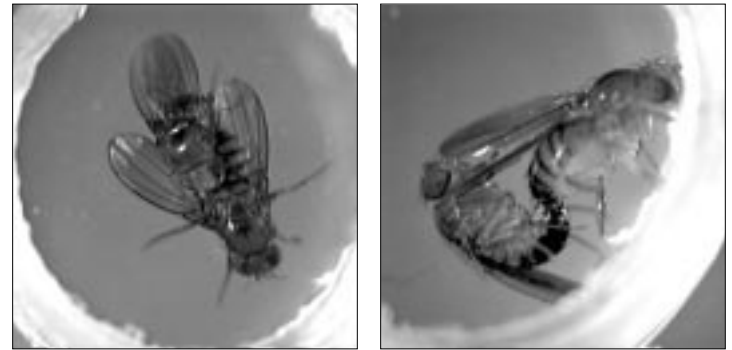
4. THE MALE'S LOVE SONG

Sequence filmed by Mary S. Tyler. Electronic recording and analysis of the male's love song provided by Becky Talyn and Harold B. Dowse. Moving collage constructed by David Tartaglia. All are at the University of Maine.

The male's **love song** is created by extending and vibrating a wing. In this sequence this movement is shown slowed down to one-half normal speed. This sequence contains a sound recording of a male's love song, played at normal speed. This recording was made by placing flies in a sound chamber and amplifying the song electronically while tracing the changes in amplitude over time with an oscilloscope. The oscilloscope trace for this song is shown in the sequence, and the scroll bar that moves across this trace is synchronized with the sound. The two components of the love song, the **sine song** and the **pulse song**, are labelled on the trace.

The sine song is created by low-amplitude wing vibrations and is heard as a humming sound. The pulse song is created by high-amplitude motions and is heard as a purring sound.

Sine song most likely functions primarily to increase receptivity of females in *Drosophila melanogaster* (von Schilcher, 1976). Though there are species-specific characteristics in sine song (Cowling and Burnet, 1981; Wheeler, Fields, and Hall, 1988), it is more likely that it is the intervals between pulses in the pulse song, with more strongly species-specific characteristics, that contribute to sexual isolation between species (von Schilcher, 1976; Cowling and Burnet, 1981; Wheeler, Fields, and Hall, 1988).

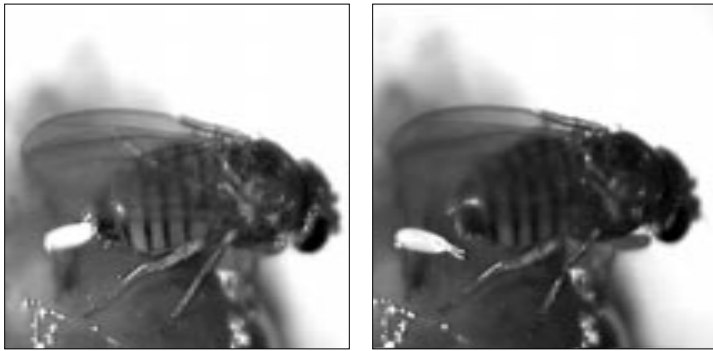


5. MATING

Sequence filmed by Mary S. Tyler and analyzed by John M. Ringo at the University of Maine.

A receptive female allows mating to occur. The female in this sequence is receptive, and the onset of mating occurs rapidly. After a short courtship, the male mounts the female posteriorly, hooks his legs over her wings, and hangs on using his sex combs. His curled **abdomen** makes contact with the female's **vaginal plates**, and a mixture of **sperm** and seminal fluid is pumped into the female's reproductive ducts where it is stored.

The transfer of sperm and seminal fluid takes approximately 20 minutes, with sperm being transmitted in the early part of this transfer. Short sequences from throughout the process have been spliced together to show typical behavior. Notice that there are quiescent periods during mating, interrupted by the female walking and kicking the male with her hind legs. Near the end of mating, the female kicks more frequently. The male then dismounts. In this sequence, almost immediately following mating, the female fends off the male, and both the male and female preen.



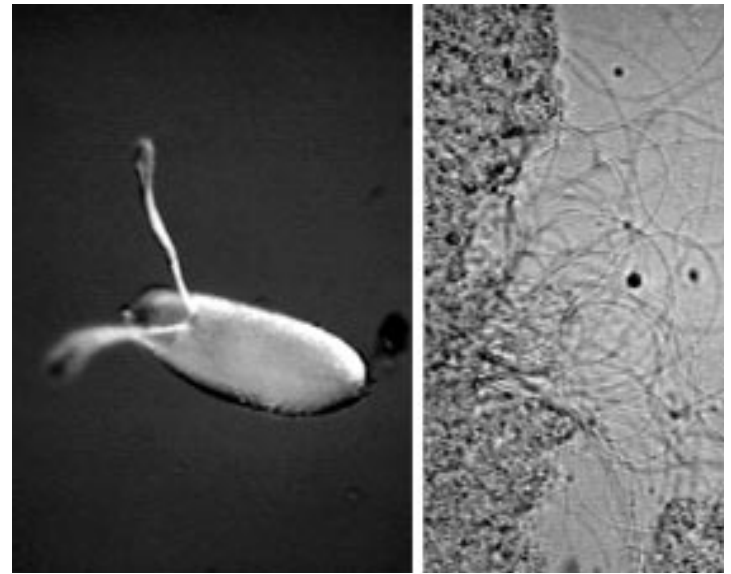
6. EGG LAYING

Sequence filmed by Mary S. Tyler at the University of Maine.

In preparation for egg laying, a female searches for a wet surface on a food source, and chooses one where the air above it is close to saturation (high humidity). In this sequence the food source was dyed blue with food coloring for better contrast, and was moistened with water.

Rhythmic contractions of genital and uterine muscles protrude the female's ovipositor and help to move the egg down the **uterus** and through the **vulva** to the outside. The female makes several attempts to extrude the egg before it fully emerges. She often preens her posterior end with her hind legs while curling her abdomen, which may help to remove the egg. A clear fluid droplet is released as the egg is emerging; this is frequently seen during egg laying. The egg emerges posterior end first, with its long slender respiratory filaments being the last to emerge.

Typically, a female embeds her egg in the food source as she lays it so that just the **respiratory filaments** protrude. In this sequence, the food medium is hard, and the egg is laid directly on the surface. The egg is coated with a sticky substance secreted by the female's accessory glands so that it attaches to surfaces.



7. THE GAMETES: EGGS AND SPERM

Sequences filmed by Mary S. Tyler at the University of Maine.

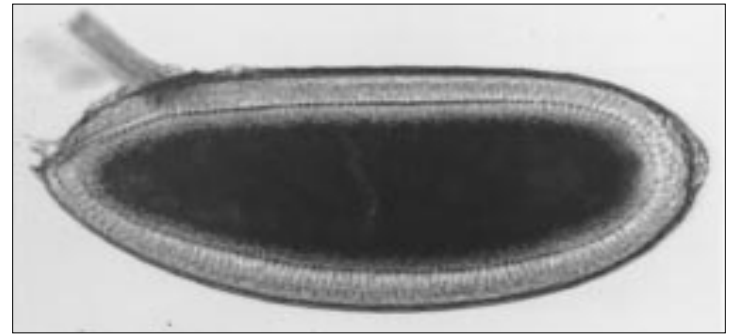
Egg: The *Drosophila* egg, about one-half mm long, is relatively large, considering a female can lay often 30, but as many as 100 eggs a day, and over 1,000 in her lifetime. The yolky, opaque egg is surrounded by a tough, protective eggshell, or **chorion**, which is modified anteriorly to form long **respiratory filaments**. The ends of these are flattened as paddles. At the anterior end, a small channel through the chorion—the **micropyle**—provides an entryway for sperm at fertilization. This is shown at a high magnification.

The chorion, especially in the region of the respiratory filaments, is designed for maximal gas exchange with minimal water loss. It consists of two layers that bound an interior layer of tiny air pockets that connect with an extensive meshwork of air spaces in the respiratory filaments. The respiratory filaments also have a water-repellent surface network that maintains a film of gas around them when submerged, allowing them to function as gills. At the anterior end of the chorion, a horseshoe-shaped ridge or collar outlines a doorway, the **operculum**, which will be used as an exit when the larva hatches. On the surface of the chorion, a pattern of ornamental markings is the imprint of ovar-

ian follicle cells that deposit the chorion around the egg prior to its ovulation.

Sperm: This sequence shows *Drosophila* sperm moving around in a balanced salt solution (insect Ringer's solution; see recipe in Tyler, 1994) just after being dissected from a female's reproductive tract. Following mating, sperm are stored in several organs of the female's reproductive tract, the paired spermathecae and the single seminal receptacle. Shown under both bright-field and phase-contrast microscopy, the sperm can be seen whipping their exceedingly long tails, or flagella. Each sperm is about 1.75 mm long, or over three times the length of the egg. Most of this length is made up of the sperm's flagellum, which is 200 times longer than the narrow, tiny head.

This footage also shows sperm still within the tubules of an intact spermatheca. These remain active for extended periods. The whole mass of sperm undulate in beautiful patterns, looking like long, whipping threads.



8. EMBRYONIC DEVELOPMENT

Sequence filmed by Rachel Fink at Mount Holyoke College in collaboration with Eric Wieschaus at Princeton University.

Preparation: This sequence shows the egg of the mutant *klarsicht* (German for “clearly seen”). This maternal-effect mutation enhances the contrast between the yolk and cytoplasm in stages past the blastoderm stage. This is most likely due to a reduced number of lipid droplets incorporated into the cells during cellularization; it does not alter development.

The egg was placed in halocarbon oil to increase transparency of the chorion. Events were filmed by time-lapse microscopy so that the final footage is 612 times faster than real time. Actual time (in hours:minutes:seconds) is indicated at the upper left in the sequence.

Events: You will see embryonic development from early cleavage to hatching. Development in *Drosophila* begins with nuclear divisions in the central yolk-filled cytoplasm. These divisions are marked by cytoplasmic contractions. The nuclei then migrate to the periphery.

The first cells to form are the pole cells at the posterior end of the embryo. These pole cells are the primordial germ cells. They will later move to the site of the developing gonad and eventually form the gametes.

While the pole cells are forming, the rest of the embryo is still a syncytium, with its nuclei lined up in the peripheral cytoplasm. As nuclei continue to divide, divisions continue to be marked by cytoplasmic contractions.

Cellularization begins as cell membranes grow inward between the nuclei that are lined up peripherally. This will be seen as a wave front of cellularization, which moves inward, first slowly and then more rapidly.

The **blastoderm**—the layer of cells formed by cellularization—becomes thicker and is clearly discernible from the yolk. Ventrally, the cells form a **germ band**, the multilayered band of germ layers (**mesoderm**, **ectoderm**, and **endoderm**) that curves around the embryo's posterior tip.

Gastrulation then begins. Gastrulation is the series of cell movements that rearrange the germ layers into their final positions for subsequent organ formation. The first signs of gastrulation occur ventrally and are obscured from view in this sequence. The **mesoderm** (the precursor to structures such as somatic and visceral musculature, the heart, and nongerm cells of the gonad) invaginates, forming the **ventral furrow**. **Germ-band extension**—the elongation of the germ band along the dorsal side of the embryo—then carries the pole cells forward.

Since gastrulation happens rapidly, a freeze frame is used to show some of these events. The sequence shows anterior **endoderm** invaginating ventrally to form the **anterior midgut**, and posterior endoderm invaginating dorsally to form the **posterior midgut**. The germ band buckles ventrally behind the head fold and is seen as a slight indentation.

The **foregut** is formed by the invagination of a region of **ectoderm** called the **stomodeum**. First the ectoderm thickens and flattens to form what is called the **stomodeal plate**. The stomodeum then invaginates. As the invagination deepens, it bends ventrally to eventually make contact with the anterior midgut. At this point the yolky region looks dark against the lighter **germ band**.

The anterior and posterior midgut regions elongate. As this is happening the **germ band retracts** by shortening and thickening, which brings the posterior end of the embryo back to its proper posterior position.

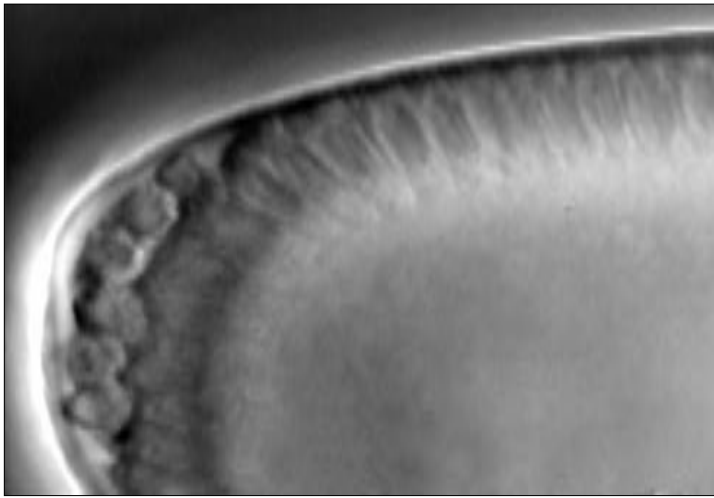
The anterior and posterior regions of the midgut continue to grow toward each other and eventually fuse. At this point, segmentation of the germ band is obvious.

Anteriorly, **head involution** causes the head region to slowly disappear to the interior. This is a remarkable event, producing a larva that appears to have no head.

The **midgut** takes on a distinct heart-shaped appearance and then becomes subdivided into three distinct regions. Soon, the first muscular contractions can be seen in the gut. Contractions then begin in somatic muscles. A short real-time sequence is used to show the contractions of the embryo at actual speed.

The **tracheal** system, a ladderlike network of tubes throughout the body which constitutes the respiratory system of the organism, becomes visible by filling with air. The two dorsal tracheal trunks can be seen ending in the posterior **spiracles**.

At this point, the embryo moves, reorienting itself, such that it is seen from its dorsal surface. All the major organ systems are established and in their final positions. The developing mouth parts can be seen; the gut which has been constricting now forms a long, convoluted tube; the **dorsal vessel**—or heart—is beating, pumping **hemolymph**; the **gonad**, which develops around the **pole cells**, is visible. Finally, the first instar larva hatches from the **chorion** by breaking open the **operculum** at the anterior end.



9. POLE CELL FORMATION, CELLULARIZATION, AND EARLY GASTRULATION

Sequences filmed by Daniel Kiehart at Duke University, Shinya Inoué, at the Marine Biological Laboratory, and Paul E. Young, Miles Laboratories.

Preparation: These high-magnification sequences, using Nomarski optics, were filmed by time-lapse microscopy so that the final footage is from 30 to 60 times faster than real time. A digital clock shows the passage of real time (in hours:minutes:seconds) during the sequences. To prepare the eggs for videotaping, the filmers first removed the chorions, then submerged the eggs in halocarbon oil in a customized chamber (Kiehart et al., 1994).

Pole cell formation: Pole cell formation is shown in two separate embryos. The pole cells are the primordial germ cells that later become the [gametes](#).

Cellularization: During [cellularization](#), cell membranes extend inward between the peripheral nuclei. As this occurs, the nuclei elongate. Once the membranes have extended inward past the level of the nuclei, their further inward extension becomes more rapid. As cellularization nears completion, the cells are pinched off from the inner yolky cytoplasm.

Early gastrulation: [Gastrulation](#) movements bring the pole cells dorsally. This is shown first in side view and then from the dorsal surface. As the posterior [endoderm](#) invaginates, the pole cells are internalized.



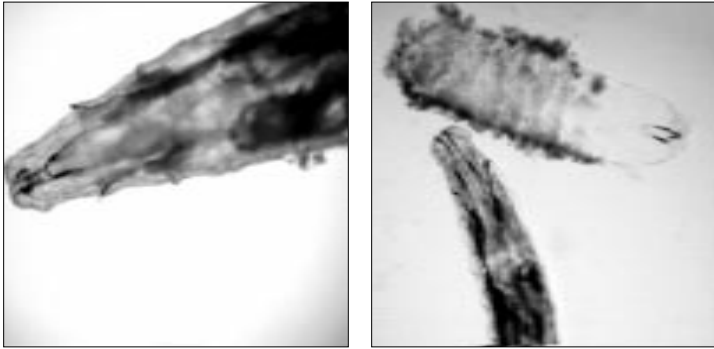
10. HATCHING

Sequence filmed by Mary S. Tyler at the University of Maine.

Prior to hatching, the fully developed larva moves actively within the [chorion](#). Soon it will push open the [operculum](#), the “door” at the anterior end of the chorion, to exit the chorion. This “door” can be seen clearly in this sequence as a region that is lighter in color than the rest of the chorion.

The severe contrast between the opaque chorion and transparent larva makes seeing the opening of the operculum difficult, but if you look carefully in the higher-magnification sequence just before the film switches back to lower magnification, you will see the operculum popping open and the larva emerging. At lower magnification, you can see the larva as it emerges from the chorion using muscular movements. This sequence was speeded up three times faster than real time.

Once the larva has emerged, the extensive ladderlike [tracheal](#) system, which ends in the posterior [spiracles](#), is easily seen (a [first instar](#) larva lacks anterior spiracles). Anteriorly, the black [chitinous](#) mouth parts are also obvious.



11. THE LARVA

Sequences filmed by Jamie W. Schnetzer and Mary S. Tyler at the University of Maine.

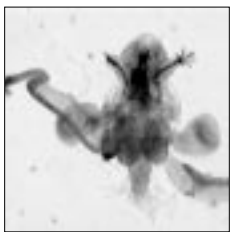
The three instars: After hatching, larvae spend about five days burrowing and feeding in a moist food source. (The timing is temperature-dependent, as is all of its development.) Enclosed in a tough, flexible **cuticle**, the larva moves by extending and contracting segmental muscles and gripping the substrate ventrally with rows of small hooks, or **denticle belts**. These are shown at higher magnifications. **Chitinous** mouth hooks bring food into the body. Growing rapidly, the larva **molts** twice, separating the larval period into three instars. The three **instars** are shown lined up, so that comparisons in size can be made. A third instar larva is seen crawling.

Molting or ecdysis: In this sequence, a larva is shown in the process of **molting**, shedding its **first instar** larval cuticle, spiracles, and mouth parts as it becomes a second instar larva. A larva just prior to molting can often be spotted by its double set of mouth hooks. The larva first pierces the old **cuticle** with its new set of mouth hooks, opening up the anterior end. A higher-magnification view shows the new and old mouth parts clearly.

With its posterior end free as well, the larva moves back and forth within the old cuticle. A higher-magnification view shows the new posterior spiracle. Eventually, the larva backs out of its old cuticle, leaving the old cuticle and mouth parts behind.

The third instar: The third instar larva is often used in studies because it is the largest of the three instars and its organ systems are more easily

located. A number of these organs can be seen through the transparent cuticle: the silvery, branched **tracheal** trunks; sheetlike **fat bodies**; yellowish-white, cordlike **Malpighian tubules** constituting the excretory system; and, in males, the large, transparent **testes**. In females the tiny **ovary** is difficult to see. This gonadal size difference allows larvae to be easily sexed.



12. DISSECTION OF THE THIRD INSTAR LARVA: SALIVARY GLANDS, IMAGINAL DISCS, AND GONADS

Sequences filmed by Mary S. Tyler at the University of Maine.

These sequences show a dissection of the salivary glands and imaginal discs of a third instar larva. Identifications of the [imaginal discs](#) and of the male and female [gonads](#) are also shown. A simple dissection is demonstrated in which the larval [cuticle](#) is torn by a straight pull using fine forceps that grip the larva at each end. This often leaves the internal structures relatively intact and in their normal relative positions. The film shows the brain and [ventral ganglion](#), imaginal discs, and the salivary glands. Imaginal discs are also shown from the ventral aspect of the brain and ventral ganglion. The film then shows how the salivary glands and imaginal discs can be separated from their attachments by dissecting with fine needles drawn against one another.

Salivary glands: Dissection of salivary glands is an important technique to learn, as these glands are often used for making [chromosome](#) squashes to see the large, [polytene chromosomes](#) typical of larval organs.

The salivary glands, viewed with transmitted light, look light against a narrow ribbon of darker [fat body](#) that is attached along one side. The salivary glands have large cells, as do other larval organs. These cells are shown at higher magnification so that you can see the prominent nuclei that contain the huge polytene chromosomes. These chromosomes have been replicating throughout the larval period without separating, and by the third instar larval stage can have as many as 1,024 copies side-by-side. Stain-absorbing regions of these copies lined up in register form distinct bands across the chromosome. It is for this reason that these cells are used for viewing chromosomes. The banding patterns shown by the staining give a visual map of each chromosome. Geneticists use these patterns to precisely map the location of genes.

To show the banding patterns of these chromosomes, the salivary glands can be stained with [aceto-orcein](#) and squashed between a slide

and coverslip. In the film, the eraser end of a pencil is used to press against the coverslip. Under the compound microscope, the red-stained nuclei are evident. If sufficient pressure is used when pressing on the coverslip, nuclear membranes are broken and the arms of the chromosomes are splayed out. This is shown at higher magnification.

In addition to being large, these chromosomes provide another advantage to geneticists: they are homologously paired (the maternal chromosome is paired with the homologous paternal chromosome). This greatly facilitates the recognition of chromosomal aberrations, so important in the study of *Drosophila* genetics (see Demerec and Kaufmann, 1986).

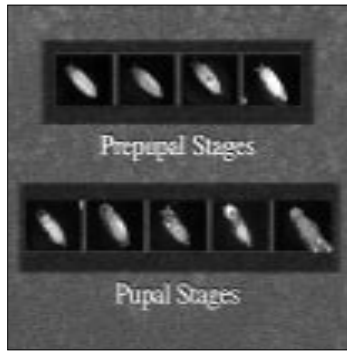
Imaginal discs and gonads: These sequences show the individual imaginal discs and gonads. Imaginal discs are the packets of folded epithelium that will form adult structures, primarily external, such as wings, [halteres](#), legs, [antennae](#), eyes, and other structures. They are carried around within the larva throughout the larval period, during which they grow in size through cell division, but do not unfold and differentiate until metamorphosis.

A wing disc is shown as it is typically found, clumped with a third-leg disc and haltere disc. The film shows a clump that was treated with trypsin, which causes partial eversion of the haltere disc and the third-leg disc. The paired first-leg discs often separate together. These are shown at higher magnification while focusing through the discs to show the folded nature of the discs.

The [eye-antennal discs](#), which are studied extensively in research, are shown from both dorsal and ventral aspects. A single eye-antennal disc is shown at higher magnification while focusing through the disc. Both discs are then shown in position where they sit dorsal to the brain, with the eye portion cupped around the anterior region of the brain and the antennal portion extending forward.

A [humeral disc](#) is shown ringing the base of the anterior [spiracle](#). The [labial discs](#) that will form the adult [proboscis](#) are tiny and difficult to find. A labial disc is shown first in isolation and then in its normal position sitting next to the black mouth parts.

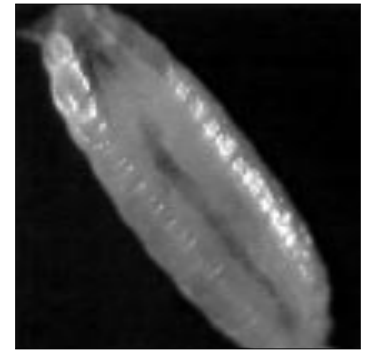
In the posterior region of the larva, the small, single, [genital disc](#), the only imaginal disc that is unpaired, can be found. For size comparisons, a genital disc is shown alongside a testis which is embedded in fat body. A pair of [testes](#) is then shown. A testis is also shown alongside an [ovary](#). The ovary, also found embedded in [fat body](#), is much smaller than the testis.



13. METAMORPHOSIS (SUMMARY)

Sequences filmed by Jamie W. Schnetzer, collage built by David Tartaglia and Mary S. Tyler at the University of Maine

When a third instar larva extends its anterior [spiracles](#), metamorphosis is about to begin. During [metamorphosis](#), most larval structures are destroyed and adult structures differentiate. This all occurs within a hard, protective [puparium](#). A collage of the process shows that it can be divided into two phases: [prepupal](#) and [pupal](#). Bainbridge and Bownes (1981) devised the staging series used here to characterize metamorphosis.

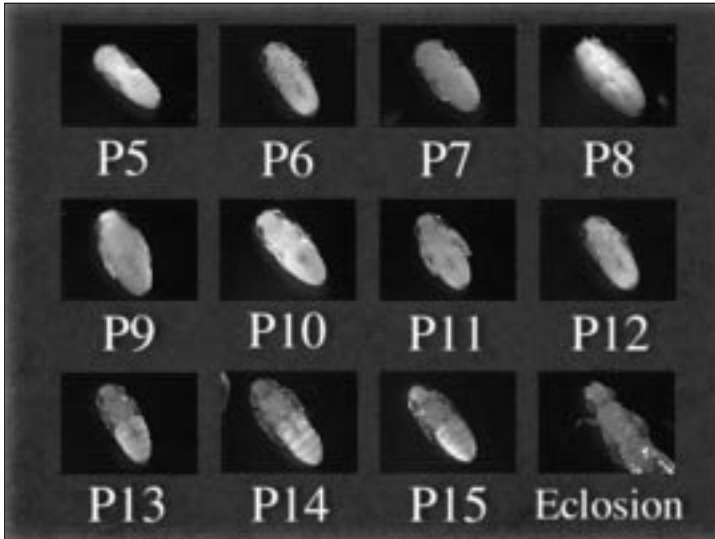
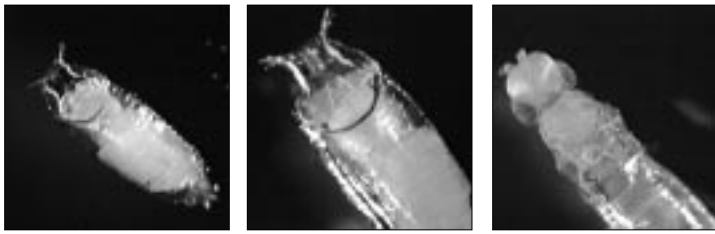


14. FIRST PREPUPAL STAGE: THE WHITE PUPA AND ITS BEATING HEART

Sequence filmed by Jamie W. Schnetzer and electronically produced sound of the heart provided by Erik C. Johnson and Harold B. Dowse at the University of Maine.

Upon entering the first [prepupal stage \(P1\)](#), the larval body shortens and becomes immobile. With the organism immobile, it is easy to see the movement of the larval mouth hooks and the pumping dorsal vessel, or heart. This stage, called the [white pupa](#), is ideal for studying heartbeat. In this footage, you will see the [dorsal vessel](#) beating as it pumps blood, or [hemolymph](#), through the organism's open circulatory system. Later in metamorphosis, the heart will stop beating as tissues are reorganized.

As you watch the heart beat, you will hear in the background the sound of the heartbeat electronically translated from an optical recording. In this method, contractions of the heart were detected by a sensing device in the ocular of the microscope through which the organism was being observed. The signals were then amplified, recorded digitally, and recreated as sounds by a digital audio processor (Dowse et al., 1995, p. 155).



15. PREPUPAL AND PUPAL STAGES

Sequences filmed by Jamie W. Schnetzer at the University of Maine.

Prepupal stages (P2–P4): After the [white pupal](#) stage, the larval [cuticle](#) tans, forming the puparium. The [prepupa](#) shown has been immersed in order to make the [puparium](#) transparent (P2). Within the puparium, most of the larval organs are beginning to self-destruct. Simultaneously, [imaginal discs](#) evert. Two everting discs are shown—an everting wing disc and an everting leg disc—both at low and then higher magnifications. The next stage is marked by the appearance of a gas bubble within the puparium below the beating heart (P3). Soon the head will evert (P4).

Head eversion: [Head eversion](#) marks the transition from prepupa to pupa (P4–P5). Muscular contractions and movement of gas bubbles

precede head eversion. The head capsule detaches from the larval spiracles, and mouth hooks are ejected. Finally the head capsule everts. This remarkable process is shown twice, first in real time and then speeded up.

A [histological section](#) of a pupa that was fixed just after head eversion and cut in a frontal plane shows some of the developing organ systems. The anterior end is facing toward the right. The section shows the everted wing discs lying alongside the [thorax](#), the eye developing next to the [optic lobes](#) of the brain, and red-stained [fat body](#) cells surrounding the brain. Distinct head, [thorax](#), and [abdomen](#) regions are visible. In the lighting used, the brain looks blue against the white fat tissue of the head capsule.

Pupal stages (P5–P15): Following head eversion, the [pupal](#) stages begin. The pupal [cuticle](#) separates from the [puparium](#), making it possible to dissect a pupa from its pupal case. As a dissection of the first pupal stage is done, you can see how the puparium can be removed easily using fine forceps. Gluing the pupal case to the substrate first using cyanoacrylate glue (“super glue”) can facilitate this dissection. Looking at the first pupal stage from the side, you can see that a translucent patch is visible in the developing eye. This feature marks this stage as P5. Legs and wings extend ventrally.

In a slightly older pupa, the [Malpighian tubules](#) developing in the [abdomen](#) have turned from white to green (P5–P6). The pupal cuticle looks like a clear bag enclosing the pupa.

Another pupa is shown being dissected from its pupal case. In this pupa, eye pigmentation has begun to develop. The eyes are first light yellow (P7–P8), then darken to amber (P9), and finally become bright red (P10). Bands of thoracic muscles are clearly visible. Malpighian tubules are bright green anteriorly and yellow posteriorly.

Later, the tips of the wings become light gray (P11). In ventral view, sex combs are visible on the male’s forelegs (P12). Wings then darken to black (P12–P13). The head, thoracic, and abdominal [bristles](#) are now black. Claws on the tips of the legs become visible (P13–P14).

At the end of metamorphosis (P15), the adult fly, fully formed within its puparium, is ready to emerge. The legs can be seen twitching in preparation for this event.

The pupal stages span one hundred hours. This is summarized in a collage showing still pictures of each of the individual stages of the pupa, dissected from the puparium. It demonstrates the gradual transition to the fully formed adult.

Eclosion: **Eclosion**, the emergence of the adult fly from the **puparium**, begins with muscle contractions. Next, the **operculum** opens, being forced open by a sac called the **ptilinum** on the front of the fly's head. The balloonlike ptilinum, highly deformable at this stage, expands with blood to break the seam of the operculum. Even after the fly's head has emerged, the ptilinum continues to expand and contract. The fly then uses abdominal contractions and movements of its legs to finish exiting from the puparium. A second eclosion sequence shows that in conditions of higher humidity emergence is quick. Once emerged, the newly eclosed fly preens. Its wings are still folded and its **cuticle** is soft and white.

Wing inflation: In this sequence, the gradual unfurling of the wings is shown. Following eclosion, blood flowing between the two epithelial layers of the wings extends and flattens them. The fly preens often as the **wings inflate**. The final stages of wing inflation are shown speeded up nine times. A higher magnification view shows the wings fully extended.

As the fly sits still, note that its cuticle is still soft, its **ptilinum** still domed, and prominent. The three **ocelli** on top of the head are easily visible. Soon, the cuticle darkens as it tans, and the ptilinum contracts.



16. GENES AND MUTANT FLIES

Sequences filmed by Mary S. Tyler. Mutants chosen in consultation with John M. Ringo and Erik C. Johnson and provided primarily by Erik C. Johnson. Collages built by David Tartaglia. All are at the University of Maine.

The identification of individual genes from new mutations was started in 1910 with Thomas Hunt Morgan's report, published in the journal *Science*, of finding a male *Drosophila* with white eyes. He called this mutation *white*. The *white* gene was the first gene to be assigned to a specific chromosome. The importance of Thomas Hunt Morgan to the study of genetics is celebrated here with a collage of photographs, superimposed on one another, each with a special meaning. You will see first the photograph—taken by a family friend, Dr. Tove Mohr—used by newspapers to announce Morgan's receipt of the Nobel Prize in 1933. Morgan had insisted that his picture be taken with the neighborhood children. As the photographs are peeled away, you will see a close-up picture showing Morgan's keen but twinkling eyes and the beard and mustache which he only shaved off occasionally to surprise his family. Next is a photograph of Morgan studying his flies in his usual pose, standing up. Only lazy investigators sat down to study flies, he would joke. The final photograph is the passport picture of T. H. Morgan and Lilian Vaughan Sampson Morgan, his wife, that they used when going over to Stockholm to receive T. H. Morgan's Nobel Prize, six months after the official ceremony. He was too busy to go at the scheduled time, he said, and, moreover, wanted to take his family abroad when he went.

Since Morgan's discovery of the *white* gene, over 4,000 other genes have been identified in *Drosophila*, primarily through mutations, and mapped to their locations on *Drosophila melanogaster's* four chromosomes. The location of a mapped gene is by tradition represented in numeric notation, where the first number represents the **chromosome** the gene is on, and the second number, separated by a hyphen, repre-

sents its position on the chromosome. The location of the *yellow* gene, for example, which is near the tip of chromosome 1, is designated as 1-0.0. (Chromosome 1 = X chromosome.)

Mutant forms of genes can help geneticists determine gene function and, once mapped to a region of a chromosome, can be used as markers for mapping other genes. Some of the mutations commonly used as markers are shown in the next segments of the film. The final picture in this introductory sequence is of a fly carrying the *yellow* mutation filmed with its mirror image being reflected in the glass slide it is standing on. (Reminiscent, perhaps, of the myth of Narcissus.)

y: yellow, location: 1-0.0: The first sequence in this gallery of mutations is that of a fly carrying the *yellow* mutation. The protein encoded by the *yellow* gene controls deposition of the black pigment **melanin**. A fly with a mutation in this gene lacks melanin in its **cuticle** and appears yellow.

Though it is not yet clear how the *yellow* gene product functions in melanin deposition, it appears during the embryonic and pupal stages. In the **pupa**, it appears 26 hours before the deposition of melanin in the regions that will be pigmented.

e: ebony, location: 3-70.7: The flies in this next sequence carry the ebony mutation. This gene codes for an enzyme used in the tanning process. The extremely dark coloration of the ebony mutant is a result of the slowed tanning of its **cuticle** following **eclosion**; this allows more **melanin** than usual to be deposited in the cuticle. A view of a newly eclosed fly with this mutation shows that initially the fly is lighter in color.

w: white, location: 1-1.5: A fly with the *white* mutation fails to deposit either the brown (ommochrome) or red (pteridine) pigments normally found in the eyes, so the eyes appear white. These pigments are necessary for proper image formation. The mutants, though sensitive to light and even phototactic, are unable to form accurate visual images.

The *white* gene is thought to code for a transport protein used to transport pigment precursors in both the brown- and red-pigment pathways. It is maximally expressed during the first few days of the **pupal** period when eye pigmentation is developing.

B: Bar, location: 1-57.0; f: forked, location: 1-56.7: The first fly in this sequence is **homozygous** for the *Bar* mutation (more technically, a chromosome aberration caused by a tandem duplication), which causes the eyes to be narrowed to a bar shape due to a reduced number of **omma-**

tidia (less than 100 as opposed to over 700 in wild-type flies). This fly is also **homozygous** for the *forked* mutation, which affects the secretion of **bristles** during the **pupal** stage and causes the bristles to be bent. A heterozygote for these mutations is also shown. It has normal bristles but a kidney-shaped eye. Looking at both **heterozygote** and wild-type flies together, you can see how the heterozygote's eye is notched on its anterior margin while the wild-type eye has a smooth margin.

ap: apterous, location: 2-55.2; cn: cinnabar, location: 2-57.5: The flies shown in this sequence are **homozygous** for two mutations, *apterous* and *cinnabar*. Since these two genes are located close to one another, this double mutant is useful to researchers who want to map another gene they suspect is located nearby. The animal's eyes are bright red due to the mutant *cinnabar* gene. This gene codes for an enzyme that is needed for synthesizing the brown pigment of the eyes, and without this brown pigment only the red pigment shows. A mutant fly is shown alongside a wild-type (on the left) so that eye color can be compared. Brown pigment is the only pigment normally found in **ocelli**, so in these mutants the ocelli are white.

Because of the *apterous* mutation, these flies appear to have no wings. This mutation causes several defects, the most obvious being a reduction of both wings and **halteres** to small stubs.

bw: brown, location: 2-104.5; vg: vestigial, location: 2-67.0: In this sequence there are flies carrying the two mutations *brown* and *vestigial*. These two markers are relatively distant from one another, but are still close enough to be useful for mapping a gene in this region. The eyes in this mutant appear brown. The *brown* gene is needed for synthesizing red eye pigment. Without the red pigment only the brown pigment shows. A wild-type fly is shown on the right for comparison.

You will notice that the size of the wings of these flies is reduced. This is due to the mutant *vestigial* gene which controls the development of the wing margins. Researchers who have looked at the wing **imaginal discs** of these mutants have found numerous degenerating cells in the region of the presumptive wing blade (see Lindsley and Zimm, 1992).

Cy: Curly, location: 2-6.1: *Curly* is another gene involved in wing formation. The fly shown carries the mutant *Curly* gene and as a result has wings that are curled upward. This curling occurred after **eclosion** when the upper wing **epithelium** contracted more strongly than the lower epithelium as the wings were drying. *Curly* is another useful dominant marker. (*Curly* is also important as a dominant marker for a balancer

chromosome, used to maintain lethal mutations in a way that doesn't require selection—see Ashburner, 1989, p. 529.)

Sb: *Stubble*, location: 3-58.22: The fly shown is carrying the dominant mutation *Stubble*. The *Stubble* mutation causes the fly's bristles to be unusually short and thick. In the heterozygote, as shown here, the bristles are less than half their normal length and are somewhat thicker, due to smaller and more numerous fiber bundles within the shaft of the bristles.

Antp: *Antennapedia*, location: 3-47.5: When a homeotic gene such as *Antennapedia* is expressed inappropriately, transformations of body regions can result. In the flies shown, *Antennapedia* has been expressed inappropriately in the head, and imperfect legs have formed where antennae should be. The variation in severity of the phenotype among the flies exemplifies the variable expressivity of the gene. Homeotic genes specify body regions. *Antennapedia* specifies the identity of the second thoracic segment. An example of the transformation caused by this dominant mutation is shown at higher magnification and is compared to a normal antenna.

shi: *shibire*, location: 1-51.5: This sequence illustrates the effects of a temperature-sensitive mutation. This type of mutation results in a trait that is normal at one temperature but not at another. The chamber in this sequence contains two flies: one of the flies carries a temperature-sensitive allele of *shibire*, the other is wild type. When exposed to temperatures of 29°C and higher, the mutant fly will become paralyzed. As you watch, the chamber is gradually heated to the restrictive temperature, and the mutant fly becomes paralyzed (*shibire* in Japanese means “paralyzed”). This temperature effect is reversible. As the chamber is slowly cooled back to a nonrestrictive temperature, the mutant fly revives.

Not surprisingly, the *shibire* gene product helps to control synaptic transmissions. The gene codes for a GTP-binding protein called dynamin, which functions in the recycling of synaptic vesicles through endocytosis. At the restrictive temperature, the mutant shows a depletion of synaptic vesicles at the neuromuscular synapses.

GETTING STARTED

If you are interested in doing your own *Drosophila* studies and need help in getting started, or would like a few hints that facilitate classroom work, the following section will be helpful.

A Few Suppliers:

For getting started, or for ordering convenient kits and mutants for classroom use, the following suppliers can be of use. If there is a university or college biology or zoology department nearby, you often can get all that you would need from them.

Carolina Biological Supply Co., 2700 York Road, Burlington, NC 27215.
Tel. 1-800-334-5551; also P.O. Box 7, Gladstone OR 97027. Tel. 1-800-547-1733.

Sells stock cultures and supplies, a number of kits for genetic experiments, as well as a number of mutant strains.

Connecticut Valley Biological Supply Co., Inc., P.O. Box 326, 82 Valley Road, Southampton, MA 01073. Tel. 1-800-628-7748.

Sells stock cultures including eggs, larvae, pupae, and adults. Must order at least three weeks in advance. Also sells a number of mutant strains.

Nasco, 901 Janesville Ave., Fort Atkinson, WI 53538-0901; also 1524 Princeton Ave., Modesto, CA 95352-3837. Tel. 1-800-558-9595.

Sells inexpensive cultures, wild-type flies and mutants. Also sells culture medium.

Culturing *Drosophila melanogaster*

(after Tyler, 2000)

- I. As you have undoubtedly noticed from any fruit basket that sat too long, *Drosophila* thrives on fermenting soft fruits. A very suitable culture medium, therefore, is crushed banana. It provides all the necessary nutrients for both larval and adult stages. The banana can be kept along with the flies in sterile pint jars with cotton or foam-rubber plugs.
- II. Another standard medium, commonly used by laboratories that raise *Drosophila*, is a cornmeal–molasses–agar mixture.
Mix and boil 3–5 minutes:
420 ml water
4.5 gm agar
Add and heat to boiling:
60 ml molasses (unsulfured)

Mix together in separate container until all lumps are removed

49 gm cornmeal

6.5 gm Brewer's yeast

145 ml cold water

Add cornmeal-yeast mixture to molasses-agar mixture.

Boil 5 minutes, stirring constantly.

Cool mixture to 60° C

Add (as mold inhibitor):

3.4 ml propionic acid

Pour culture medium one-inch deep into sterile culture jars with sterile plugs. Pint milk bottles work well, but any wide-mouthed jar fitted with a plug made of cotton covered with cheesecloth or foam-rubber should work well.

A sprinkle of active baker's yeast (from a salt shaker) may be added to each jar before adding flies.

It is important when maintaining cultures not to overcrowd (about 100 flies/pint-culture jar is ideal) and to subculture (transfer to fresh food) every several weeks. This keeps the flies healthy, large, and mold-free.

Collecting eggs:

Collecting fertilized eggs is easy, but it is not easy to catch the very early stages of development, since eggs can be held within the female's uterus after fertilization for a period of time, even as late as just prior to larval hatching. When a female is laying rapidly, however, the uterus is being cleared fast, and eggs in their early stages of development can be obtained. To achieve this, it is best to use cultures of flies that are five days old. A female is within her peak laying period at this time, and is laying eggs as quickly as one every three minutes.

Collecting chamber: A simple collecting chamber consists of an empty culture bottle (any wide-mouthed bottle will do) plugged with cotton or foam-rubber. Wet toweling may be stuffed in the bottom (for humidity). Place approximately forty pairs of flies in the chamber by inverting a culture bottle containing flies over the mouth of the empty bottle. Holding the bottles together, bang the empty bottle against padding on a table top to cause the flies from the upper culture bottle to drop into the empty bottle. Quickly replace the plugs of both bottles.

Use plastic spoons whose handles have been cut so they fit in the culture chamber without touching the plug. Put culture medium on the spoon (the same culture medium that the flies have been grown in), and place one or two of these spoons in the collecting chamber with the flies.

Since flies will quickly flee your fingers and escape from the bottle during this process, it helps to face the bottom end of the bottle towards a bright light. The flies will be attracted away from the mouth of the bottle. Place the bottle on its side so the medium won't slip off the spoons.

After several hours, a suitable number of eggs will have been laid on the spoons, and they can be removed and replaced with fresh spoons containing medium. If you prefer to have a number of different stages of development, including advanced stages, represented on a single spoon, leave the spoon in the collecting chamber for an extended period (up to 24 hours). Females produce the greatest number of eggs in the late afternoon and evening.

Anesthetizing adults:

Often one wants to study or manipulate adults when they are relatively stationary. Laboratories often use ether or CO₂ to anesthetize the animals; however, a cheaper method more suitable for classroom use is to cool the flies. If you surround vials of flies with crushed ice, flies will be immobilized within a short period (about 10 minutes) and can be manipulated easily.

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GLOSSARY

- Abdomen** The posteriormost part of the insect body, behind the head and thorax.
- Aceto-orcein** A staining solution made of 1% natural orcein in a 1:1 solution of glacial acetic acid and 85% lactic acid.
- Alleles** Alternative forms of a gene that occupy a specific position, or locus, on a chromosome.
- Anal plates** Sclerotized plates surrounding the anus. In male *Drosophila*, these are heavily bristled.
- Antenna** Segmented appendage on the head carrying sensory receptors.
- Antennal lobe** Region of the brain servicing the antenna.
- Antennal organ** Known as Johnston's organ, this is the special sensory apparatus of the antenna, found near its base.
- Anterior midgut** In *Drosophila* this is the region of the gut that is just posterior to the foregut and is formed in the embryo by an endodermal invagination. This anterior endodermal invagination will form the anterior midgut and gastric caeca of the larva.
- Arista** (pl. *aristae*) A feathery extension of the antenna through which sound vibrations can be detected.
- Blastoderm** The layer of cells formed in the embryo during cleavage. In *Drosophila* this layer is formed in the periphery of the yolkly egg.
- Bristles** Sclerotized, stout, hairlike projections from the adult cuticle, classified as two main types, larger macrochaetae and smaller microchaetae.
- Cellularization** The process during early development whereby the syncytial blastoderm is turned into a cellular blastoderm. Cell membranes grow inward between the peripheral nuclei, separating off individual cells from the inner yolkly cytoplasm.
- Chitin** The principle component of the cuticle; made up primarily of sugars, often complexed with proteins.
- Chorion** A tough, protective eggshell. In *Drosophila* and other terrestrial insects, it is specially designed for maximal gas exchange with minimal water loss.
- Chromosome** A structure in the cell nucleus made up of the genetic material, DNA, complexed with protein. *Drosophila* has four chromosomes.
- Compound eye** The type of eye found in insects. It is made up of many individual facets, or ommatidia, each with its own corneal lens.
- Corneal lens** In the compound eye, the lens that sits at the distal end of each ommatidium. In *Drosophila*, it is a chitinous structure, biconvex, and approximately 5- μ m thick at its center.
- Cuticle** The hardened outer covering of flies and other arthropods, made of chitin and hardened with the tanned protein called sclerotin, forming a protective layer that prevents water loss.
- Decamp** To walk away. A female *Drosophila* that is unreceptive to a courting male decamps as part of her rejection behavior.
- Denticle belt** Rows of small hooks found on the ventral surface of the larva that help the larva move by gripping the substrate.
- Dorsal vessel** The simple tubular heart of *Drosophila*.
- Ecdysis** Molting; the process of shedding an old cuticle and forming a new one.
- Ecdlosion** The emergence of the adult fly from the puparium.
- Ectoderm** One of the three germ layers in an embryo. The ectoderm gives rise to epidermis, nervous tissue, and foregut. The imaginal discs arise from invaginations of the ectoderm.
- Endoderm** One of the three germ layers in an embryo. In the fly, the endoderm gives rise to the epithelium of the midgut and midgut derivatives.
- Epithelium** A major type of tissue in an organism found lining body cavities and covering the outside of the body. The epidermis is an example of a specific type of epithelium.
- Esophagus** A region of the foregut, just posterior to the pharynx. In *Drosophila* the esophagus passes through the brain.
- Eye antennal disc** The imaginal disc that gives rise to both the compound eye and the antenna of the adult fly.
- Fat body** A sheet of fat cells that serves as site for fat storage. In *Drosophila* these sheets are creamy white.
- First instar larva** The larva that hatches from the egg. This feeding stage grows rapidly and soon molts into the second instar larva.
- Flick** A rapid movement of the wings. An unreceptive female *Drosophila* uses flicks when rejecting the male during courtship. Males also use wing flicks directed at other males that try to court them.
- Foregut** The anterior part of the gut formed from ectoderm and comprising the buccal (mouth) cavity, pharynx, esophagus, proventriculus, and salivary glands of the larva.
- Gamete** An egg or sperm.
- Gastrulation** The stage in embryonic development that follows cleavage. During gastrulation, cells rearrange themselves, with the endoderm and mesoderm cells moving inward and the ectoderm cells spreading to the outside, with some ectoderm invaginating to form structures such as the foregut and hindgut.
- Genital disc** The only imaginal disc that is unpaired, this disc gives rise to the genital ducts, accessory glands, and external genitalia of the adult fly.
- Germ band** The multilayered band of germ layers on the ventral side of the embryo that curves around the embryo's posterior tip. Created during gastrulation.
- Germ band extension** This is the elongation of the germ band along the dorsal side of the embryo so that, eventually, the embryo's posterior end meets its head end. Occurs during early gastrulation.
- Germ band retraction** This is the shortening and thickening of the germ band which brings the posterior end of the embryo back toward the posterior pole of the egg. Occurs during later gastrulation.
- Gonad** The ovary or testis; the organ in which germ cells reside and differentiate. In the embryo, the gonad develops around the pole cells, or primordial germ cells, the population of cells which later become the gametes.
- Haltere** A fleshy, club-shaped organ found posterior to the wings and used for balance. Halteres are homologous to the hind wings of nondipteran insects.
- Head eversion** The turning outward of the head capsule, which had previously been turned inward. This event marks the transition from prepupa to pupa in *Drosophila*.
- Head involution** This is the folding inward of the head region, during gastrulation, causing it to slowly disappear to the interior. Because the head involutes during development, the *Drosophila* larva superficially appears to be headless.
- Hemolymph** The oxygen-carrying and nutritive fluid, equivalent to blood, which bathes the organs of organisms with open circulatory systems (such as *Drosophila*).
- Heterozygous** Having two different alleles of a particular gene.
- Hindgut** The posterior part of the gut. Ectodermally derived, it forms the hindgut and Malpighian tubules of the larva.
- Histological section** A thin slice of a tissue or organism that is typically prepared for sectioning by being fixed and embedded in a hard medium such as paraffin or plastic. Histological sections are usually stained with colored dyes.
- Homeotic gene** A gene whose expression determines the specification of a body region. The expression of the homeotic gene *Antennapedia*, for example, specifies the identity of the second thoracic segment in the fly.

Homozygous Having two of the same allele of a particular gene.

Humeral disc The imaginal disc that in the adult fly forms two dorsal thoracic plates, just posterior to the head, called the humerus and pronotum. In the larva this disc is found ringing the base of the anterior spiracle.

Imaginal discs These are packets of folded epithelium that eventually differentiate into many of the structures of the adult, such as the wings, legs, antennae, eyes, and proboscis. They form in the embryo as epidermal invaginations. They are carried around in the larval stages, growing in size, but do not unfold and differentiate until metamorphosis.

Imago The adult; the sexually mature stage of an insect.

Instar Any of the larval stages in an insect separated by a molt. *Drosophila* goes through three instar larval stages before the onset of metamorphosis.

Labial discs The imaginal discs that will form the proboscis of the adult fly.

Labial palps The fleshy, swollen structures on the distal end of the proboscis that make contact with the food when the adult is feeding.

Larva Early stage(s) in the life cycle, differing significantly in morphology and ecology from the adult.

Love song Courtship song; a series of sounds made by rapid movements of the male's wings, which he extends and vibrates one at a time during courtship.

Malpighian tubules Tubules attached to the gut that have an excretory function. They form as evaginations of the hindgut.

Melanin A black pigment common in many animals.

Mesoderm One of the three germ layers in an embryo. The mesoderm gives rise to organs and tissues such as the heart, somatic and visceral muscles, fat body, and the regions of the gonads not derived from primordial germ cells.

Metamorphosis The transformation from the larval to the adult stage.

Micropyle A small channel through the chorion at the anterior end of the egg that provides an entryway for sperm at fertilization.

Midgut The region of gut in the larva between the foregut and hindgut. It is embryonically derived from anterior and posterior endodermal invaginations.

Molting The shedding of an old cuticle and the formation of a new one. Also called ecdysis.

Ocellus (pl. *ocelli*) A simple eye containing a single light-perceiving element covered by a lens. In the adult *Drosophila*, there are three ocelli on top of the head.

Ommatidium (pl. *ommatidia*) The separate light-sensitive elements of the compound eye, each with its own corneal lens. In *Drosophila* each eye has approximately 750 ommatidia.

Operculum Literally, a "lid" or "cover." In *Drosophila*, an operculum in the chorion at the anterior end of the egg provides an exit door for the larva at hatching. In the pupal stage, the operculum at the anterior end of the puparium is the door through which the adult fly will exit.

Optic lobes Lateral enlargements in the brain, that flank the central brain and service the eyes.

Ovarian follicle cells Cells in the ovary that surround an egg, nourishing it and laying down its chorion.

Ovary The female gonad. In the *Drosophila* larva, the paired ovaries are quite small and are found embedded in fat body. In the adult, the ovaries are large.

Ovipositor A modification at the hind end of an adult female fly through which the eggs are laid. It can be extended and retracted. A female extends her ovipositor when laying an egg and also when rejecting a male during courtship.

P1–P4 The prepupal stages, according to the staging series of Bainbridge and Bownes (1981).

P5–P15 The pupal stages, according to the staging series of Bainbridge and Bownes (1981).

Phenotype The detectable feature in an organism that is the manifestation of a genetic trait.

Prepupa Used here to refer to the stages between pupariation and head eversion; technically, it is the stages between pupariation and the retraction of the epidermis from the previous instar cuticle. (See Ashburner 1989, for full discussion.)

Pole cells In *Drosophila*, these are the primordial germ cells that later become the gametes. They are the first cells to form in the embryo, forming posteriorly. They are moved anteriorly by germ band extension, and then sink to the interior along with the invagination of the posterior midgut.

Polytene chromosomes Chromosomes that replicate without separating. They occur in *Drosophila* larvae in most of the differentiated cells, which grow throughout the larval stages, replicating their chromosomes without dividing.

Posterior midgut In *Drosophila*, this is the region of the gut that is posterior to the anterior midgut and is formed in the embryo by an endodermal invagination.

Proboscis The extensible mouth parts of the adult fly.

Ptilinum A sac on the front of the fly's head that expands with blood to break the seam of the operculum when the fly exits its puparium during eclosion.

Pulse song Part of the *Drosophila* male's love song. It is a purring sound created by high-amplitude motions of the wing. Intervals between pulses and qualities within a pulse in the pulse song are species-specific and contribute to sexual isolation between species.

Pupa Used here to refer to the stages between head eversion and eclosion. (See Ashburner, 1989, for full discussion.)

Pupariation Formation of the puparium. This process occurs at the end of the third instar larval stage and includes shortening of the body, ever-

sion of the anterior spiracles, and tanning of the larval cuticle.

Puparium The tanned, or hardened, larval cuticle that surrounds the organism during metamorphosis and from which the adult fly will eclose.

Pupation Formation of the pupa.

Respiratory filaments Anterior extensions of the chorion of the egg. Respiratory filaments have a water-repellent surface network that maintains a film of gas around them when submerged, allowing the filaments to function as a physical gill.

Sclerotized plates Hardened plates made of cuticular protein.

Second instar larva The larval stage between the first and second molts. In *Drosophila* it is a feeding stage that lasts 24 hours.

Sex combs Fringes of tiny black, stout bristles on the end of the first segment of the *Drosophila* male's front legs. They help the male pull himself up onto the female's back during mating.

Sexually dimorphic Having two sexes that are distinguishable from one another.

Sine song Part of the *Drosophila* male's love song. It is a humming sound created by low-amplitude vibrations of the wing.

Somatic contractions Contractions in body musculature as opposed to musculature of the gut.

Sperm The male gamete. In *Drosophila* these are exceedingly long, measuring about 1.75 mm, over three times the length of the egg.

Spermatheca (pl. *spermathecae*) A region of the female's reproductive tract where sperm can be stored. In *Drosophila*, the pair of spermathecae are mushroom-shaped organs lying embedded in fat tissue and connected to the uterus by narrow ducts.

Spiracle An external opening to a trachea. In *Drosophila* larvae all instars have posterior spiracles, but only the second and third instars have anterior spiracles. In the adult the spiracles, though more numerous (there are nine pairs along the thorax and abdomen), are not as obvious.

Stomodaeal plate In the embryo, the flattening and thickening of the ectoderm in the oral region in preparation for invagination to form the stomodeum.

Stomodeum In the embryo, the invaginated ectoderm in the oral region that forms the foregut posterior to the pharynx. The pharynx invaginates later and joins the rest of the foregut.

Sucking pump A muscular structure at the base of the proboscis that sucks food into the esophagus.

Syncytium A multinucleate tissue in which cell membranes don't completely separate the nuclei. In the *Drosophila* embryo, during cleavage, the blastoderm first consists of a syncytium in which many nuclei are lined up in the peripheral cytoplasm.

Temperature-sensitive mutant A mutant that exhibits a normal trait at a permissive temperature, but fails to exhibit this trait if the temperature is shifted to a higher restrictive temperature. The wild-type displays the trait at both temperatures.

Testis The male gonad. In the *Drosophila* larva, the paired testes are large, spherical structures, embedded in fat body, and easily seen. In the adult, they are long and coiled.

Third instar larva The larval stage following the second molt. In *Drosophila* the third instar larva feeds and then crawls out of the food source to pupariate. The stage lasts about two and a half days.

Thorax In the fly, the region between the head and the abdomen which bears the wings, legs, and halteres.

Trachea The tubelike invaginations of the body wall that serve as air channels to allow internal tissues to exchange respiratory gases with the outside air. They open to the outside at the spiracles.

Uterus In the adult female *Drosophila*, this is the muscular, unpaired structure that receives eggs from the oviducts and moves them toward the outside.

Vaginal plates In the adult female *Drosophila*, these are sclerotized plates surrounding the vulva.

Ventral furrow In the *Drosophila* embryo, this is a ventral invagination that marks the onset of gastrulation and represents the inward folding of the mesoderm.

Ventral ganglion In the *Drosophila* larva, this is the region of the central nervous system that is connected to the two brain hemispheres, lies ventral to them, and extends further posteriorly.

Vulva In the adult female *Drosophila*, this is the opening of the reproductive tract to the outside, serving both as an entrance for sperm during mating and as an exit for eggs during egg laying.

White pupa The first prepupal stage in *Drosophila* when the larval body shortens, everts its anterior spiracles, and becomes immobile.

Wing inflation The gradual unfurling of the folded wings in the adult fly following eclosion. Blood flowing between the two epithelial layers of the wings unfurl the wings by extending and flattening them.