chapter 8 Development of the Fruit Fly *Drosophila melanogaster*

Life Cycle

The fruit fly *Drosophila melanogaster* is the familiar visitor on your overripe bananas and an organism of choice in genetics laboratories. As bridges between genetics and developmental biology are both built and traveled upon, it becomes imperative that developmental biologists study *Drosophila* to aid in the union of these two disciplines. Some of the major questions in developmental biology can only be answered with genetics. So we must learn about the geneticists' organisms and make them ours as well. An embryologist well-schooled in *Drosophila* development is both rare and valuable. Study this laboratory exercise well and it could make you some bucks in the future.

Drosophila is a **holometabolous** insect—that is, an insect that has a larval and a pupal stage prior to the adult stage. (Hemimetabolous insects, on the other hand, have nymph stages preceding the adult.) The adult *Drosophila* may live for more than 10 weeks. During this time, mating takes place. Fertilization is internal, and sperm are stored within the female's body in a **seminal receptacle** and the paired **spermathecae**. Females reach the peak of their egg production between the fourth and seventh day after their emergence. During this time, they lay eggs almost continuously at a rate of 50–70 eggs per day.

The eggs are approximately one-half mm in length, white, oval, and slightly flattened in lateral view (they look much like a kernel of rice). The ovum is surrounded by an inner, very thin **vitelline envelope** and an outer, tough extracellular coat called a **chorion**. At its anterior end two small filaments, extensions of the chorion, extend from the dorsal surface. These are **respiratory filaments** and serve for gas exchange, as their name implies. Eggs are laid half-buried in rotten fruit or the medium in your culture jars, and the filaments protrude into the air.

Eggs hatch in 22–24 hours at 25°C. The larva that emerges looks like a tiny worm and is called the **first instar larva**. It feeds on the substrate that the eggs were laid in and, after another 25 hours, molts into a larger wormlike form, the **second instar larva**. This feeds as well and, after about 24 hours, molts into the **third instar larva**. This is the largest of the larval forms. It feeds, but it also starts to climb upward out of its food, so that it will be in a relatively clean and dry area to undergo pupation. The third instar molts into a **pupa** after 30 hours. The pupa is stationary, and in its early stages is yellowish-white. As it develops, the pupa becomes progressively darker. During the pupal stage, the larva is **metamorphosing** into the adult fly, also called the **imago**. In doing so, it lyses most of the larval structures, although some larval organs are preserved. The larval nervous system, for example, is not lysed, but even it undergoes major restructuring; the Malpighian tubules (excretory structures), fat bodies, and gonads are kept as well. Most of the adult structures, however, form anew from two sets of cells that have been carried as undifferentiated, mitotic cells within the larva throughout its instar stages: these are the **imaginal discs** (*imaginal* since they are for the *imago*) and the **histoblasts**.

Imaginal discs These are small, almost teardrop-shaped packets of epithelial cells that will form the epidermal structures of the adult, such as the wings, legs, eyes, mouthparts, and genital ducts. Imaginal discs are carried around within the larva, growing in size but not differentiating. During the pupal stage, they evert and differentiate into their adult structures.

Histoblasts These cells are found in small groups (nests) within the larva. They form the abdominal epidermis and the internal organs of the adult. They, too, grow by mitosis during the larval stages and then differentiate during the pupal stage. They are recognizable within the larva as clumps of small cells nestled among the huge differentiated polytene larval cells.

The pupal stage lasts for 3–4 days, after which the adult fly, or imago, emerges from the pupal case (**eclosion**). Adult male flies are sexually active within hours of emerging, females don't have ripe eggs until two days after eclosion, and the cycle begins again.

Pause for a minute. Think about what you've just read. It's weird! What does the *Drosophila* do during its life cycle? It has a larval form: a fully differentiated, feeding organism, that carries around, as extra baggage, cells that will replace it—cells that will become *another* fully differentiated, feeding organism. The larva is only a vessel, a nurturing culture environment for these cells that become the adult. At the appointed time, the larva self-destructs as these "passenger cells" differentiate. It is an astonishing way to make an adult.

Culturing Drosophila melanogaster

As you have undoubtedly noticed from the fruit basket that sat too long, *Drosophila* thrive on fermenting soft fruits. A very suitable culture medium, therefore, is crushed banana. It provides all the necessary nutrients for both the larval and adult stages. The banana can be kept along with the flies in sterile pint jars with cotton or foam rubber plugs.

Another standard medium, commonly used by laboratories that raise *Drosophila*, is a cornmeal-molasses-agar mixture. While the batch brews, it fills the scientific hallways with the smells of Grandpa's favorite cookies.

Cornmeal-molasses-agar culture for Drosophila

water	420 ml	*Mix and boil water and agar 3–5 minutes. Add unsulfured molasses and heat to boiling again*
agar	4.5 gm	
unsulfured molasses	60 ml	
cornmeal	49 gm	*Mix together cornmeal, brewer's yeast, and cold water in a separate container until all lumps are removed.*
brewer's yeast	6.5 gm	
cold water	145 ml	
propionic acid	3.4 ml	*Add cornmeal-yeast mixture to molasses-agar mixture. Boil 5 minutes, stirring constantly. Cool mixture to 60°C. Add propionic acid (as mold inhibitor). Pour culture medium 1-inch deep into sterile culture jars with sterile plugs. Pint milk bottles work well, but any widemouthed jar fitted with a plug made of cotton covered with cheesecloth or foam rubber should work well. Add a sprinkle of active baker's yeast (from a salt shaker) to each jar before adding flies.*

It is important when maintaining cultures not to overcrowd (about 100 flies per pint culture jar) and to subculture approximately every other day. 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 5 days old. A female is within her peak laying period at this time and is laying eggs as quickly as one every 3 minutes.

Collecting chamber

A simple collecting chamber consists of an empty culture bottle (any widemouthed bottle will do) with wet toweling stuffed in the bottom (for humidity) and a cotton or foam rubber plug. Place approximately 40 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 bottom bottle against padding on a tabletop to cause the flies from the upper culture bottle to drop into it. 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), score it to make grooves (female flies like to lay their eggs in moist grooves), paint a light coating of baker's yeast suspension (a slurry of bread-making yeast in water) on the surface of the scored medium, and place one or two of these spoons in the collecting chamber with the flies. Since flies will outfly your quick fingers and escape from the bottle during this process, it helps to face the bottom end of the bottle toward 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 about an hour, a suitable number of eggs will have been laid on the spoons, and they can be removed and replaced with fresh spoons containing medium. Again, attract the flies away from the bottle mouth with a bright light. If you prefer to have a number of different stages, including advanced stages, 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.

Mating behavior of adult flies

Before removing the spoons from the bottle, observe the adult flies through the glass, or place males and females in a small covered petri dish and observe them under the dissecting scope. To remove flies easily, first place the bottle in the refrigerator or keep it on ice for at least 20 minutes. This numbs the flies, and you can remove them without their escaping into the room. (Numbing with cold is an easier and safer way than anesthetizing with ether or CO_2.)You can distinguish males from females by looking for the black pigmentation on the posterior abdominal segment of the males; it is absent from females. Also, males have a shorter abdomen with six segments rather than eight, and they have a **sex comb** (a fringe of ten or so black, stout bristles) on the end of the first segment of the front legs.

Watch for **courtship behavior**. A female is very much in control of whether she is inseminated, being larger and stronger than a male. She must give an **acceptance signal** by slowing down, extruding her ovipositor, and spreading her wings, in order for mating to occur. There is no known incidence of

rape among these organisms. A female rejects a male by kicking with her hind legs, fending with her middle legs, flicking her wings, producing a rejection buzzing sound by fluttering her wings, or moving away rapidly. If she has already mated, she also will extrude her genitalia to reject the male. A male courts anything that produces the right "taste" or "smell" (even other males if they are immature). He orients himself toward the female's head, taps her with his forelegs, "tasting" her to make sure she is the right species, and then pursues her when she moves, extending and vibrating one wing producing a **courtship song**. Though the "love song" of the *Drosophila* is species-specific, females do respond to the songs of other species as well. Females "hear" the song through their antennae; the **aristae** (feathery extensions of the antennae) augment the vibrations, and they are sensed by Johnston's organ in the second segment of the antenna. Later in the courtship, the male extends his proboscis to touch the female's genitalia. If all the active courtship of the male has stimulated the female enough to accept the male, the two mate with the male positioned on top of the female.

Sperm travel through the male penis into the female uterus and then swim into the female **seminal receptacle** and **spermathecae**, where they are stored for fertilization. During **oviposition**, the eggs emerge from the female's **ovipositor** posterior end first. The female oviposits preferentially on a moist food surface in a humid atmosphere. If the air is too dry, the female may feed, but she won't oviposit.

Observations of the Egg (Use Sterile Technique)

After a suitable waiting period, remove a spoon from the collecting chamber, and look at the surface of its medium under a dissecting scope. You should see a number of white eggs, often with just their respiratory filaments sticking out of the culture medium. You may find the eggs clustered in patches, laid preferentially on the moister regions of the medium. Scan the entire surface of the medium. Use a microknife or fine forceps to remove the eggs to a small petri dish containing insect Ringer's solution. (This is a balanced salt solution that will allow continued development of the egg until hatching.)

Insect Ringer's solution

NaCl	7.5 gm	*Make up to 1 liter with distilled water.*
KCl	0.35 gm	
$CaCl_2$	0.21 gm	

The chorion

Observe the eggs under a dissecting microscope and record your observations with diagrams. Your initial observations will have to be of superficial structures until you remove the chorion from the egg. Notice that the **chorion** is thick and tough, and that the two **respiratory filaments** and a **micropyle** are extensions of this chorion (Figure 8.1). The micropyle is a tiny channel that leads to the ovum, and it is through this channel that a sperm must swim in order to fertilize the egg. Though there have been reports of polyspermy in *Drosophila*, normally only one sperm is successful in making the trip. What do you think the purpose of the micropyle is? Record your answer in your notebook.

Now place several of the eggs in a drop of Ringer's solution on a slide and observe these under a compound microscope. Focus at the surface of the chorion as well as deeper. Place a small drop of toluidine blue over the eggs. This dye will help to show the ornamental markings of the chorion. These markings are beautiful. They are the impressions ("footprints") left by the **ovarian follicle cells** that deposited the chorion prior to ovulation.

The chorion is a complex structure, consisting of inner and outer laminae bounding a layer of tiny air pockets that connect anteriorly with an extensive meshwork of airspaces in the respiratory filaments. This design maximizes gas exchange and minimizes water loss. The respiratory filaments are the major region of gas exchange. By restricting gas exchange to such a small surface area, the egg is able to minimize water loss when it finds itself stuck in the drying winds of a dry summer. In addition to a meshwork of air pockets, the respiratory filaments have a water-repellent surface network that maintains a film of gas (a **plastron**) around them when submerged. The plastron allows the respiratory filaments to function as a physical gill if the egg gets trapped in a rain puddle. The egg's chorion, therefore, allows survival of the egg through the rainy season and the dry.

To see some of the complexities of the chorion and respiratory filaments, put a footed coverslip over the egg and adjust your microscope to increase contrast. (A **footed coverslip** is made by nicking the corners of the coverslip against some hard paraffin so that crumbs of paraffin remain attached at each corner. This will give just enough spacer between the slide and the coverslip to avoid crushing the egg. Adjust the size of the crumbs to the size of the eggs. Add more Ringer's solution underneath the coverslip as needed.)

Examine the respiratory filaments under higher power. What differences do you see between them and the rest of the chorion? Include your answers in your notebook with diagrams of what you see. Focus deeper to the level of the embryo within the chorion, and try to stage your embryo using the staging series provided. You will be correcting your stagings after you remove the chorion.

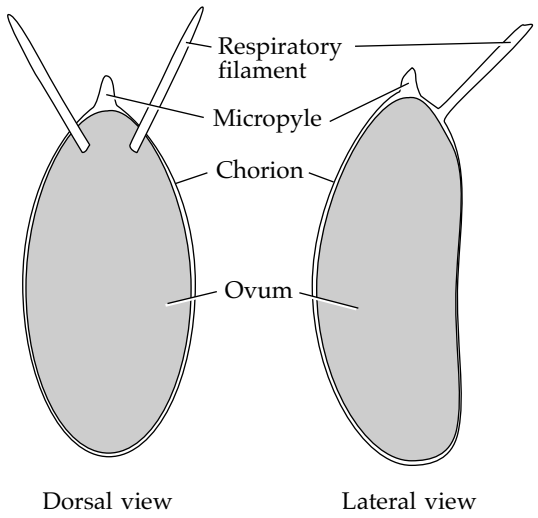

Figure 8.1
Diagrams of the *Drosophila* egg.

Dechorionating an egg

Soak an egg in undiluted bleach (such as Clorox®) for 5 minutes. Agitate the solution slightly with a pipette. You should see remnants of chorion as it separates from the egg. Rinse the egg in Ringer's solution. If the chorion is not gone, repeat the operation.

Note: In moving eggs from one solution to another, a micropipette can be used, but the egg often sticks to the glass and remains within the pipette. Better ways are: to transfer the egg using a hairloop; to catch the egg in the meniscus between half-closed tongs of fine forceps; or to let the egg stick to the side of a needle.

Place the dechorionated egg in a drop of Ringer's solution on a slide, and observe it under a compound microscope. Determine its stage of embryogenesis using the descriptions and staging series below.

Embryogenesis

Study the written description of embryogenesis below, and then proceed with your observations. Look at several embryos to see as many stages of embryogenesis as possible.

Cleavage

The eggs you collect will be in various stages of development. It is difficult to see what is going on inside the egg both because of the chorion (if you haven't removed it) and because the egg is very yolky. It is a **centrolecithal** egg, meaning that the yolk is concentrated centrally and the cytoplasm is pushed to the periphery. Cleavage is unusual in that nuclear division (**karyokinesis**) occurs many times before the cytoplasm cleaves (**cytokinesis**). The nuclei of early cleavage are centrally located through the first seven divisions (Figure 8.2A), after which they start migrating to the periphery (Figure 8.2B). By the time there are about 5000 nuclei all lined up in the peripheral cytoplasm, cell membranes are laid down between them, making a peripheral layer of separate cells, and the embryo goes from being a **syncytial blastoderm** (Figure 8.2C) to a **cellular blastoderm** (Figure 8.2D). This pattern of cleavage is called **superficial** or **peripheral cleavage**.

Cellularization of the blastoderm does not occur simultaneously around the egg. The cells that form first are at the posterior end. They are relatively large and are called the **pole cells** (Figures 8.2C, D). The pole cells form the **primordial germ cells**, which give rise to the gametes. It is interesting that they should be set aside so early in development. Do you have any suggestions as to why?

Gastrulation

Following cleavage, gastrulation proceeds primarily by the infolding of a midventral band of cells. First a **ventral furrow** appears as the mesoderm folds inward (Figure 8.2E). At the anterior and posterior ends of this furrow, the endoderm invaginates forming the anterior and posterior **midgut** (Figure 8.2F). Later the ectoderm will also invaginate to form the anterior **stomodeum** (foregut) and posterior **proctodeum** (hindgut) (Figures 8.2G, H). In addition to these ventral infoldings, there is also a lateral infolding toward the anterior end which extends around the circumference of the embryo. This is the **cephalic furrow** (Figure 8.2F) and roughly delineates the boundary of the future head.

Gastrulation creates a multilayered band of germ layers on the ventral side of the egg that curves around the egg's posterior tip. This band is called the **germ band**. It elongates along the dorsal side of the egg so that eventually, like an acrobat with her back arched and her legs gracefully curved back to touch her head, the embryo's posterior end meets its head end (Figures 8.2G, H). The germ band then shortens and thickens, bringing the posterior end of the embryo back toward the posterior pole of the egg (Figures 8.2I, J). As the germ band shortens, definitive **segmental boundaries** appear, marking off head regions (**mandibular**, **maxillary**, **labial**), **thoracic segments** (t1–t3), and **abdominal segments** (a1–a10). (In the larva you will find only eight abdominal segments. Abdominal segments 9 and 10 have formed the **telson** of the larva, a tail-like structure.).

During gastrulation, a peculiar thing happens. The developing head disappears from view—it turns inward, or **involutes** (Figure 8.2K). Meanwhile the thoracic segments expand forward, overgrowing the region that used to be "head." Only a tiny external head will remain. So when you finally look at your *Drosophila* larvae, don't be too surprised when they appear to be headless.

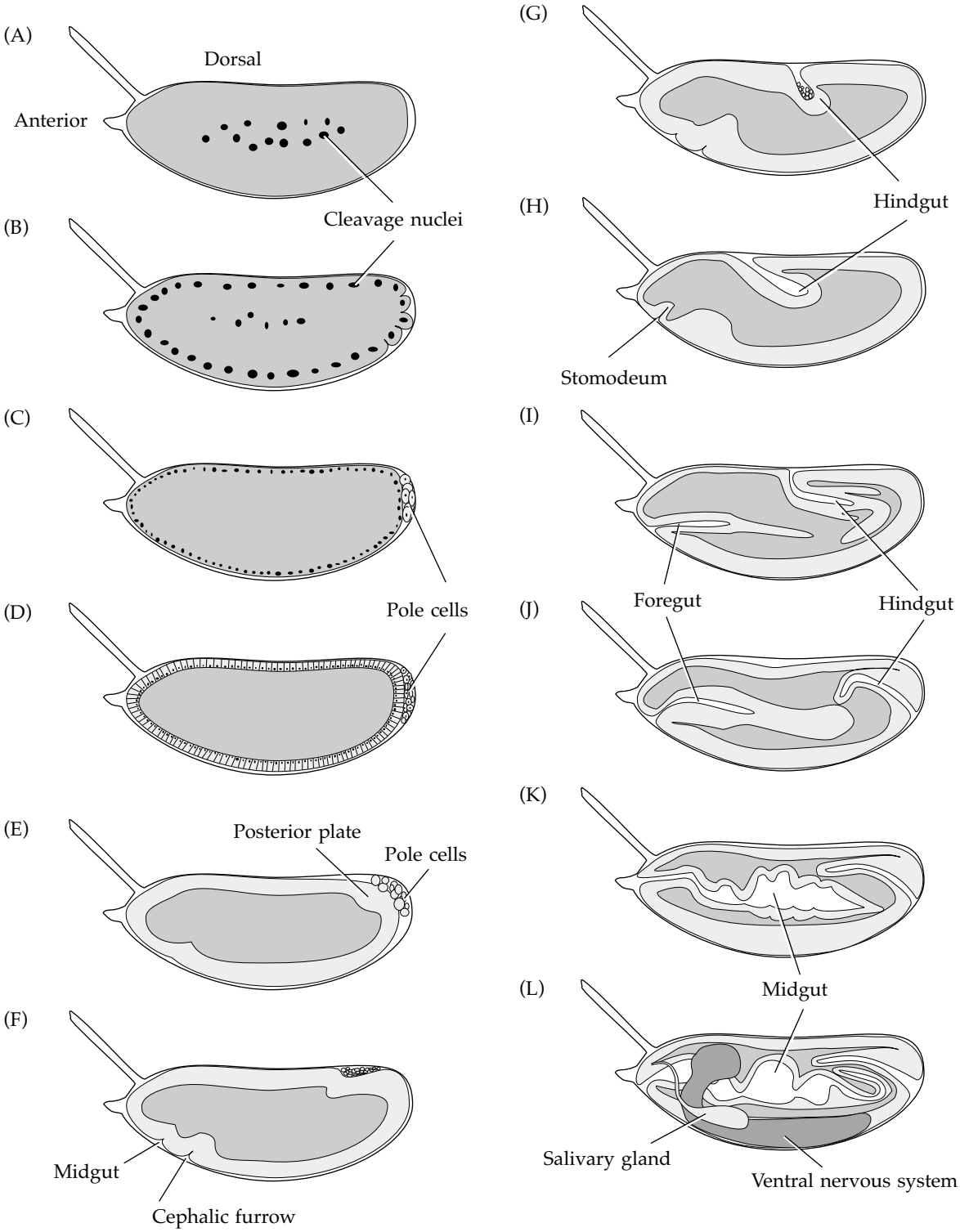
(A)
Dorsal
Anterior
Cleavage nuclei
(B)
(C)
Pole cells
(D)
(E)
Posterior plate
Pole cells
(F)
Midgut
Cephalic furrow
(G)
Hindgut
(H)
Stomodeum
(I)
Foregut
Hindgut
(J)
(K)
Midgut
(L)
Salivary gland
Ventral nervous system

Figure 8.2 ▲
Stages in embryonic development of *Drosophila*. (A) Early cleavage (15 minutes–1.5 hours). Nuclei cleave in the central region. (B) Migration of cleavage nuclei (1.5 hours). Nuclei migrate to the periphery. (C) Formation of syncytial blastoderm (2 hours). Pole cells form posteriorly. (D) Cellular blastoderm (2.5 hours). Cell membranes form between the nuclei. (E) Early gastrulation (3.5 hours). The ventral furrow forms. Thickening of the posterior plate below the pole cells. (F) Midgut invagination (3.5–4 hours). The midgut invagination can be seen ventrally, as can the cephalic furrow. (G) Germ band extension (4–5 hours). Invagination of the hindgut can be seen dorsally. (H) Stomodeal invagination (5–7 hours). Invagination of the stomodeum can be seen ventrally. (I) Shortening of the germ band (9–10 hours). Foregut and hindgut invaginations are deep. (J) Shortened embryo (10–11 hours). Hindgut is now fully posterior. (K) Dorsal closure (13–15 hours). The ectoderm closes dorsally. The midgut broadens. The head involutes. (L) Condensation of ventral nervous system (15 hours–hatching). The gut regions are joined. The nervous system forms ventrally.

Later development

By 16 hours of development, muscular movement will be apparent (Figure 8.2L). Just before the embryo hatches as the first instar larva at 22–24 hours, you will be able to see air-filled **tracheae** and other internal organs.

Embryonic staging series

You can use the staging series shown in Table 8.1 to stage your embryos; it is one of the more widely used series for *Drosophila* embryonic development. (Whenever you refer to a particular stage, you must cite the source of the staging series you've used, since a number of different ones exist.)

Table 8.1 Embryonic stages of *Drosophila*

Stage	Time (hours)	Developmental event (at 25ºC)
1	0–0:25	First two nuclear divisions. Egg uniformly dark in center and light at periphery.
2	0:25–1:05	Nuclear divisions 3–8. Egg cytoplasm retracts considerably from vitelline envelope, leaving empty space at anterior and posterior poles.
3	1:05–1:20	At posterior end, three polar buds form (later to pinch off and become pole cells), and divide once. Nuclear division 9. Dividing blastoderm nuclei cause granulated appearance in wide zone in periphery. Empty space at anterior pole disappears.
4	1:20–2:10	Blastoderm nuclei in periphery making a bright peripheral rim. Nuclear divisions 10–13, just prior to cellularization. Two more divisions in polar buds.
5	2:10–2:50	Cellularization of the blastoderm occurs, and nuclei elongate considerably. Pole cells begin to shift dorsally. Midventral blastoderm cells look irregular and wavy, preceding their invagination.
6	2:50–3:00	Early gastrulation: ventral furrow forms, from which mesoderm and endoderm originate; at posterior pole, cells shift dorsally to form a dorsal plate to which pole cells adhere; cephalic furrow becomes visible as a lateroventral slit.
7	3:00–3:10	Endoderm of anterior and posterior midgut and ectoderm of hindgut invaginate; dorsal folds appear.

(continued)

Table 8.1 (*continued*)

Stage	Time (hours)	Developmental event (at 25°C)
8	3:10–3:40	Amnioproctodeal invagination, rapid phase of germ band elongation.
9	3:40–4:20	Transient segmentation of the mesodermal layer, visible in region of germ band as prominent bulges protruding into yolk sac.
10	4:20–5:20	Stomodeum invaginates ventrally at anterior pole. Germ band continues to expand. Interior of egg occupied by yolk sac which is a dark, uniform mass. Periodic furrows in epidermis appear. Pole cells leave cavity of posterior midgut and locate themselves dorsally outside yold sac. Primordia of Malpighian tubules form. Neuroblasts divide.
11	5:20–7:20	Growth with no major morphogenetic changes. Intersegmental furrows form in epidermis; mandible, maxilla, and labium visible as protuberances. Germ band extension reaches its maximum extent. Posterior pole becomes withdrawn from vitelline envelope.
12	7:20–9:20	Shortening of the germ band so that opening of hindgut becomes located at dorsal side of posterior pole. Width of germ band increases. Anterior and posterior midgut clearly visible and fuse. Germ band segmentation very prominent.
13	9:20–10:20	Germ band shortening completed. Conspicuous triangular gap ventrally due to retraction of clypeolabrum. Labium moves to ventral midline, displacing opening of salivary gland and duct. Yolk sac protrudes dorsally, has characteristic convex shape. Dorsal fold (ridge) appears. Head involution begins.
14	10:20–11:20	Head involution continues. Dorsal closure and closure of midgut. Anal plate ventrally displaced from posterior tip. Dorsal spiracles evident.
15	11:20–13:00	Dorsal closure and dorsal epidermal segmentation. Gut forms closed tube containing yolk sac. Supraoesophageal ganglia and pharynx evident.
16	13:00–16:00	Intersegmental grooves distinguishable mid-dorsally. Dorsal ridge overgrows tip of clypeolabrum. Constrictions appear in midgut. Shortening of ventral cord.
17	16:00–24:00	Tracheal tree contains air. Retraction of ventral cord continues. Embryo hatches as first instar larva.

Source: After Campos-Ortega and Hartenstein, 1985.

Larval Development

You will be maintaining cultures over the week. Place some of your eggs in a small petri dish containing culture medium. Place this dish in a larger petri dish containing sterile water; this will humidify the culture through the week. During the week, keep a record of the developmental stages you see. Record, for example, when you see first instar larvae, second instar, and so on. If one is available, keep a recording thermometer in the room and make a record of the daily temperatures. You may also keep embryos at other temperatures by using the refrigerator (4°C) or an incubator with variable heat settings. (At 22–25°C, development to an adult takes about 9 days. At 10°C, it is slowed down to 57 days. At 29°C, it is speeded up to 8 days. Temperatures continuously above 29°C can be lethal.) By next week, you will have a developmental timetable that you can compare with a standard timetable (Table 8.2). Make comparisons and suggest reasons for any differences you note. Why is development slowed at lower temperatures? Why are high temperatures lethal? Record your answers in your laboratory notebook.

The sections that follow may be done next week.

Table 8.2 Larval stages of *Drosophila*

Time after fertilization		
Hours	**Days**	**Developmental event (at 25°C)**
24	1	Hatching from egg; first larval instar begins
49	2	First molt; second instar begins
72	3	Second molt; third instar begins
120	5	Puparium formation; puparium white
122	5.1	Puparium fully colored
124	5.2	"Prepupal" molt
132	5.5	Pupation; cephalic complex, wings, legs everted
169	7	Eye pigmentation begins
189	7.9	Bristle pigmentation begins
216	9	Adult ready to emerge from pupa case

Source: After Doane, 1967.

Anatomy of the Larva (Nonsterile Technique Can Be Used)

Put your culture dish or a scoop of medium from the laboratory stocks containing larvae under a dissecting scope. You should see three different sizes of larvae, representing the three larval instars. The first two instars should be found burrowing through the medium. A late third instar will be climbing up, away from the food, getting ready to pupate. Watch the behavior of the larvae as they burrow and eat their way through their food. Make notes in your laboratory notebook. Notice that as the larva feeds, it extends a pair of **mouth hooks** that bring food to the mouth. Look at the head end again. What feature is particularly conspicuous by its absence? Right—the larva has no eyes. Does this mean that the larva is blind? Test its sensitivity to light in various ways, and record your results in your laboratory notebook. You can use a box with a hole at one end to test migration toward or away from light. Don't "toast" your larva—that is, don't place the lamp so close to the larva that you are testing a response to heat rather than a response to light. What other behavioral responses do you note? How does the larva respond when poked, for example? Record all your observations in your laboratory notebook. Note any differences in behavior among the three larval instars.

A few minutes before each larval molt, the larva will stop feeding and lie motionless. The mouth hooks then start biting through the old cuticle. With active muscle contractions, the larva ruptures and leaves its old cuticle, discarding mouthparts and spiracles as well, which are replaced by new structures of the next larval instar. If you are very lucky, or very patient, you may see a larval molt. If you do, time the molt and make whatever observations you can, recording them in your laboratory notebook. (Hint: double mouth hooks and double spiracles [see definition of spiracle below] are evidence of an approaching molt.)

Use a moist paintbrush or partially closed forceps to move larvae from the culture medium to a drop of Ringer's solution on a slide. Try to find all three instar stages. Put the slide over ice in a petri dish to anesthetize the larvae, then observe them again under the dissecting scope.

External anatomy

Look for the tiny external **head**, three **thoracic segments**, eight **abdominal segments**, and a **telson** extending beyond the anus. These segments are delineated by ventral rows of tiny hooks, **denticle belts**, which prevent the larva from slipping backward as it moves forward with waves of extension and contraction. You should also see a number of **sensory bristles** in the first instar larva distributed over the cuticle.

Internal anatomy

The larvae are transparent, and, with the proper lighting, you should be able to distinguish a number of internal structures (Figure 8.3). Use both your dissecting scope and compound microscope, and play with the lighting until you are satisfied. Transmitted light, light coming from below, shining up through the larva, should be best.

The two **fat bodies**—long, whitish sheets running the length of the body—are the most obvious structures. Embedded in the fat body in the fifth abdominal segment are the transparent, vesicular **gonads**. Since the testis is several times larger than the ovary, you can sex the larvae simply on the basis of gonad size.

A tree of beautifully pearl-white **tracheae** (hollow tubes) start anteriorly at the two **anterior spiracles** (tufted openings to the outside) and end in the telson at the two **posterior spiracles**. This is the respiratory system for the larva.

The gut starts anteriorly as a muscular **pharynx** and continues as a narrowed **esophagus**, which runs smack through the middle of the brain in the thoracic region. (There must be a joke or two you can come up with about animals with guts through their brains.) In the region of the esophagus, you should see two lateral transparent **salivary glands**, which you will be dissecting later. The esophagus empties into the heavy-walled, bulblike **proventriculus**, which in turn empties into the **gastric area** that has fingerlike, blind-ending pouches, the **gastric caecae**. The gastric region continues into the long coiled **midgut** or **midintestine**, which doubles back on itself and empties into the straighter **hindgut** or **hind intestine**. You should also be able to distinguish the two yellowish **Malpighian tubules**, the excretory organs that carry urinary waste from the body, emptying it into the posterior midgut.

Focus dorsally and try to see a pulsating blood vessel. This is the **heart** (**dorsal vessel**), which extends anteriorly as an **aorta**. The circulatory system is **open**, and the hemolymph bathes the internal organs. Using a clock with a second hand, time the beats of the heart. How does it compare to your own? Does it speed up when the larva is warm? Is there a difference among the three instar larvae? Are there other differences that you note that distinguish the three larval stages from one another? Note these in your laboratory notebook.

Focus again on the region near the brain. Concentrated in this region are the **imaginal discs**. They should appear as tiny teardrop-shaped packets, each one with a connection to the tracheal system. It is these that you will be dissecting. Check Figure 8.3 carefully to determine the position of each type of imaginal disc.

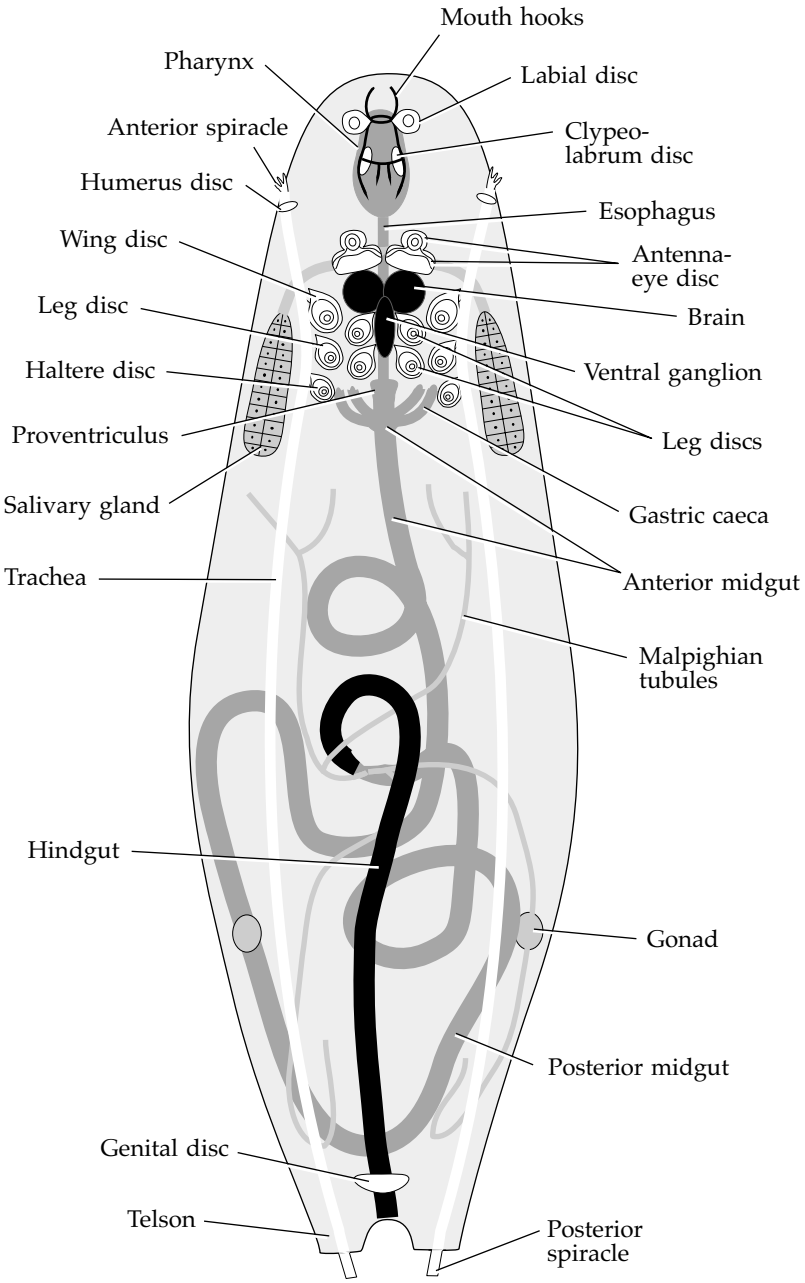

Figure 8.3
Schematic diagram of a third instar larva. The fat bodies and circulatory system are not shown for the sake of clarity.

Development of imaginal discs

It is important to understand the development of imaginal discs before dissecting them, so you can appreciate more fully what you will be seeing. The imaginal discs have their origins in the embryo where they start out as epidermal thickenings that then invaginate to become vesicles. They never separate

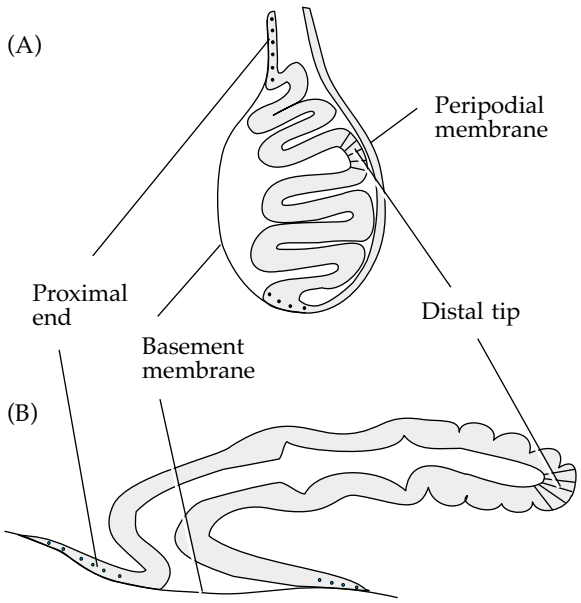

Figure 8.4
Schematic diagram of eversion in a leg imaginal disc. (A) During development of the disc, an epithelial tube forms that becomes folded back upon itself as it is forced into the confines of the tiny disc, bounded by a basement membrane. The epithelium on the far side of the disc does not fold and forms the peripodial membrane. (B) During pupation, the disc everts. The folded epithelium pushes outward against the peripodial membrane, extending into an elongate tube. The distal tip of the tube is shown by five cross lines and the proximal end by small dots in the diagrams of the uneverted and everted discs. (After Poodry and Schneiderman, 1970, and Condic et al., 1991.)

entirely from the epidermis, but maintain a narrow connection to it. Though there is variation among the types of discs, the general scheme of development is that once the vesicles form, one side of the vesicle starts growing considerably, bending inward as it grows, forming a short, wide tube. With nowhere to expand, squashed into the confines of its little vesicular package, the tube must fold back upon itself as it grows. The opposite side of the vesicle remains smooth and thin as the **peripodial membrane** (Figure 8.4A). The original basement membrane, on the outside of the vesicle, does not bend inward with the growing side and remains as an envelope around the imaginal disc. The final effect is a small, transparent package with what looks like a coiled structure (the tube) within. Though not actually coiled, but rather folded like a telescope, this tube will be what forms the final adult structure for which the imaginal disc is determined.

There are nine pairs of discs: **labial**, **clypeolabrum**, **humerus**, **antenna** that is attached to the **eye** disc, **wing**, **haltere**, and three pairs of **leg** discs. There is also a single **genital** disc that will form the genital ducts, accessory glands, and external genitalia, but not the gonads (Figures 8.3 and 8.5). The imaginal discs are suspended within the larval body, looking casually placed, like Christmas ornaments dangling from the tracheal tree. Here they undergo growth but no differentiation.

It is not until the pupal stage, the stage of metamorphosis, that the imaginal discs will take on their adult form. During pupation, each disc everts and its cells **elongate**. The result is that the folded tube within the disc pushes outward against the peripodial membrane, becoming long and extended (Figure 8.4B). (Imagine an old-fashioned top hat with its crown pushed down being snapped to its full height, and you'll have an approximate image of evagination of an imaginal disc.) **Differentiation** of the discs then begins.

Dissection of Imaginal Discs (Nonsterile Technique Can Be Used)

The removal of imaginal discs from the larva requires skill and patience, and it is truly in the realm of microdissection. The imaginal discs you will be dissecting are so small that students before you have dubbed them the "imaginary" discs. Do not despair. Just take a deep breath, don't drink a lot of caffeine before beginning, and be confident in your skills.

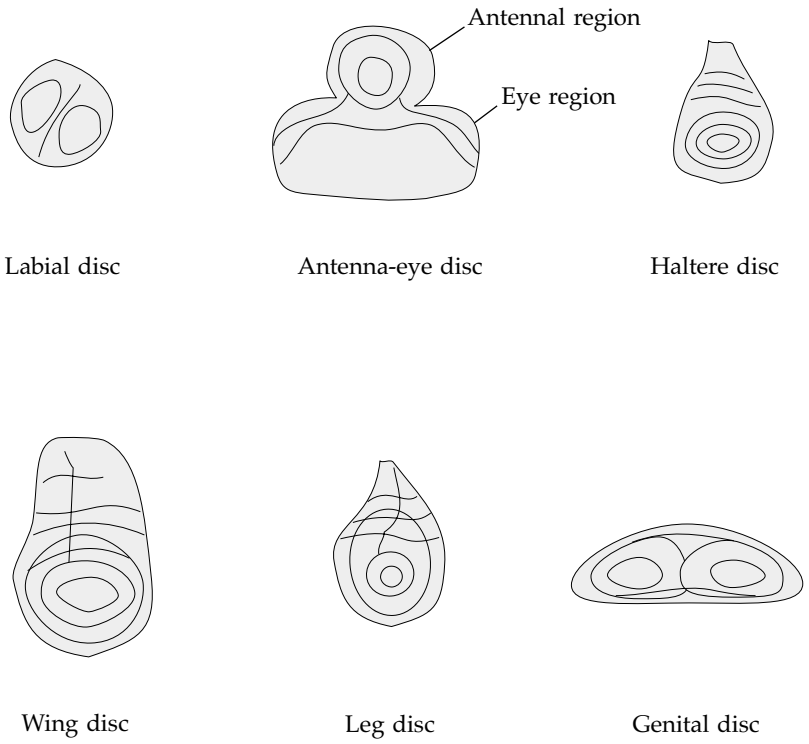

Figure 8.5
Schematic diagram of imaginal discs from a third instar larva.

Collect late third instar larvae from either your own cultures or the stock cultures kept in the laboratory. Third instar larvae will be the largest and the ones crawling up the sides of the bottles. You could dissect imaginal discs from any of the three instar larval stages, but since the discs have been growing all through the larval stages, they will be largest in the third instar. Rinse one of the larvae in tap water, and transfer it to a drop of insect Ringer's solution on a slide. Place the slide over ice to anesthetize the organism. Your dissection of the discs will be done directly on the slide. Everything but the discs then will be removed from the slide, and a permanent whole-mount preparation will be made of the discs that remain.

Remove your slide from the ice and place it under your dissecting scope. It will be easier to dissect on a lower than higher power (trust me). Use two pairs of fine forceps. With one, hold on to the anterior end of the larva in the region of the mouthparts. Hold on *firmly*. With the second pair, pull on the posterior third of the larva without closing the forceps all the way. A lucky pull will break the body wall just behind the mouthparts and pull it away like a sleeve. The inner parts will be displayed for you in their correct anterior-to-posterior orientation. Use Figure 8.3 to determine what you are looking

at. Identify the strands of trachea (tough, white, branched, stringlike structures), the various regions of the gut, the salivary glands, the fat bodies, and the many pairs of imaginal discs hanging to the tracheae. Remember, the discs will look like small, teardrop-shaped packets with a coiled inner structure. (Keep track of the salivary glands; you will be using them later for chromosome squashes.)

Detach the discs using two microneedles. Draw one needle across the other in a scissorlike fashion. Keep manipulating the freed discs to a single, clean spot on your slide until you have collected as many as you can find. Remove the salivary glands to a drop of Ringer's solution on a fresh slide using a microneedle (or pipette, but remember, pipettes have been known to swallow and never relinquish small, soft objects). Invert a petri dish over the slide containing the salivary glands so that they won't dry out before you get to them. Return to your slide of imaginal discs and push the remaining debris off to the side. Remove this debris carefully with a Kimwipe™. Place a drop of 70% alcohol on top of the discs, and record the time in your laboratory notebook. The alcohol will start fixing the discs. Now observe the discs under the dissecting scope, identifying as many as you can (Figure 8.5). Make diagrams of the discs in your laboratory notebook. ***Do not let the discs dry out***. Add more 70% alcohol when necessary.

Eversion of imaginal discs

If you have extra leg imaginal discs that you are not saving for whole mounts, and have both time and energy, you can try causing premature eversion of them by soaking them in 0.1% trypsin (an enzyme that acts on proteins) made up in Ringer's solution and adjusted to pH 7.0. (The enzyme solution may be made ahead of time and kept frozen in small aliquots. Unfreeze only the amount you need. The enzyme quickly loses its activity at room temperatures.) You should see eversion within 5–10 minutes. Why do you think this treatment induces eversion?

Whole mount preparations of imaginal discs

It is not easy to mount an entire set of discs. Be satisfied with less. Even a few discs will be adequate. The steps will involve first fixing the discs so they will not deteriorate over time, clearing them so that they are transparent, and finally mounting them in a clear, permanent mounting medium.

You will be using 70% alcohol as a fixative, not because it is the best fixative, but because it will leave no toxic residues in the laboratory. If you want to stain the discs as well, a few drops of toluidine blue can be added to 10 ml of 70% alcohol; this can be used in place of straight 70% alcohol on your discs. The discs should fix for one hour starting from the time you first put alcohol on them (recorded above). ***Do not let them dry out*** during this period. Put a petri dish cover or some other suitable top over the slide to cut down on evaporation whenever you leave the slide unattended.

You will be clearing the discs in glycerin. This is an excellent clearing agent and is miscible with alcohol and water. Either draw off some of the alcohol surrounding the discs (dangerous) or let some evaporate (much safer) before adding glycerin. Place a drop of glycerin over the discs, then ring the discs with glycerin jelly that has been melted in a warm-water bath. The glycerin jelly is the permanent mounting medium. Now place a footed coverslip over the discs. If there is not enough mounting medium to fill the space under the coverslip, use a pipette to add more glycerin jelly from the side. If there is too much glycerin jelly, leave it until the next lab when it will be thoroughly solid. Then you can clear away the excess by cutting it away with a razor blade.

Kaiser's glycerin jelly

distilled water	52 ml	*Heat to 75°C and stir until dissolved.*
gelatin	8 gm	*Do not heat above this temperature.*
glycerin	50 ml	
1 crystal of thymol (to retard mold)		

Observe your slide under the compound microscope, and record what you see with diagrams. You may need to amend your previous identifications, since the imaginal discs now will be much clearer then they were before.

Put your slide face up in a covered box until next week. At that time, use fingernail polish to ring the edge of the coverslip, making an airtight seal. This will prevent the glycerin jelly from drying out.

Chromosome squash from salivary glands

It is common to make chromosome preparations from *Drosophila* larval salivary glands. The reason is that these glands are soft and easily squashed and their cells are large with huge **polytene chromosomes**, chromosomes that replicate without separating. By the third instar, the chromosomes can have as many as 1024 chromatids. Most of the differentiated larval cells are polytene, in fact. Larval cells grow not by mitosis, but by duplicating their chromatin and increasing cell size. Only the imaginal discs, histoblasts, and gonadal cells undergo mitosis and remain nonpolytene.

Use the salivary glands on the slide already set aside. Place a drop of aceto-orcein stain on the glands, and cover the slide with a petri dish to avoid evaporation. Stain for 2–5 minutes. Then, without removing any of the stain, place a 22-mm square coverslip over the glands, and with your thumb press down firmly (but not hard enough to break the coverslip). The glands must be completely squashed. Observe under a compound microscope and make a diagram of what you see. A good squash will burst open the cells and splay out the arms of the chromosomes. The banding pattern you see gives a visual map of each chromosome. Geneticists use it to precisely define the location of mapped genes.

Aceto-orcein stain

1% natural orcein in a 1:1 solution of glacial acetic acid and 85% lactic acid	*Warm gently (do not boil), then cool and filter.*

Pupation

When the third instar larva is ready to pupate, it leaves the medium, its anterior spiracles evert, its body shortens and ceases to move, and it attaches to a firm substrate (such as the side of your bottle). The cuticle then transforms into a **puparium**, which is initially soft and white but soon hardens, turning tan and eventually brown and brittle. Shortly after the puparium forms, the larva detaches from the inside of the puparium by molting a fourth time. Metamorphosis then takes place.

Look at the sides of a culture bottle to see the white-to-brown pupal cases stuck to the side of the glass. These can be released from the glass with a needle or microknife. Examine several under a dissecting scope to see how many stages you can observe.

If time allows, peel a pupa. This can be done by using superglue to glue the pupa to the bottom of a plastic petri dish. When the pupa is secure, use microknives to chip back the pupal case to uncover the tender body of the metamorphosing pupa within. It is well worth the trouble. Remember what is

happening during pupation: the larval organs are self-destructing, and the imaginal discs and histoblasts are differentiating to form the adult. To witness this will inspire you, or at the very least change your opinion of these rough brown packages.

Eclosion marks the end of pupation and the beginning of adult life. The insect cracks open the puparium anteriorly and laterally at its seams and emerges from the pupal case. It almost invariably occurs around dawn, when leaves are still damp with dew (*Drosophila* means "lover of dew") and the emerging fly can unfold its new wings and harden its cuticle without the risk of desiccation. The timing of this is controlled by circadian rhythm. If the pupa misses dawn by even a few hours, eclosion will be delayed until the next morning. The dedicated among you will undoubtedly want to rise with the sun and watch this event.

Use Table 8.3 to acquaint yourself with the stages of metamorphosis and stage your pupae.

Table 8.3 Stages of metamorphosis in *Drosophila*

Stage	Hours[a]	Developmental event (at 25°C)
P1	0–1	White puparium: wriggling stops completely
P2	1–3	Brown puparium: oral armature stops moving permanently, heart stops pumping, gas bubble becomes visible within abdomen
P3	3–6.5	Bubble prepupa: puparium becomes separated from underlying epidermis; bubble in abdominal region is large, causing prepupa to become positively buoyant at end of this stage (it floats)
P4	6.5–12.5	Buoyant and moving bubble: prepupa is buoyant, and bubble moves, first appearing in the posterior of the puparium, displacing pupa anteriorly, and then appearing in the anterior, displacing the pupa posteriorly. Imaginal head sac is everted and oral armature of larva is expelled
P5	12.5–25	Malpighian tubules migrating and white: legs and wings extend; Malpighian tubules move from thorax to abdomen and become visible as white structures in dorsal anterior abdomen
P6	25–43	Green Malpighian tubules: Malpighian tubules turn green, and dark green "yellow body" appears between the anterior ends of the two Malpighian tubules
P7	43–47	"Yellow body": "yellow body" (actually dark green) moves back between the Malpighian tubules; transparent pupal cuticle separates from underlying epidermis; eye cup becomes yellow at its perimeter
P8	47–57	Yellow-eyed: eyes become bright yellow
P9	57–69	Amber: eyes darken to deep amber
P10	69–73	Red-eye Bald: eyes become bright red; orbital and ocellar bristles and vibrissae darken
P11	73–78	Head and thoracic bristles: head bristles, followed by thoracic bristles, darken
P12	73–78	Wings grey: wings become gray; sex comb darkens
P13	78–87	Wings black: wings become black; tarsal bristles darken and claws become black
P14	87–90	Mature bristles: green patch (the meconium–waste products of pupal metabolism) appears dorsally at posterior tip of abdomen
P15	90–103	Meconium and eclosion: tergites become tan, obscuring Malpighian tubules and "yellow body"; legs twitch; flies able to walk prematurely if puparium removed; eclosion completed

[a]Times start on approximately day four of larval life when the larva is still white but is no longer able to crawl. Timing is variable among individuals, and the times given are a simplification from the Bainbridge and Bownes paper.

Source: After Bainbridge and Bownes, 1981.

Accompanying Materials

Vade Mecum: "Fruit Fly." This chapter of the CD shows the entire life cycle of *Drosophila melanogaster*, including mating behaviors and ways of sexing the adult and larva. The movies on development include color-codings to indicate germ layers.

Tyler, M. S. and R. N. Kozlowski. 2003. *FlyCycle-II*. Sinauer Associates, Sunderland, MA. This CD-ROM is an adaptation of the 45-minute film, *Fly Cycle: The Lives of a Fly*, Drosophila melanogaster, 1996 by M. S. Tyler, J. W. Schnetzer and D. Tartaglia, Sinauer Associates, Sunderland, MA. This covers the life cycle of the fruit fly as well as a number of the mutants used in research.

Gilbert, S. F. 2003. *Developmental Biology*, 7th Ed. Sinauer Associates, Sunderland, MA. In several chapters you will find excellent discussions of *Drosophila* development, larval polytene chromosomes, imaginal discs, and metamorphosis. The diagrams and photographs throughout are extremely useful.

Fink, R. (ed.). 1991. *A Dozen Eggs: Time Lapse-Microscopy of Normal Development.* Sinauer Associates, Sunderland, MA. Sequence 6. This shows *Drosophila* embryogenesis from cleavage to hatching.

Selected Bibliography

Ashburner, M. et al. (eds.). 1976–1986. *The Genetics and Biology of* Drosophila. Academic Press, New York. This is a series of volumes of collected papers. Sophisticated and technical, it is well worth browsing through. Volumes 2a–2e, edited by Ashburner and T. R. F. Wright, concentrate on developmental and biochemical studies.

Bainbridge, S. P. and M. Bownes. 1981. Staging the metamorphosis of *Drosophila melanogaster. J. Embryol. Exp. Morph.* 66: 57–80. This well-illustrated paper gives detailed descriptions of each stage of metamorphosis.

Campos-Ortega, J.A. and V. Hartenstein. 1985. *The Embryonic Development of* Drosophila melanogaster. Springer-Verlag, Berlin. This is the authoritative reference on *Drosophila* development. It is well illustrated and explains each stage of development.

Condic, M. L., D. Fristrom and J. W. Fristrom. 1991. Apical cell shape changes during *Drosophila* imaginal disc elongation: A novel morphogenetic mechanism. *Development* 111: 23–33. Though dealing primarily with the details of cell-shape changes, the paper also provides a good start on a bibliography for imaginal discs.

Demerec, M. (ed.). 1994. *Biology of* Drosophila. Cold Spring Harbor Laboratory Press, New York. This is a facsimile edition of the original 1950 publication. It is the *Drosophila* bible, an excellent classic, and a comprehensive guide to the histology and development of all stages in the *Drosophila* life cycle.

Demerec, M. and B. P. Kaufmann. 1986. Drosophila *Guide.* Carnegie Institute of Washington, Washington, D.C. A very inexpensive, short paperback guide to the life cycle, breeding methods, and genetic techniques for *Drosophila.*

Doane, W. W. 1967. *Drosophila*. In *Methods in Developmental Biology,* F. H. Wilt and N. K. Wessells (eds.). Thomas Y. Crowell Co., New York, pp. 219–244. The entire text is superb, covering a number of different species and describing rearing and experimental methods for them.

Hall, J. C. 1994. The mating of a fly. *Science* 264: 1702–1715. A lengthy review of courtship and mating in *Drosophila*, including details on genetics and molecular biology.

Hipfner, D. R. and S. M. Cohen. 1999. New growth factors for imaginal discs. *BioEssays* 21: 718–720. A brief and excellent review of how imaginal discs grow during the larval stages.

Lawrence, P. A. 1992. *The Making of a Fly: The Genetics of Animal Design*. Blackwell Scientific, Oxford. This book is beautifully illustrated, making it useful beyond its genetic emphasis.

Leptin, M. 1994. *Drosophila*. In *Embryos, Color Atlas of Development*, J. B. L. Bard (ed.). Wolfe Publ., London, pp. 113–134. A well-written review of *Drosophila* development that is beautifully illustrated.

Poodry, C. A. and H. A. Schneiderman. 1970. The ultrastructure of the developing leg of *Drosophila melanogaster*. *Wilhelm Roux Arch*. 166: 1–44. This is an excellent paper on the eversion process in a leg imaginal disc.

Roberts, D. B. (ed.). 1986. Drosophila: *A Practical Approach*. IRL Press, Oxford. This is a gold mine of well-explained techniques and general information. One chapter, "Looking at Embryos," is particularly useful for this laboratory study.

Spieth, H. T. and J. M. Ringo. 1983. Mating behavior and sexual isolation in *Drosophila.* In *Genetics and Biology of* Drosophila, Vol. 3c, M. Ashburner, H. L. Carlson and J. N. Thompson, Jr. (eds.). Academic Press, New York, pp. 223–284. This very thorough review describes each mating behavior, discusses variations among species, includes pictures of courtship behavior, and analyzes its adaptive significance.

Treisman, J. E. 1999. A conserved blueprint for the eye? *BioEssays* 21: 843–850. A clear review of the recent evidence that the genetics of eye development in *Drosophila* and vertebrates share a number of common features.

Wilkins, A. S. 1986. *Genetic Analysis of Animal Development*. Wiley-Interscience, New York. An excellent book with several descriptive chapters on the embryonic and larval development in *Drosophila.*

Suppliers

Most biology or zoology departments have someone with a supply of *Drosophila* cultures "in the cupboard" (which they must constantly clean out to keep active). A kind word can often get you all the *Drosophila* material that you need for this lab. If the source is not in-house, however, a number of supply companies provide cultures.

Nasco
901 Janesville Ave.
Fort Atkinson, WI 53538-0901
1-800-558-9595

www.nascofa.com

Sells inexpensive *Drosophila* cultures, wild-type and mutants. Also sells *Drosophila* medium, though it is certainly easy enough to make your own.

Connecticut Valley Biological Supply Co., Inc.
P.O. Box 326
82 Valley Road
Southampton, MA 01073
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.

Any good chemical supply company such as:

Sigma Chemical Company
P.O. Box 14508
St. Louis, MO 63178-9916
1-800-325-3010

www.sigma-aldrich.com

brewer's yeast (also from natural food stores)
glacial acetic acid
glycerin
lactic acid
natural orcein
propionic acid
salts for Ringer's solution
thymol

Glossary

Abdomen: In an insect, the posterior-most part of the body, behind the head and thorax.

Acceptance signal: In the *Drosophila*, the signal given by the adult female that she will accept the courtship of a male. She slows down, extrudes her ovipositor, and spreads her wings so that mating can occur.

Aceto-orcein: A staining solution made of 1% natural orcein in a 1:1 solution of glacial acetic acid and 85% lactic acid.

Agar: A gelatinous substance found in red algae. It can be extracted and used as a stiffening agent to produce a gel. In the laboratory, it is often used to make an agar-based culture medium. A 1.5% solution makes a solid but elastic gel when cooled to below 40ºC that will not melt again until it reaches temperatures above 85ºC. Agar is also widely used in the food industry for gelling and thickening; baking recipes may include agar instead of gelatin for thickening in making puddings, etc.

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: In an insect, the 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. It will form the anterior midgut and gastric caeca of the larva.

Arista (aristae, pl): In *Drosophila*, a feathery extension of the antenna through which sound vibrations can be detected.

Balancer: A structure on the side of an organism that helps the organism to stabilize during flight (or swimming). In *Drosophila*, the balancers sit posterior to the wings and represent a modified pair of wings.

Basement membrane: The extracellular matrix (acellular) found below any epithelium. It creates a cellular barrier between the epithelium and its underlying mesenchyme and is of major importance to the differentiation and maintenance of an epithelium. It is made up primarily of type IV collagen, laminin, fibronectin, and heparan sulfate proteoglycan.

Blastoderm: The layer of cells formed in the embryo during cleavage. In *Drosophila* this layer is formed in the periphery of the yolky egg. In the chick, it is formed as a disc-shaped area at the animal pole of the yolky egg.

Bristles: In insects, sclerotized, stout, hairlike projections from the adult cuticle, classified as two main types, larger macrochaetae and smaller microchaetae.

Cellular blastoderm: In the *Drosophila* embryo, the stage at which the blastoderm has gone from being a syncytium to a being cellular. Cell membranes grow inward between the peripheral nuclei, separating off individual cells from the inner yolky cytoplasm.

Cellularization: In the *Drosophila* embryo, 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 yolky cytoplasm.

Centrolecithal: Having yolk that is concentrated in the central region of the egg. Centrolecithal eggs are also macrolecithal eggs, having a large quantity of yolk. The insect egg is an example of a centrolecithal egg.

Cephalic furrow: Literally, a furrow in the head region. In the *Drosophila* gastrula, it is a lateral infolding that occurs toward the anterior end and extends around the circumference of the embryo; it roughly delineates the boundary of the future head.

Chitin: Made up primarily of sugars often complexed with proteins, it is the principle component of a cuticle.

Chorion: A tough, protective eggshell. In *Drosophila* and other terrestrial insects, it is specially designed for maximal gas exchange with minimal water loss. In fish, it is a clear, tough enveloping layer.

Chromosome: A structure in the cell nucleus made up of the genetic material, DNA, complexed with protein.

Clypeolabrum: In the *Drosophila*, one of the head segments that is situated anterior to the mouth parts. It is broad and flat, and hangs down in front of the mandibles.

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 5μm 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.

Cytokinesis: Division of a cell's cytoplasm, as opposed to karyokinesis (division of a cell's nucleus). Typically, cytokinesis follows karyokinesis. In the early cleavage stages in *Drosophila*, however, nuclear division (karyokinesis) occurs many times before the cytoplasm cleaves.

Decamp: To walk away. A female *Drosophila* that is unreceptive to a courting male decamps as part of her rejection behavior.

Dechorionation: Removal of the chorion. The chorion is a tough extracellular coat that surrounds the eggs of many species (e.g., *Drosophila*, zebrafish). A chorion can usually be removed either physically with fine forceps or chemically with an enzyme capable of dissolving the chorion (e.g., Clorox®, in the case of the *Drosophila* chorion and pronase in the case of the zebrafish chorion).

Denticle belt: On the ventral surface of the *Drosophila* larva, rows of small hooks that help the larva move by gripping the substrate.

Dorsal vessel: The simple tubular heart of *Drosophila*.

Drosophila melanogaster: The species name for the common fruit fly.

Ecdysis: Molting; the process of shedding an old cuticle and forming a new one.

Eclosion: The emergence of the adult fly from the puparium.

Ectoderm: The germ layer in an embryo that gives rise to the epidermis and nervous system. In many cases, as in vertebrates, an anterior and posterior invagination of ectoderm gives rise to the stomodeum and proctodeum, respectively.

Endoderm: The germ layer in an embryo that gives rise to the epithelium (lining) of the gut and gut 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.

Eye-antennal disc: In the fly, 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 serve in site fat storage. In *Drosophila* these sheets are creamy white.

First instar larva: In *Drosophila*, 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. In vertebrates, this is formed from endoderm and includes the pharynx and esophagus-trachea regions. In insects it is formed from ectoderm and comprises the buccal (mouth) cavity, pharynx, esophagus, proventriculus and salivary glands of the larva.

Gamete: An egg or sperm.

Gastric caecae: In the *Drosophila* larva, a ring of four blind-ending fingerlike pouches that extend from the gastric region. They are secretory, as is the rest of the gastric region, and aid in digestion.

Gastrulation: The stage in embryonic development that follows cleavage. During gastrulation, cells rearrange themselves, the endoderm and mesoderm cells moving inward and the ectoderm cells spreading around the outside, with some ectoderm invaginating to form such structures as the stomodeum and proctodeum.

Genital disc: In the fly, 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: In *Drosophila*, created during gastrulation, this is the multilayered band of germ layers on the ventral side of the embryo that curves around the embryo's posterior tip.

Germ band extension: In *Drosophila*, occurring during early gastrulation, 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.

Germ band retraction: In *Drosophila*, occurring during later gastrulation, 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.

Glycerin jelly: A mounting medium used in making whole-mounts of specimens that have not been dehydrated. It contains glycerin, gelatin, and water. It is heated before use to make it liquid; when it cools, it becomes a firm gel.

Gonad: The organ that forms gametes. In males, this is the testis, a sperm-forming organ, and in females this is the ovary, an ovum-forming organ. In the embryo, the gonad begins developing in the absence of germ cells, and secondarily, becomes populated by the primordial germ cells, the cells that will become the gametes.

Haltere: In the fly, a fleshy, club-shaped organ found posterior to the wings and used for balance. Halteres are homologous to the hind wings of non-dipteran insects.

Head eversion: In the fly, marking the transition from prepupa to pupa in *Drosophila*, this is the turning outward of the head capsule which had previously been turned inward.

Head involution: In the fly, during gastrulation, this is the folding inward of the head region causing it to slowly disappear to the interior. Because the head involutes during development, the *Drosophila* larva appears superficially to be headless.

Hemolymph: The oxygen-carrying and nutritive fluid, equivalent to blood, which bathes the organs of an organism such as a fly which has an open circulatory system.

Heterozygous: Having two different alleles of a particular gene.

Hindgut: The posterior part of the gut. In vertebrates, it refers to the endodermal gut posterior to the colon. In insects it is ectodermally derived, and forms the hindgut and Malpighian tubules of the larva.

Histoblasts: In the fly larva, the population of cells that will give rise to the abdominal epidermis and internal organs of the adult. They are recognizable within the larva as clumps of small cells nestled among the large differentiated cells 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.

Holometabolous: A term referring to insects that have a larval and pupal stage prior to the adult stage. *Drosophila* is an example of a holometabolous insect.

Homeotic gene: A gene whose expression determines the specification of a body region. In *Drosophila*, the expression of the homeotic gene *Antennapedia*, for example, specifies the identity of the second thoracic segment.

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

Humeral disc: In the fly, the imaginal disc that in the adult 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: In the fly, 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.

Involution: A type of gastrulation movement in which a sheet of cells folds inward and spreads over an inner surface.

Kaiser's glycerin jelly: A specific recipe for glycerin jelly, containing gelatin, glycerin, water, and a mold retardant such as thymol. It is used as a mounting medium for whole-mounts.

Karyokinesis: Division of a cell's nucleus, as opposed to cytokinesis (division of a cell's cytoplasm). Though these two events normally both occur during cell division, there are exceptions. In the early

cleavage stages in *Drosophila*, for example, nuclear division (karyokinesis) occurs many times before the cytoplasm cleaves.

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

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

Larva (larvae, pl.): Early stage or stages in the life cycle, differing significantly in morphology and ecology from the adult.

Love song: In the fruit fly, the 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 having an excretory function. They form as evaginations of the hindgut.

Mandibular: Of the mandible. In insects, a mandible is one of the first pair of mouthparts, or "jaws." In vertebrates, the mandible is the lower jaw.

Maxillary: Of the maxilla. In insects, a maxilla is one of a pair of mouthparts, lying beneath the mandible. In vertebrates, the maxilla is the upper jaw.

Melanin: A black pigment common in many animals.

Mesoderm: The germ layer in an embryo that gives rise to the muscular and circulatory systems and most of the skeletal and urogenital systems.

Metamorphosis: The transformation from the larval to the adult stage.

Microknife: A knife with a small blade, suitable for microdissection under a dissecting microscope. It can be made inexpensively from chips of razor blade, a wooden dowel, and superglue.

Microneedle: A needle that is very thin, suitable for microdissection under a dissecting microscope. It can be made inexpensively from inset pins, a wooden dowel, and superglue.

Micropyle: Found in the eggs of certain organisms such as the fruit fly, 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 between the foregut and hindgut.

Molting: Or ecdysis, is the shedding of an old cuticle and formation of a new one.

Mouth hooks: In the *Drosophila* larva, a pair of hooks that articulate with a chitinized H-shaped sclerite posteriorly. The mouth hooks are used to bring food to the mouth.

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

Open circulatory system: A circulatory system in which blood is not confined to vessels. Blood, after leaving the heart through major vessels, enters the hemocoel, the complex of blood-filled spaces surrounding the organs. Typical of arthropods and molluscs.

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: In the brain of the fly, lateral enlargements, flanking the central brain, that service the eyes.

Ovarian follicle cells: Cells in the ovary that surround an egg, nourishing it.

Ovary: The female gonad.

Oviposition: The laying of an egg.

Ovipositor: A modification at the hind end of 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: In *Drosophila*, the prepupal stages, according to the staging series of Bainbridge and Bownes (1981).

P5-P15: In *Drosophila*, the pupal stages, according to the staging series of Bainbridge and Bownes (1981).

Peripodial membrane: In an imaginal disc of the *Drosophila* larva, the thin, smooth wall on one side of the disc. It is the unfolded region of epithelium of the vesicle that forms an imaginal disc.

Pharynx: In the *Drosophila* larva, the region of the digestive tract between the oral hooks of the mouth and the esophagus.

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

Plastron: A thin film of air maintained around a structure when submerged. It can act as a "physical gill."

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 brought anteriorly by germ band extension, and then sink to the interior along with the invagination of the posterior midgut.

Polyspermy: Fertilization of an egg with more than one sperm.

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 the *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.

Prepupa: In *Drosophila*, used to refer to the stages between pupariation and head eversion, though technically, it is the stages between pupariation and the retraction of the epidermis from the previous instar cuticle. (See Ashburner, 1989, for full discussion.)

Primordial germ cells: The early population of cells that give rise to the germ cells. These are typically identifiable early in development.

Proboscis: The extensible mouth parts of the adult fly.

Proctodeum: The posterior-most region of the gut that is formed from an ectodermal invagination. The posterior opening created by the invagination is the anus.

Proventriculus: In the *Drosophila* larva, a heavy-walled, muscular, bulb-like region of the gut posterior to the esophagus. It's inner intima layer is chitinous and is used for mechanical break down of food.

Ptilinum: A sac, on the front of the fly's head, which 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: In *Drosophila*, used to refer to the stages between head eversion and eclosion. (See Ashburner, 1989, for full discussion.)

Pupariation: Formation of the puparium. In *Drosophila*, this process occurs at the end of the third instar larval stage and includes shortening of the body, eversion 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: In the *Drosophila,* anterior extensions of the chorion of the egg. They have a water-repellent surface network which maintains a film of gas around them when submerged, allowing them to function as a physical gill.

Salivary glands: Glands associated with the anterior end of the digestive system that secrete saliva. In the *Drosophila* larva, they are large and soft, and their chromosomes are polytene (as are most of the differentiated cells of the larva), making them suitable structures for creating chromosome squashes.

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.

Seminal receptacle: In the adult female *Drosophila*, a compactly coiled tube attached at the anterior end of the uterus that stores sperm received from the male during mating.

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.

Spermatheca (spermathecae, pl): A region of the female's reproductive tract of certain organisms 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.

Stomodeal 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.

Sucking pump: In the fruit fly, a muscular structure at the base of the proboscis that sucks food into the esophagus.

Superficial cleavage: A pattern of cleavage that occurs in centrolecithal eggs, in which only the peripheral cytoplasm cleaves to form individual cells, leaving the central yolky cytoplasm uncleaved. The type of cleavage found in *Drosophila* embryos. Also called peripheral cleavage.

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.

Telson: In the *Drosophila* larva, a tail-like structure that is formed from the posterior-most segments, abdominal segments 9 and 10.

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 which germ cells form sperm.

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 adult fly, the region between the head and the abdomen, bearing the wings, legs, and halteres.

Trachea: In the fly, the tube-like invaginations of the body wall serving as air channels to allow internal tissues to exchange respiratory gases with the outside air. They open to the outside at the spiracles. In vertebrates, the tube that leads from the oral cavity to the lungs.

Uterus: The portion of a female reproductive tract lying caudal to the oviducts. In the adult female *Drosophila*, it is a 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, it is the region of the central nervous system that is connected to the two brain hemispheres, lies ventral to them, and extends further posteriorly.

Vitelline envelope: An extracellular coat surrounding an ovum.

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: In *Drosophila* this is the first prepupal stage 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.