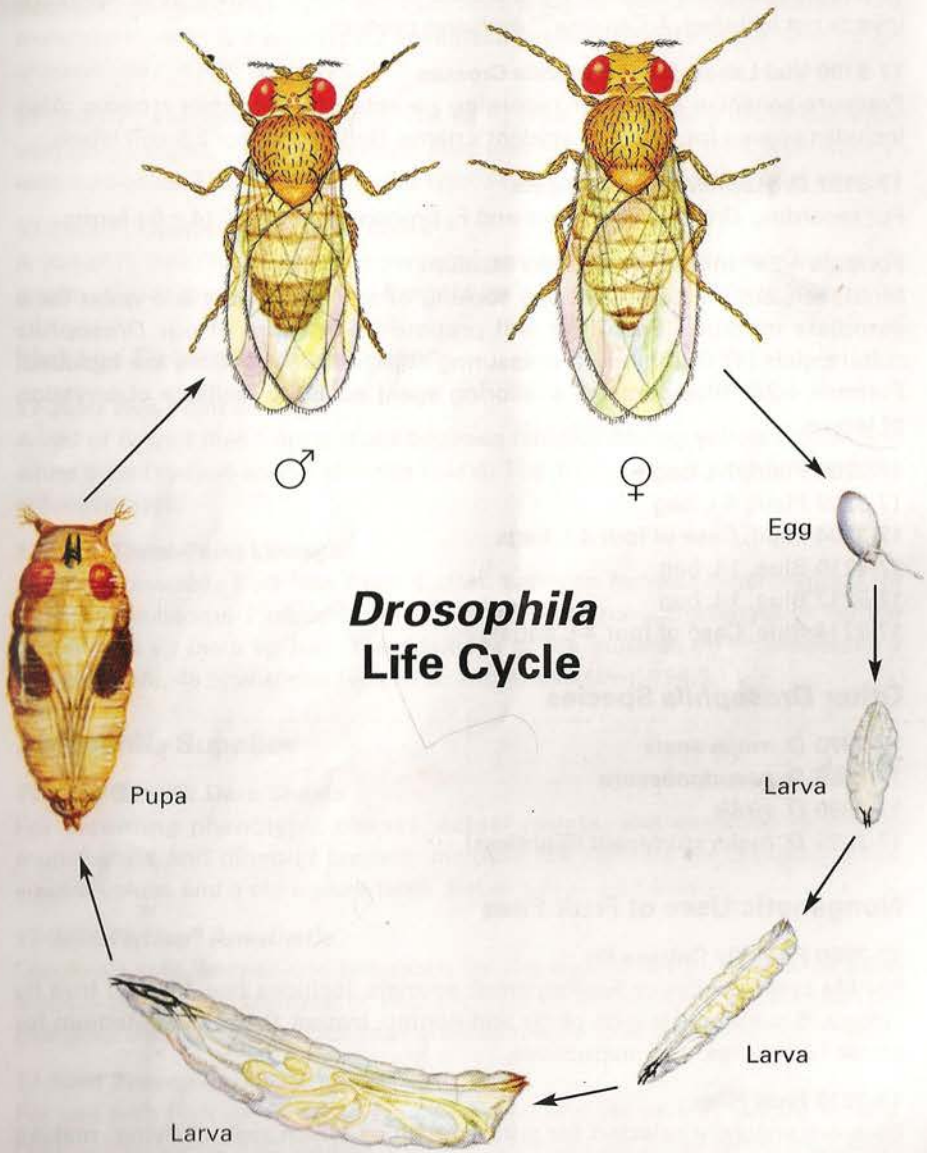

Drosophila **Life Cycle**

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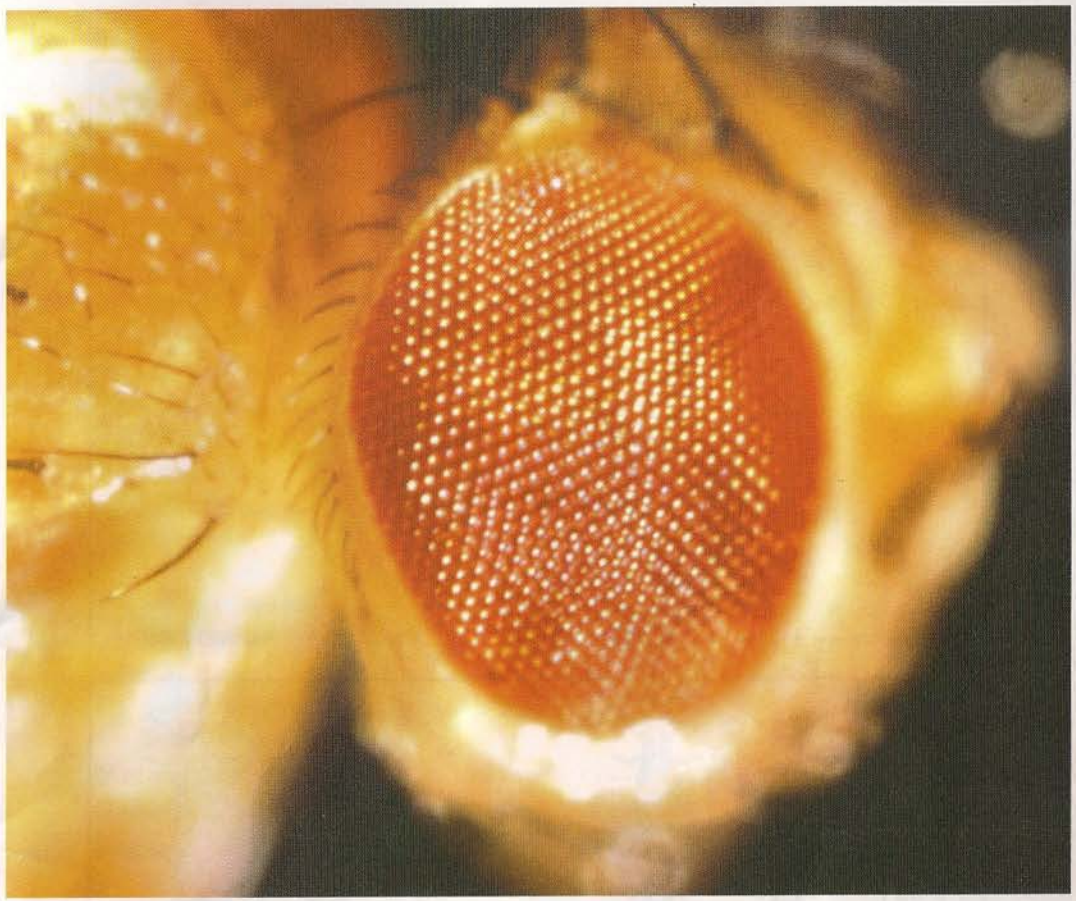

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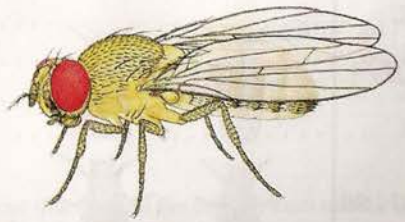
Carolina™ *Drosophila* Manual



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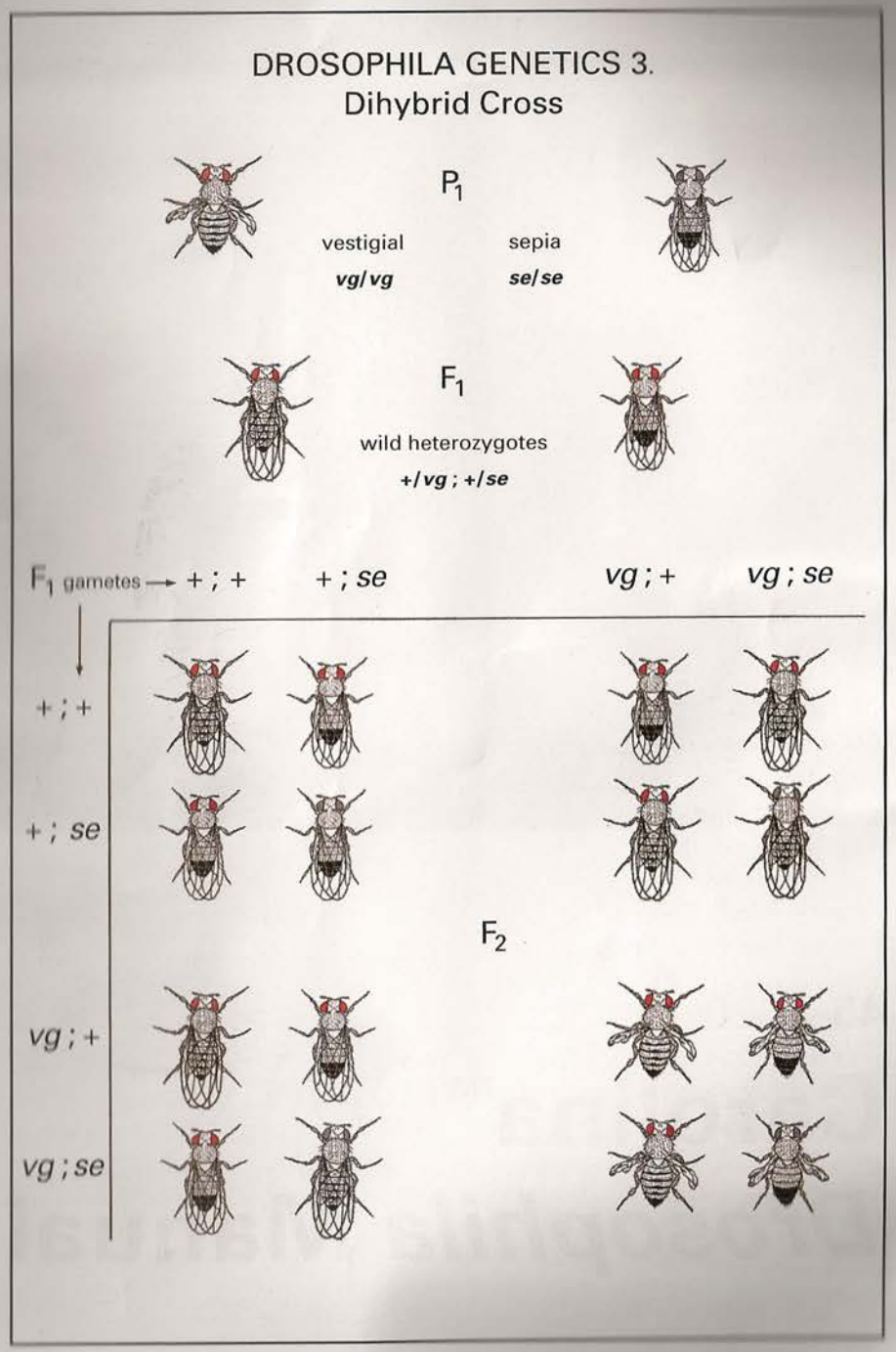

Carolina™ *Drosophila* Manual

Raymond O. Flagg, Ph.D.
Carolina Biological Supply Company

For your convenience, we have listed throughout this manual the catalog item numbers of products available from Carolina Biological Supply Company. For pricing and culture information, please refer to the most recent *Carolina™ Science and Math* catalog, call toll free 800-334-5551, or visit the Carolina Biological Supply Company Web site at www.carolina.com.

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Acknowledgement
We are indebted to the Literary Executor of the late Sir Ronald A. Fisher, F.R.S., and to Oliver & Boyd, Edinburgh, for their permission to reprint Table III from their book, *Statistical Methods for Research Workers.*

Introduction

Basic genetic mechanisms arose early enough in primitive organisms (or were so superior to alternatives) that most organisms share them. It is, therefore, possible to study the principles of genetics in one organism and gain an understanding of the modes of inheritance in many.

The animal most widely used for genetic studies is the common fruit fly, *Drosophila melanogaster*. This model organism possesses many attributes which have contributed to its popularity. The fruit fly is easily cultured and its generation time is only two weeks at 21°C. A single female may lay as many as 500 eggs in 10 days. The small size of *Drosophila* cultures allows the organism to be studied in laboratories and classrooms, even those with limited space; however, each individual fly is large enough for rapid notation of mutant phenotypes.

The fruit fly has been the subject of genetic studies since about 1909. Myriad spontaneous mutations have been found and many others have been induced with radiation. *D. melanogaster* has a tremendous number of genes for study, but practicality dictates the selection of a few readily identifiable phenotypes for use in instruction.

Culturing

When cultures arrive, remove the caps but leave the plugs in place. Put the cultures in a clean location not exposed to direct sunlight. Cultures should be kept at 20 to 25°C (68 to 77°F). While the life cycle of *Drosophila* may be shorter at higher temperatures, it is generally best to carry the cultures at approximately 21°C (70°F). Higher temperatures may contribute to male fly sterility, growth of bacteria and fungi, and mite infestation. Lower temperatures greatly slow the development of the flies.

The minimal equipment for raising fruit flies and for making crosses includes culture vessels, plugs, and medium; marking pencils or permanent pens and labels; anesthetic; white sorting cards (17-3102); fine sorting brushes (17-3094); and a magnifying lens or microscope (Fig. 1).

Culture Vessels

Transparent vials or glass or plastic bottles can be used as culture vessels for *Drosophila*. For most classrooms and research, the optimal vessel size is 50 cm^3 to 100 cm^3. Vessels should be clean, but they need not be sterilized if the medium is properly prepared. Plastic vials should not be autoclaved, and may be used directly from our shipping cartons. Plastic vials should be cleaned with a 10% bleach solution before they are reused.

Plastic (polyurethane) foam or nonabsorbent cotton can be used to plug vials. However, plastic plugs are neater, easier to handle, and last longer than cotton plugs. Plastic plugs may also be used directly from the shipping package. Plastic

Figure 1. Minimal equipment for studying fruit flies.

plugs can be cleaned for reuse using a 70% ethanol solution, and both plastic and cotton plugs may be autoclaved before reuse.

Continued use and pressure may compress plastic plugs. Compressed plugs will expand when wetted with isopropyl alcohol. Reexpanded plugs should be free of fumes before they are used with cultures. Plastic plugs can be held in place with plastic breathing caps. The caps are useful shipping aids, as they control moisture and secure cultures against accidental opening.

Media

Fruit flies can be raised on a variety of fermenting plant materials. The first cultures were raised on grapes or ripe banana with yeast added, but excess moisture and molding were serious problems. Cooked preparations with agar added resulted in media with greater firmness. Later, the use of mold inhibitors in *Drosophila* media—such as methylparaben (also known as Tegosept®; 87-6163), and propionic acid—greatly simplified the culture of fruit flies.

Instant Drosophila *Medium*

The ultimate development in fruit fly culture medium is Formula 4-24® Instant *Drosophila* Medium (17-3200), which needs neither cooking nor sterilizing. Each liter will prepare 75 or more of our *Drosophila* culture vials (17-3120). Formula 4-24® Blue (17-3210) is also available. It contains a blue coloring agent, added to facilitate observation of larvae (which are white). Equal volumes

of Instant *Drosophila* Medium and cool water are dumped into a vial and a few grains of dry viable yeast (17-3235) are sprinkled on top (Fig. 2). The metabolic byproducts of yeast include CO_2. Large amounts of yeast in a culture can produce enough CO_2 to render *Drosophila* sterile or even cause death. Five to eight grains of yeast per culture are sufficient. After one minute, flies can be introduced and the vial plugged. In half-pint bottles and larger vessels, it is generally advisable to use less water than the volume of Instant *Drosophila* Medium. Eight to ten grains of yeast are appropriate for such larger vessels.

Figure 2. Formula 4-24® Instant *Drosophila* Medium is easily prepared.

Early in the history of *Drosophila* culture, it became practice to place a paper strip in the culture medium to hold anesthetized flies so they would not stick in soft or wet medium. As better media were developed, those in the habit of using the paper inserts continued to do so with the idea that paper served other uses, such as a surface for pupation. In fact, larvae pupate on the side of the culture vessel just as well as on paper; further, the paper can collapse into the medium, drowning the pupae. If paper is used in *Drosophila* cultures, the potential for mold problems can be reduced by soaking the paper in 0.1% Tegosept® in isopropanol and allowing it to dry before use. It is not necessary to use paper inserts in *Drosophila* cultures containing Formula 4-24® Instant *Drosophila* Medium. The cultures of fruit flies that we ship contain plastic inserts to hold the medium in place during shipment.

Cooking Medium

Mold inhibitors are used in *Drosophila* culture media to reduce the growth of undesirable fungi that may contaminate cultures and can retard the development of the flies. It is important not to use more mold inhibitor than necessary, as it also inhibits the growth of yeast and flies.

Various published formulae call for concentrations of Tegosept® ranging from 0.07% to 0.2%. Although most references call for dissolving the mold inhibitor in alcohol, we have found it simpler to add the mold inhibitor to boiling water.

Dissolve 15 g of agar and 2 to 3 g of Tegosept® mold inhibitor in 1 L of boiling water. Add 130 mL of sulfur-free molasses and again bring to a boil. Mix 100 g of dry yellow cornmeal (fine grain) with 250 mL of cold water; pour this mixture into the boiling solution and cook for a few minutes. While the

medium is still thin enough to pour easily, pour it 2 to 3 cm deep in the culture vessels. Sterilizing is not necessary.

Cooked *Drosophila* Media (Five alternate formulae)

Ingredient	Amount				
Water	750 mL	750 mL	750 mL	750 mL	500 mL
Tegosept®	1 g	1 g	1 g	1 g	1 g
Agar	15 g				15 g
Molasses (sulfur-free)	130 mL	100 mL	100 mL	30 mL	
Cornmeal (yellow)	100 g		150 g	60 g	
Cream of wheat		100 g			
Oatmeal (not instant)			16 g	60 g	
Brewer's yeast				10 g	
Banana pulp					500 mL

Bacterial Infection

Bacterial contaminants sometimes infect *Drosophila* cultures, causing a reduction in fly hardiness, sterility, and often death. The infections appear as a gray or drab yellow-green viscous sheen or yellow discoloration on the surface of white medium, and as a gray, yellow, or dark green area of color on the blue medium. Using filtered or distilled water in cultures and periodic cleansing of laboratory workspaces and utensils with a diluted bleach and 70% ethanol solution will help prevent accidental introduction of bacteria into cultures. If infected cultures do occur, daily applications of an aqueous solution of 0.5% penicillin G or 0.1% tetracycline should eradicate the infection. If transferred, fruit flies from an infected vial will carry the bacteria to other cultures; therefore, isolate infected cultures while treating them. If the infection persists or kills all immature and mature flies, discard or sterilize the culture vessel and plug.

Controlling Mites

The single largest threat to *Drosophila* stocks is mite (Fig. 3) infestation. While some mites eat only fly media, others consume embryos and pupae. Any infested culture should be removed immediately from the laboratory and sterilized. Infestation may be difficult to detect, however, as an adult mite is smaller than a *Drosophila* embryo. Mite embryos are even smaller, and can often be found in strands of 10 to 20 eggs on *Drosophila* pupal cases.

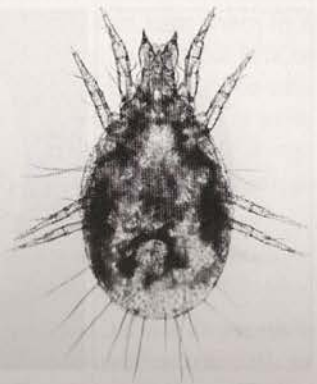

Figure 3. A typical long-haired mite.

Maintaining clean utensils and working areas is the best defense against mite infestation. Mites and their eggs are susceptible to ethanol,

so wipe down laboratory areas and equipment regularly with a 70% ethanol solution. Quarantining incoming stocks and limiting culture length to one month will also decrease the chances of a mite infestation.

Our odorless anti-mite shelf paper (17-3115) and anti-mite vial plugs (17-3091) will help prevent mite infestations. Treated shelf paper will kill mites that walk across it as well as roaches, ants, and silverfish. If culture vials of *Drosophila* are placed on anti-mite paper, mites cannot cross the paper and the fruit flies in the culture vials are in no way injured. Replace anti-mite paper every three months. Treating work surfaces with 1 part benzyl benzoate in 5 parts isopropanol also helps to control mites. If it is necessary to rescue a mite-infested stock, carefully select two or three females and one or two males; make sure that no mites are attached to their bodies and place them in a clean, quarantined vial.

Life Cycle

There are four distinct stages in the life of the fruit fly: egg (Fig. 4), larva (Fig. 5), pupa (Fig. 6), and adult (Fig. 7). At 21°C (72°F), a fresh culture of *D. melanogaster* will produce new adults in two weeks: eight days in the egg and larval stages, and six days in the pupal stage. Adult fruit flies may live as many as eight weeks under optimal conditions.

The day after the egg is laid, the larva hatches. The larva molts twice; that is, it sheds the cuticle, mouth hooks, and spiracles. During the periods of growth before and after molting, the larva is called an instar. The fruit fly has three instars. The cuticle of the third instar hardens and darkens to become the puparium. At this point (at around 11 days), salivary-gland chromosomes can be removed, stained, and observed (see page 25).

Metamorphosis occurs within the puparium. The pupa begins to darken just prior to the emergence of an adult fly. About one day before emergence, the folded wings appear as two dark, elliptical bodies, and the pigment in the eyes is visible through the puparium.

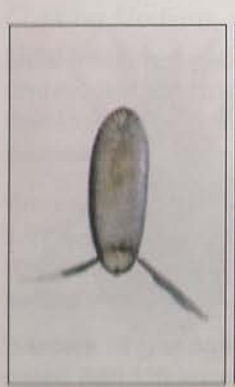

Figure 4.
Fruit fly egg.

Figure 5.
Fruit fly larva.

Figure 6.
Fruit fly pupa.

Figure 7.
Fruit fly adult.

When metamorphosis is complete, the adult emerges (ecloses) by forcing its way through the anterior end (operculum) of the puparium. At first the fly is light in color, the wings are unexpanded, and the abdomen is long. In a few hours the wings expand, the abdomen becomes more rotund, and the color gradually darkens.

Two days after emerging, a female can start laying eggs. After maturity, fruit flies are fertile as long as they live.

Virgin Flies

A female *Drosophila* can store and use the sperm from a single insemination for the major portion of her reproduction. Thus, it is necessary to select virgin females for genetic crosses. The males need not be virgin.

Older males will mate with newly emerged females. Therefore, it is extremely important that all adult flies be removed (cleared) from a culture 8 to 12 hours before it is used for the selection of virgin females.

When pupae appear to be ready for emergence (Fig. 6), clear all adult flies from the culture vessel as late in the evening or as early in the morning as is practical. The flies tend to emerge in greater numbers during the early part of the day.

To insure virginity, females should be selected before they are 15 hours old. The virginity of the flies can be tested by keeping the females isolated in a culture vial for 3 to 4 days before transferring them to another vial with the males. Do not be alarmed if you see eggs on the surface of the media, because females can lay eggs even if they are not inseminated. If larvae appear in the vial, however, then the females are not all virgin.

Anesthetizing

Investigators have used many means to immobilize fruit flies for examination. They have crowded them into constricted glass tubes, chilled them, knocked them out with ether and methoxyflurane, and kept them in a continuous flow of carbon dioxide. Using these techniques, the novice kills or loses many flies. Carolina's FlyNap® anesthetic (17-3025) is an easy-to-use, safe, effective, and inexpensive way to anesthetize fruit flies. FlyNap® is safer than ether for students and instructors, as well as for the flies. It is not explosive like ether, and a single exposure to FlyNap® safely anesthetizes **young** *D. melanogaster* for 50 minutes to several hours.

FlyNap®

Fruit flies can be anesthetized in an empty vial (Figs. 8 and 9), in a *Drosophila* anesthetizer (such as that found in the 17-3015 FlyNap®/Anesthetizer Kit), or in their culture vessel; however, anesthetizing flies in culture vials containing media risks drowning unconscious flies. If it is not possible to transfer flies to

Figure 8. Young fruit flies can be anesthetized in an empty culture vial with FlyNap®.

Figure 9. FlyNap® safely anesthetizes *D. melanogaster* for 50 minutes and longer.

an empty vial, lay the culture vial containing medium on its side while anesthetizing the flies, taking care not to insert the anesthetizing wand too far into the vial. The anesthetic wand is a primary component of the FlyNap® Kit (17-3010; patent No. 4,224,898). To transfer flies to an empty vial, first tap the bottom and sides of the culture vial on a padded surface to move the flies to the bottom of the vial. Quickly transfer the flies from the culture vial into an empty vial and plug the vial. Dip the absorbent end of the anesthetic wand in the FlyNap® solution, making sure to remove excess liquid by running the wand across the lid of the bottle. Use one finger to push the plug in the anesthetizing vial slightly to one side. Quickly stick the anesthetic end of the wand into the vial; position it beside the plug so that the anesthetic tip is below the plug. Keep the culture vial upright with the wand in place. Watch the flies closely and remove the wand when all of the flies are anesthetized. Do not over-anesthetize the flies, as this may kill them. Immediately spill the flies onto a sorting card. If the plug from the anesthetizing vial is to be reused, do not reinsert a part of the plug that may have FlyNap® on it into a culture vial.

The length of time the flies will remain anesthetized depends on the amount of FlyNap® on the wand and on the number and age of the flies that are to be anesthetized. When removed of excess FlyNap®, the wand dispenses the correct amount of anesthetic (35 to 100 L) for our standard *Drosophila* culture vial. Less anesthetic is needed for smaller vessels; more is needed for larger ones. Use two wands with FlyNap® for 250-mL vessels.

In the amount transferred on the wand, FlyNap® has no ill effect on *Drosophila* eggs, larvae, pupae, or young adults. Some flies may exhibit trembling of the legs and/or wings immediately after being anesthetized; this is harmless and will stop after a minute or two.

Ether

To use the *Drosophila* Anesthetizer (Fig. 10; item 17-3040), remove the hollow stopper from the top and remove the cap from the bottom. Fill the hollow stopper one-third full with ether. Pour the ether on the foam pad in the bottom of the anesthetizer. Replace the cap and put the stopper back in the top of the anesthetizer. **Caution:** *Ether is highly flammable.*

Figure 10. *Drosophila* Anesthetizer.

If you have not etherized flies before, familiarize yourself with the process before beginning. Use an empty culture vial to practice transferring the flies. When ready to etherize flies, remove the stopper from the top of the anesthetizer. Tap the bottom of the culture vessel against the palm of your hand to knock the flies down. Remove the plug from the culture. Invert the culture over the anesthetizer and tap the flies into the chamber. After the adults have been tapped into the chamber, quickly right the culture vessel so its base covers the top of the anesthetizer. Plug the culture vessel. Tap the base of the anesthetizer on the table, remove the culture vessel, and plug the anesthetizer with the stopper. Watch the behavior of the flies in the chamber. About 20 seconds after the flies stop moving, they can be dumped onto a white card for examination with a hand lens or microscope.

When the novice etherizes fruit flies, there is a tendency to over-etherize them; therefore, the *Drosophila* Anesthetizer is made to release the ether slowly. Individuals experienced in handling fruit flies may wish to etherize them rapidly. It is a simple matter to speed the release of ether into the inner etherizing chamber by adding holes with the end of a red-hot teasing needle (if you do so, be certain that there is no ether in the anesthetizer).

Usually, the flies remain etherized for 5 to 10 minutes. With the stopper removed, the anesthetizer can be inverted over the flies to re-etherize them if necessary. Note that flies are killed or sterilized if they are re-etherized too often in a short period of time.

Flies that extend their wings and legs at right angles to their bodies are over-etherized and should be considered dead. Pale-colored flies with incompletely expanded wings have just emerged from the pupal case. As flies of this age may be sterilized by ether, they should be avoided in selecting for a cross.

Carbon Dioxide

Flies may also be anesthetized using a carbon dioxide anesthetizer (17-3034). This method safely anesthetizes *Drosophila* without harmful chemicals, and allows regulation of immobilization time, from minutes to hours, without the

risk of fly sterility or death. Flies may be anesthetized while in their culture vessels as well. Replacement carbon dioxide tablets are also available (17-3037).

Sorting and Selecting

The anesthetized flies should be placed in a row on a white card. The flies can be moved about with a teasing needle, a fine brush, or any suitable tool. The flies should be examined with a hand lens or with a microscope at a magnification of at least 12× to 15× unless the strains carry special sex markers. With the flies strung out along the card, one type can be sorted to one side and a second kind to the other side.

Flies that are to be discarded are dropped into a morgue—a jar of alcohol or oil, or a jar of water and detergent.

Sexing

In selecting flies for genetic mating, it is absolutely essential that the sex of each fly be properly identified. The sex of *Drosophila* is most reliably distinguished through examination of the genital organs with magnification (Fig. 11). The male genitalia are surrounded by heavy, dark bristles which do not occur on the female. This characteristic is quite distinct even in a fly that has just emerged from the pupal case.

In older flies, the posterior part of the abdomen is quite dark in males and considerably lighter in females. The tip of the abdomen is more rounded in males than in females, and the female has more sternites. In general, male fruit flies are smaller than females of the same strain, but size is not a reliable character for sorting the sexes.

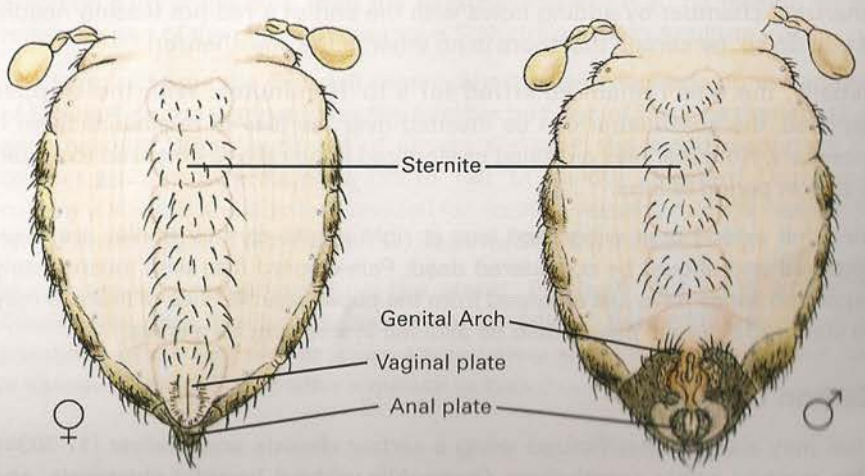

Figure 11. Ventral posterior view of female and male fruit flies.

With care, the sexes can be distinguished by examination of the front legs. There are sex combs (Fig. 12) on the front legs of the male but not on those of the female. This characteristic can even be used to identify the sex of the individual while it is still within the pupal case.

Figure 12. Sex combs on front legs of male fly.

Sexing Pupae

Use a fine brush to select two or three mature, darkened pupae (Fig. 6) from the side of the culture vessel. Examine pupae from one strain at a time; do not mix strains. Space the pupae on a microscope slide and examine the dorsal and ventral surfaces at 100× magnification.

The dorsal surface of a pupa is readily recognized by the long, black bristles on the thorax. The pupa should be positioned with the ventral surface up (Fig. 13). The eyes and the mouth hooks are readily visible at the anterior end of the pupa. The legs, which will be used for identifying the sex, are posterior and mediad to the eyes. The wing buds are seen as large, darkened areas lateral to the legs. If the legs lie so close together that it is difficult to distinguish one pair from another, that pupa should be rejected.

The sex of the pupa is determined from examination of the first pair of legs. The male (Fig. 14) has dark sex combs which are not found on the female. It is essential that the hairs, bristles, and tarsal claws, which are common to both sexes, not be confused with sex combs. If the sex of a pupa is not clearly distinguishable, that pupa should be discarded. After hybrid larvae are developing from crosses set up from selected pupae, the parental adults can be anesthetized and examined to confirm proper sexing of the desired phenotypes.

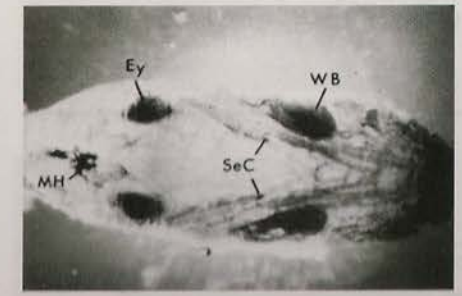

Figure 13. Ventral view of male *Drosophila* pupa. Ey, Eye; MH, Mouth Hook; SeC, Sex Comb; WB, Wing Bud. Photography: J. Hadden and J.A. Cunningham.

Figure 14. Mid-ventral view of male *Drosophila* pupa. SeC, Sex Comb; TC, Tarsal Claw.

Sex Markers

There is an unusual mode of inheritance, attached-X, in which distinctive sex-linked phenotypes, such as body color and eye color, can be used to identify the sex of *D. melanogaster*. In such cases, these phenotypes serve as sex markers. In attached-X strains, daughters inherit any sex-linked traits (such as yellow) directly from their mothers, and sons inherit any sex-linked traits (such as white) directly from their fathers. Our stocks of attached-X are homozygous for yellow, forked, attached, and white: all the females have yellow bodies and forked bristles.

Under normal diploid circumstances, a female fruit fly has two X chromosomes, a male has an X and a Y, and the X chromosomes reassort between the sexes from generation to generation. The Y chromosome has been shown to be insignificant in determining the sex of a fruit fly, unlike humans for instance, where the presence of a Y chromosome induces maleness. In *Drosophila*, sex is determined by the ratio of X chromosomes to the individual's "ploidy"—the number of chromosome sets. This is the X:A ratio. If the ratio is greater than or equal to 1, the individual is female. If the ratio is less than or equal to 1/2, the individual is male. Those with ratios that fall between these values are "intersex."

In an attached-X strain, a female has a pair of X chromosomes attached at the centromere region and a Y chromosome; a male has the usual X and Y. The attached-X chromosomes cannot segregate in meiosis and the Y chromosomes criss-cross the sexes between generations with no apparent effect.

Daughters result from fertilization of attached-X eggs by Y-carrying sperm. Sons develop from Y-carrying eggs fertilized by X-carrying sperm. Although the Y chromosome is important to male fertility in fruit flies, a combination of Y chromosomes from both egg and sperm does not develop beyond the earliest embryonic stage. That is, a zygote without an X chromosome never matures. Triple-X individuals rarely live; however, on rare occasions the combination of three X chromosomes from both egg and sperm produces a viable superfemale (Fig. 15).

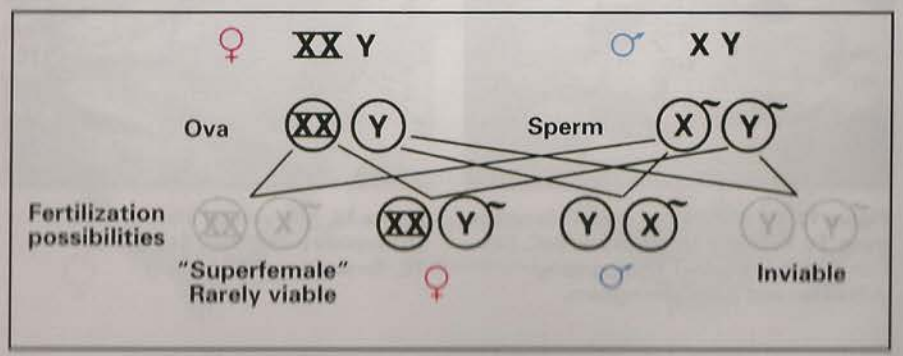

Figure 15. Attached-X inheritance.

Figure 16. Wild-type *Drosophila*.

Figure 17. Homozygous aristapedia.

Figure 18. Recessive sex-linked white.

Figure 19. Recessive autosomal eyeless.

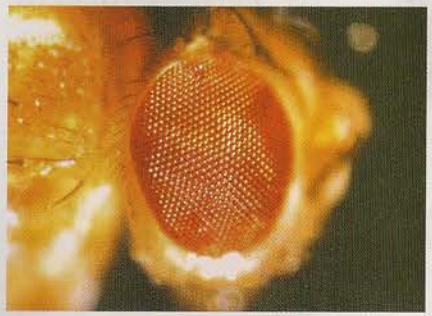

Figure 20. Red eye of wild type.

Figure 21. Recessive autosomal sepia.

Figure 22. Dominant sex-linked Bar.

Figure 23. Dominant autosomal Lobe.

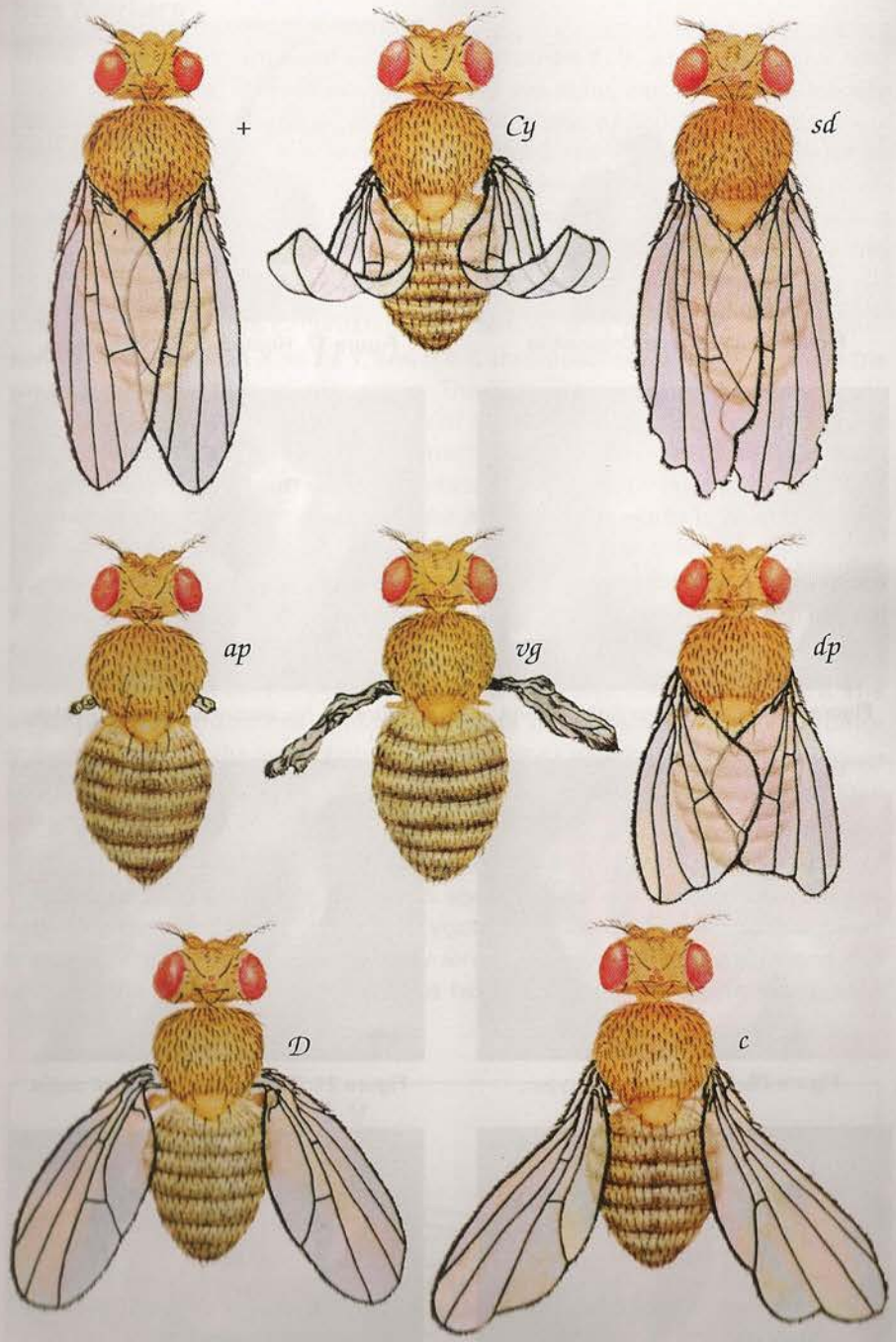

Figure 24. Wing mutations: +, wild; *Cy*, Curly; *sd*, scalloped; *ap*, apterous; *vg*, vestigial; *dp*, dumpy; *D*, Dichaete; *c*, curved.

Mating and Counting

Every vial should be clearly labeled with the characteristics of each parent. The first cross between strains is called the parental generation, the P_1. The progeny of the first cross is the first filial generation, the F_1. The next generation is the F_2 and so on.

Sweep the virgins selected for a cross onto a card that has been folded down the center. Tap about six virgins into a culture vial containing approximately the same number of males of another strain. For reciprocal crosses, set up additional cultures but reverse the sex of each strain.

Seven to ten days after a cross is started, remove the parents from the culture vial. This is to preclude breeding between generations and to avoid confusion when counts are made.

Choose about six pairs of flies, which need not be virgin, from the F_1. Place them in a fresh culture vial to produce an F_2. After 7 to 10 days, remove the F_1 flies from the culture.

Begin a count of the F_2 progeny on the day after the new generation emerges. On the first day of emergence, a culture generally will produce more females than males. On successive days, the proportion of males tends to increase until the sex ratio balances.

Anesthetize and count the progeny every other day for 10 days. Counts made for fewer than 10 days may omit individuals with slow developmental rates due to their sex or a mutation. Counts beyond 10 days risk including flies of the next generation. Do not return counted flies to the same culture vessel.

Genetic Notation and Phenotypes

A fruit fly with red eyes (Fig. 16) and other normal characteristics is called wild type. Either a wild-type fly or a single wild-type gene is designated by the + symbol. A standard name and abbreviation are designated for each mutation. The abbreviation of a recessive gene appears only in lower case letters, while the name and abbreviation of a dominant gene begin with a capital letter.

A mutation is classed as recessive if it is used in separations as the homozygous mutant type vs. the heterozygote, even though a certain intermediate effect may be produced. A mutation generally used as a heterozygote vs. homozygous wild is treated as dominant.

The location of a mutation is often given numerically in parentheses following the name or abbreviation of the gene. For example, *ss*^{a}(3-58.5) designates the recessive mutation spineless-aristapedia (Fig. 17), which is on the third chromosome at position 58.5 on the linkage map.

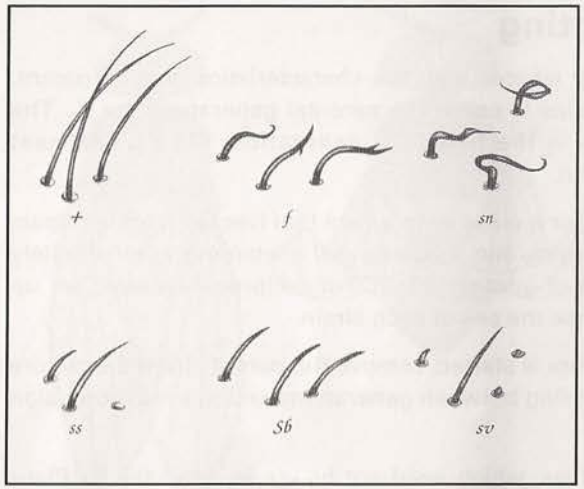

Figure 25. Bristle phenotypes: +, wild; *f*, forked (1-56.7); *sn*, singed (1-21.0); *ss*, spineless (3-58.5); *Sb*, Stubble (3-58.2); *sv*, shaven (4-3.0). Bristle phenotypes can be readily distinguished with the magnification of a stereomicroscope.

Figure 26. Wild type.

Figure 27. Yellow.

Figure 28. Ebony.

The phenotypes illustrated in this manual show the flies as they usually appear under standard culture conditions at room temperature. Some phenotypic variation within populations is to be expected. For example, the wings of vestigial may be considerably longer than the wings shown in Figure 24. Often, the expressed character will be different on opposite sides of the same fly, as differences in eye size on an individual of Lobe or eyeless.

Eyes: Selected phenotypes for color, shape, and size of the eye are presented in Figures 18 through 23.

Antennae: The wild-type antennae are shown in Figure 16. The good viability of spineless-aristapedia (Fig. 17) recommends that mutant over many that are more widely studied.

Bristles: The wild bristle and five useful mutations are depicted in Figure 25.

Body Color: Wild type and the most widely used body colors (yellow and ebony) are pictured in Figures 26 through 28. The effects of the mutations are also visible on the wings.

Wings: There are many readily recognizable mutations in wing venation (Fig. 29), wing shape, and wing size (Fig. 24).

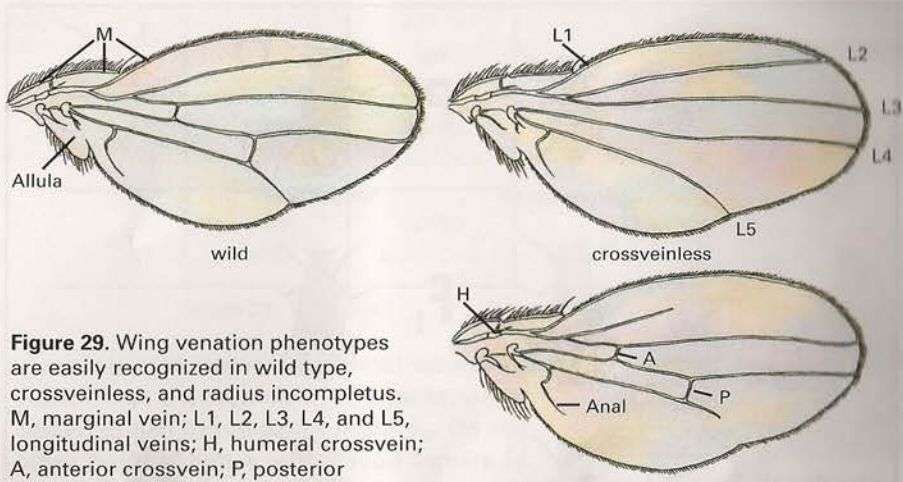

Figure 29. Wing venation phenotypes are easily recognized in wild type, crossveinless, and radius incompletus. M, marginal vein; L1, L2, L3, L4, and L5, longitudinal veins; H, humeral crossvein; A, anterior crossvein; P, posterior crossvein; Anal, anal crossvein.

Experimental Crosses

Monohybrid Crosses

Introductory Drosophila *Set*

This set (17-1900) includes two fruit fly cultures, one winged (yellow, forked, attached, and white) and the other wingless (yellow, forked, attached, white; apterous). Marker characteristics are tied to sex, so no magnification is needed for separating males and females. The females are yellow-bodied and red-eyed, and the males are gray-bodied and white-eyed.

The "normal" wing condition is a wild characteristic and the gene is symbolized as +. The wingless condition is a mutant characteristic caused by the gene apterous, abbreviated *ap*.

When the winged flies (+/+) are mated with the apterous flies (*ap*/*ap*), the offspring (the F_1) all have the genotype +/*ap* and are winged. Because all the F_1 flies are winged, the wild gene + is termed dominant and the mutant gene *ap* is called recessive.

Matings between the F_1 flies produce offspring (the F_2) in a ratio of 3 winged to 1 apterous (Fig. 30).

The Introductory *Drosophila* Set involves an unusual mode of inheritance, attached-X, which locks certain grossly visible traits to the sex of the fly (see *Sex Markers*). This is intended to simplify recognition of the sexes by the students and is not intended for use in teaching the inheritance of attached-X. Students should be told that the body and eye colors are locked to the sex of the fly by a very unusual chromosomal arrangement; we recommend that you place the emphasis on the Mendelian inheritance of apterous.

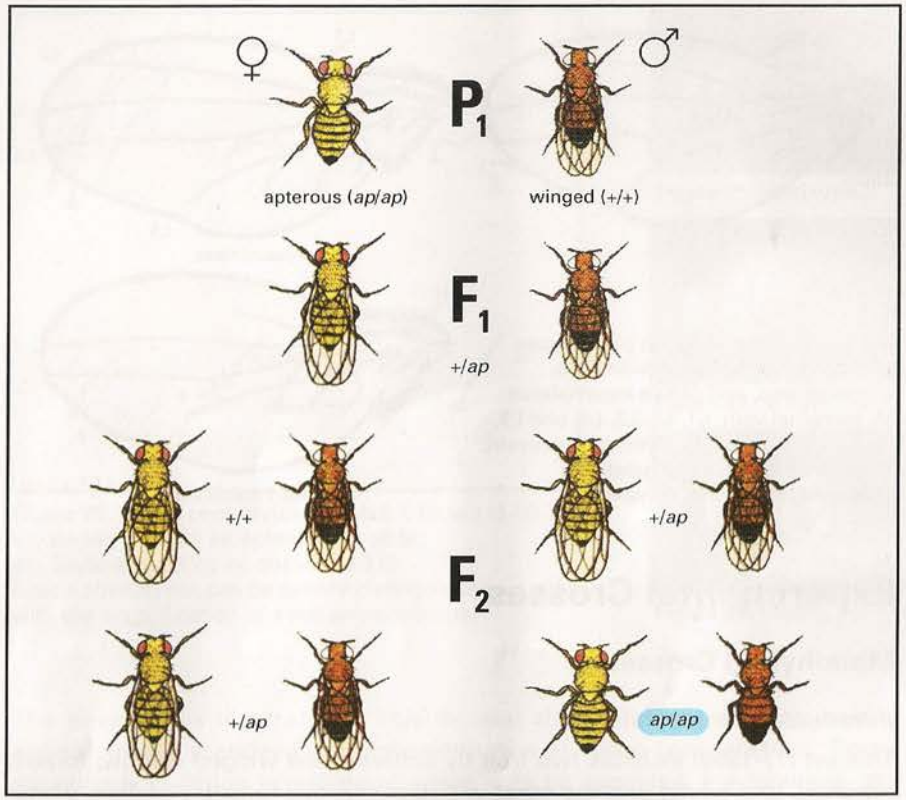

Figure 30. A cross between sex-marked flies involving genes for apterous and wild-type wings. (The females carry yellow forked attached. See page 14.)

Recessive Gene: Set up reciprocal crosses between wild and a stock of apterous, or vestigial, or sepia, or ebony. Following the techniques described in "Mating and Counting," record the phenotype of the F_1, and cross the F_1 flies among themselves to produce an F_2 (3:1).

Testcross: Backcross any of the F_1 flies from the above procedure with their parental mutant strain (not wild). The offspring (1:1) will reveal the heterozygous nature of the phenotypically wild F_1 (Fig. 31).

Dominant Gene: Make reciprocal crosses between wild and either Lobe or Wrinkled and carry through to the F_2 (3:1).

Sex-Linked Recessive Gene: Sex-linked inheritance in *Drosophila* was first reported in 1910 by Thomas Hunt Morgan (Fig. 32). Morgan found a white-eyed male, developed a true-breeding strain for white eye, and demonstrated that the gene for white eyes is linked to the X chromosome.

Set up and carry through the F_2 reciprocal crosses between wild and a stock of white. The phenotypic results from mating a wild female with a male having a recessive sex-linked mutation are the same as those from a cross

P_1 \ F_1	+	*ap*
ap	+/*ap* wild	*ap*/*ap* apterous
ap	+/*ap* wild	*ap*/*ap* apterous

Figure 31. Testcross.

Figure 32. Thomas Hunt Morgan. Photograph courtesy of the Division of Biology, California Institute of Technology.

involving a recessive autosomal mutation, except that expression of the mutation is limited to half of the males in the F_2. On the other hand, when a white-eyed female is mated to a wild male, all the males in the F_1 have white eyes and all the females have red eyes; the F_2 has a 1:1 ratio with half of each sex having white eyes (Fig. 33).

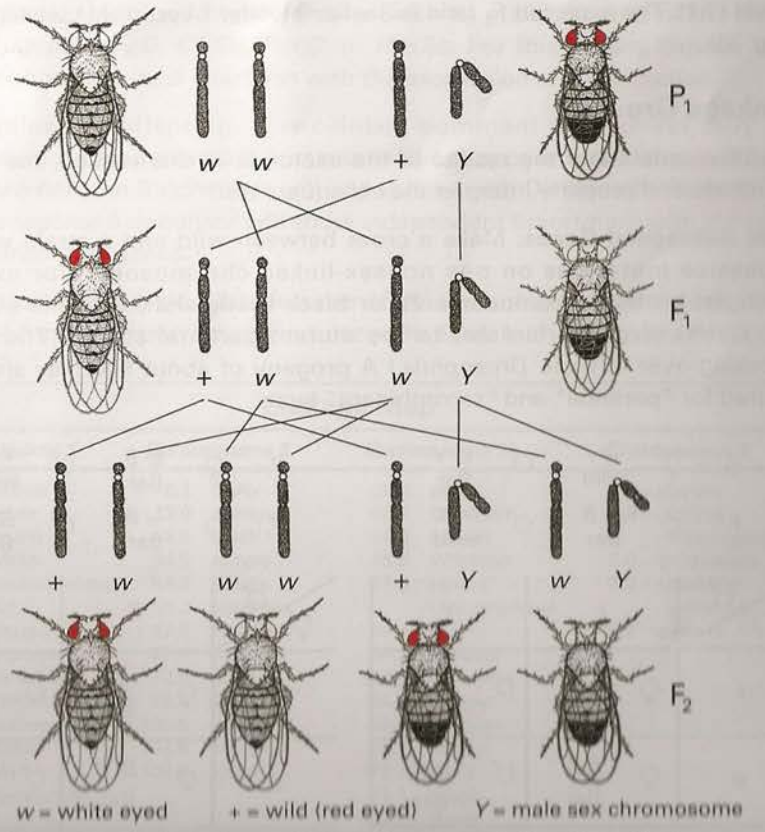

Figure 33. Sex-linked cross. Schematic representation of results of breeding white (*w*) and wild (+).

Sex-Linked Dominant Gene: Set up and carry through the F_2 reciprocal crosses between wild and Bar. When a wild female is mated with a Bar-eyed male, all the females in the F_1 show Bar and all the males are wild; the F_2 ratio is 1:1 with half of each sex showing Bar. When a homozygous Bar female is mated with a wild male, all of the F_1 show Bar and the F_2 ratio is 3 Bar to 1 wild (half of the males are wild). Bar produces a narrow eye in males and homozygous females, while heterozygous female flies have kidney-shaped eyes (Fig. 34).

Dihybrid Crosses

Autosomal Genes: Set up and carry through the F_2 crosses between vestigial (chromosome 2) and ebony (chromosome 3), or between apterous (2) and sepia (3). The expected F_2 ratio is 9:3:3:1 (see inside front cover).

Gene Interaction: Produce an F_2 from a cross between brown (eye color, chromosome 2) and scarlet (eye color, chromosome 3). Flies homozygous for both brown and scarlet will have white eyes. Because these white-eyed flies have reduced viability, the expected 9:3:3:1 ratio may not be obtained.

Autosomal and Sex-Linked: Obtain an F_2 from a cross between female white(1);vestigial(2) and male wild. The F_1 females are wild and the males are white (1:1). The expected F_2 ratio is 3 wild: 3 white: 1 vestigial: 1 white;vestigial (Fig. 35).

Linkage Groups

To fully understand the results of the exercises in this section, the student must use and properly interpret the chi-square test.

Two Autosomal Genes: Make a cross between wild and a strain with two recessive mutations on one nonsex-linked chromosome; for example, vestigial brown (chromosome 2), or black vestigial(2), or sepia ebony(3). Backcross virgin F_1 females to the mutant parental strain. (There is no crossing-over in male *Drosophila*.) A progeny of about 500 flies should be scored for "parental" and "recombinant" types.

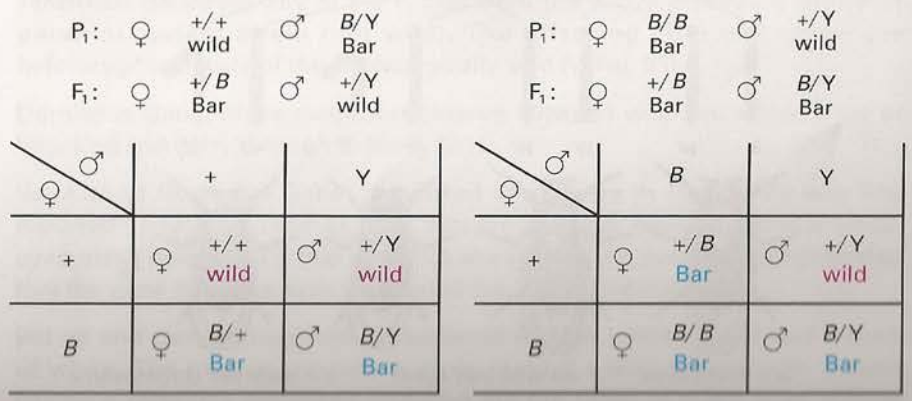

Figure 34. Reciprocal crosses for sex-linked dominant gene.

Two Sex-Linked Genes: Obtain an F_2 from a cross between yellow miniature or white-eosin females and wild males. If very tight linkage is desired, use yellow white females.

Double Crossovers: If students are to work with three or more genes in the same linkage group, have them consult genetics texts for information on double crossovers, interference, and coincidence.

Breed the flies as in the two preceding exercises according to whether or not the genes are sex-linked. Some useful strains are: white miniature forked(1), yellow crossveinless vermilion forked(1), yellow white miniature(1), black vestigial brown(2), and spineless-aristapedia(3).

Determining Linkage Groups: It is relatively easy to determine the chromosome on which a mutation (other than dumpy or black) is located with the balanced marker stock Curly/Plum dp b(2);Dichaete/Stubble(3).

1. Mate females of the mutant strain with *Cy/Pm;D/Sb*. If only F_1 males show the mutation, it is a sex-linked recessive. If all the F_1 flies show the mutation, it is dominant (Step 2). Follow Step 3 if the mutation is an autosomal recessive.

2. Dominant. Mate wild-type females with F_1 males of one of the four phenotypes: *Cy;D*, *Cy;Sb*, *Pm;D*, or *Pm;Sb*. For this mating choose the phenotype that least interferes with the expression of the mutation.

   Examine the offspring. A sex-linked dominant will appear only in females. A chromosome 2 dominant will not appear with Curly or Plum. A chromosome 3 dominant will not appear with Dichaete or Stubble. A chromosome 4 dominant will show independent assortment with the two dominant test genes.

3. Autosomal recessive. Mate females of the mutant strain with F_1 males of one of the four phenotypes produced in Step 1: *Cy;D*, *Cy;Sb*, *Pm;D*, or

Linkage Map

Chromosome 1		Chromosome 2		Chromosome 3		Chromosome 4	
0.0	yellow	6.1	Curly	26.0	sepia	0.0 +	shaven
0.0 +	scute	13.0	dumpy	40.7	Dichaete	0.0 +	cubitus interruptus
0.8	prune	48.5	black	44.0	scarlet		
1.5	white	54.5	purple	46.0	Wrinkled	0.0 +	grooveless
13.7	crossveinless	54.8	Bristle	47.0	radius incompletus	0.0 +	sparkling-polished
20.0	cut	55.2	apterous				
21.0	singed	57.5	cinnabar	52.0	rosy	0.2	eyeless
27.7	lozenge	67.0	vestigial	58.2	Stubble		
33.0	vermilion	72.0	Lobe	58.5	spineless		
36.1	miniature	75.5	curved	64.0	kidney		
51.5	scalloped	100.5	plexus	69.5	Hairless		
56.7	forked	104.5	brown	70.7	ebony		
57.0	Bar	107.0	speck	79.1	bar-3		
64.8	maroonlike			91.1	rough		
				100.7	claret		

Pm;Sb. For this mating, choose the phenotype that least interferes with scoring the mutation of unknown linkage.

Examine the offspring. A chromosome 2 recessive will not appear with Curly or Plum. A chromosome 3 recessive with not appear with Dichaete or Stubble. A chromosome 4 recessive will show independent assortment with the two dominant test genes.

Drosophila F_1 Crosses

The F_1 crosses of *Drosophila* that we ship contain only F_1 offspring. The date on the label indicates when the parent (P_1) flies were crossed in the vial. The P_1 flies were removed from the vial before shipment unless otherwise noted on your shipment. F_1 flies should begin emerging 12 to 14 days after the date on the label.

Except in cases of sex linkage or dominance, all the F_1 flies have wild phenotypes—they are heterozygous. If the female parent carried a homozygous sex-linked mutation, that character (such as white eye) will appear in the F_1 males.

To produce an F_2, transfer 12 to 15 F_1 flies (there is no need to select virgins) to a vial that contains fresh medium. After 7 to 10 days, remove the F_1 flies from the F_2 culture.

At 21°C, the F_2 progeny should begin emerging 12 to 14 days after the F_2 vial is set up. Begin a count of the F_2 progeny on the day after the new generation emerges. On the first day of emergence, a culture generally will produce more females than males. On successive days, the proportion of males tends to increase until the sex ratio balances.

Anesthetize and count the progeny every other day for 10 days. Counts made for fewer than 10 days may omit individuals with slow developmental rates due to their sex or a mutation. Counts beyond 10 days risk including flies of the next generation. Do not return counted flies to the same culture vessel.

DROSOPHILA RECORD SHEET

Name: Lee Ziston | Ex. No.: 5 | P. Female: white; vestigial | × | P. Male: wild

F. Female: wild | F. Male: white | P. Paired: 10 Sept. | P. Removed: 17 Sept.

F. Paired	F. Removed (from F_1 vial)	Date Counted	F_2 Phenotypes and Numbers of Flies: wild	white	vestigial	w;vg
24 Sept.		8 Oct.	17	9	5	3
	1 Oct.	11 Oct.	36	28	12	8
		13 Oct.	28	30	11	9
		16 Oct.	31	32	9	11
Carolina Biological Supply Company		Total	112	99	37	31
		Inferred Ratio	3	3	1	1

Figure 35. *Drosophila* Record Sheet (17-3192). Actual size is 4 × 6 inches.

GENETIC DATA SHEET

	1	2	3	4	Total
Phenotype Class	wild	white	vestigial	w;vg	4
Number of individuals (actual count)	n_1 112	n_2 99	n_3 37	n_4 31	279
Expected number	e_1 105	e_2 105	e_3 35	e_4 35	280

$$\chi^2 = \frac{(n_1-e_1)^2}{e_1} + \frac{(n_2-e_2)^2}{e_2} + \frac{(n_3-e_3)^2}{e_3} + \frac{(n_4-e_4)^2}{e_4}$$

$$\chi^2 = \frac{49}{105} + \frac{36}{105} + \frac{4}{35} + \frac{16}{35} = 1.38$$

χ^2 TABLE

df	P= .95	.90	.80	.70	.50	.30	.20	.10	.05	.01
1	.004	.016	.064	.148	.455	1.07	1.64	2.71	3.84	6.64
2	.103	.211	.446	.713	1.39	2.41	3.22	4.60	5.99	9.2
3	.352	.584	1.00	1.42	2.37	3.66	4.64	6.25	7.82	11.3

Carolina Biological Supply Company

Figure 36. Genetic Data Sheet (17-1510). Actual size is 4 × 6 inches.

Records and Analysis

It is important to keep clear, concise records. We suggest a form such as the *Drosophila* Record Sheet (Fig. 35). The inferred ratio shown in this record was derived by scanning the raw data and preparing a genetic grid for an F_2 of white;vestigial crossed with wild.

The goodness of fit of the inferred ratio to the raw data is tested by the chi-square method. Keep in mind that the chi-square method does not alone prove that the inferred ratio is the correct ratio; it merely indicates whether or not the experimental data fit a given theoretical expectation. An example of this is shown in the Genetic Data Sheet (Fig. 36). With 3 d.f. (degrees of freedom), a chi-square of 1.38 indicates that by random chance the difference between the actual count and the expected numbers would occur more than 70% of the time. This result indicates that in the Record Sheet the inferred ratio is a good statistical fit with the raw data. If the probability had been 0.05 or less, it would have indicated a significant deviation of the raw data from the inferred ratio.

Salivary-Gland Chromosomes

Salivary-gland chromosomes are large and incapable of division (Fig. 37). Their centromeres are united in a region called a chromocenter. The maternal and paternal chromosomes in each pair are so closely appressed that a pair of arms appears as one.

Cultures intended for salivary-gland preparations should be kept at room temperature or cooler (16 to 20°C). The flies should be well fed and uncrowded; cultures should be somewhat more moist than cultures used for stocks and crosses.

Figure 37. Salivary-gland chromosomes.

Third-instar larvae should be selected at the time they crawl out of the medium onto the sides of the vessel and evert the anterior ends of the tracheal system, but before the cuticle hardens. This usually occurs at 11 days.

To remove the salivary glands from a larva, place it in a drop of 2% aceto-orcein (84-1451) in 45% acetic acid or, alternatively, place the larva in a drop of 0.7% aqueous sodium chloride solution, on a microscope slide. A stereomicroscope and a pair of dissecting needles are needed for the operation. Use one needle to hold the larva in place. Place the second needle directly behind the mouthparts and use it to detach the head and pull out the

salivary glands. If the dissection is performed in aceto-orcein stain, the nuclei take up the stain and, in 5 to 10 minutes, the salivary glands (under low power) will be speckled with red.

Transfer the salivary glands to a drop of aceto-orcein stain on a clean slide. Do not allow the glands to dry. After the glands have been in aceto-orcein for 5 to 10 minutes, apply a coverslip. To spread the chromosomes, place a piece of bibulous paper over the coverslip and press down with the ball of the thumb or with a blunt instrument. The amount of pressure must be determined by trial and error. The chromosomes should be well spread, but not broken. Do not allow the slide to dry. It may be helpful to temporarily seal the edges of the coverslip with fingernail polish or melted wax. The slide is then ready for examination with a compound microscope.

The larvae of larger fruit flies, such as *D. virilis* (17-2890), have larger salivary glands than *D. melanogaster* and may give better results for less-experienced students. Alternatively, blow fly larvae salivary-gland preparations can be useful options for beginners. Blow flies are larger than fruit flies and their salivary glands are therefore easier to isolate. Using our Giant Chromosomes Kit (17-1310), students prepare stains as described above, and then observe the banding of the giant chromosomes.

Resources

Web Sites

At the time of this printing, the following Web sites are active. You may wish to perform an independent search for related sites.

Database for molecular and genetic data on *Drosophila*:
http://flybase.bio.indiana.edu/

The Berkeley *Drosophila* Genome Project Online: http://www.fruitfly.org/

Printed Material

Ashburner, A., and E. Novitski (editors). 1976. *The Genetics and Biology of* Drosophila, Vol. Ia–Ic, Academic Press, New York.

Demerec, M. (editor). 1950. *Biology of* Drosophila. Hafner Publishing Co., New York.

Lindsley, D.L., and E.H. Grell. 1967. *Genetic Variations of* Drosophila melanogaster. Carnegie Institution of Washington, Washington, D.C.

Strickberger, M.W. 1962. *Experiments in Genetics with* Drosophila. John Wiley and Sons, Inc., New York.

Available Stocks

Large, vigorous cultures of *D. melanogaster* are shipped in shatterproof 4 × 1 1/4-diameter vials. Order cultures by item number and name. For pricing and culture information, please refer to the most recent *Carolina™ Science and Math* catalog, call toll free 800-334-5551, or visit the Carolina Biological Supply Company Web site at www.carolina.com.

Extra-Large *Drosophila* Cultures

Extra-large vials containing 70 to 100 flies are available. Each culture is shipped in a 120-mL container. With **two weeks** notice, we can supply an extra-large culture of any type in our collection (not including F_1's or F_2's). Contact Carolina's *Drosophila* Lab at 800-227-1150 ext. 5424.

17-2100 wild. Flies with red eyes and other normal standard characteristics. Mutant types are inherited departures from this standard phenotype.

Chromosome 1 Mutants (sex-linked)

apricot. See **white-apricot.**

17-2110 Bar. Narrow eye in male and homozygous female, kidney-shaped in heterozygous female.

17-2120 Biny. Balanced lethals: an X-deficient for scute; other X carries lethal yellow, Y carries translocated wild allele at yellow locus. When females are mated to males with standard Y, only females are produced.

17-2122 Crossveinless forked. Crossveins absent or greatly reduced; bristles shortened and split.

17-2124 cut-6 miniature. Stable wing phenotype; wings miniature.

17-2130 lozenge. Eyes reduced, almond shaped, glossy.

17-2140 maroonlike. Eyes brownish purple

eosin. See **white-eosin.**

17-2150 miniature. Wings just slightly longer than abdomen and proportionately narrower.

17-2160 Basc: Muller-5. Used in detecting X-chromosome lethal mutations; inversion in the X-chromosome inhibiting crossovers; genetic markers of Bar and white-apricot.

17-2170 prune. Eyes transparent brownish red, darkening with age.

17-2180 scalloped. Wing margins scalloped and veins thickened.

17-2190 scute crossveinless vermilion forked. Number of bristles reduced; crossveins absent; eyes vermilion; bristles shortened with ends bent or split.

17-2200 singed. Bristles curled.

17-2210 vermilion. Eyes vermilion; ocelli colorless.

17-2220 white. Eyes white.

17-2225 white-apricot. Eyes apricot; allele of white.

17-2235 white-coffee. Eyes deep ruby but darken to nearly sepia with age.

17-2240 white-eosin. Eyes eosin.

17-2260 white miniature forked. Eyes white; wings miniature; bristles reduced and bent or split.

17-2265 white crossveinless.

17-2270 yellow. Yellow body, wing hairs, and veins.

17-2277 yellow forked attached and white. Females yellow with forked bristles; males with white eyes.

17-2280 yellow Bar. Recessive, dominant, and sex linkage can be demonstrated in a single cross with a wild male.

17-2285 yellow crossveinless vermilion forked.

17-2290 yellow white. Tight linkage.

17-2295 yellow white miniature.

Chromosome 2 Mutants

17-2310 all over Curly purple. Balanced lethal stock; curly wings; purple eyes; several heterozygous recessives, *al dp b c px sp.*

17-2320 apterous. No wings.

17-2330 black. Black on body, tarsi, wing veins; darkens with age.

17-2335 black curved.

17-2338 Adh (n1).

17-2340 black vestigial.

17-2345 black vestigial brown.

17-2360 brown. Eyes pale red-brown, darkening with age.

17-2370 cinnabar. Eyes bright red; ocelli colorless.

17-2375 cinnabar brown. Eyes white

17-2380 curved. Wings divergent, thin, uplifted, and curving down.

17-2390 dumpy. Wings truncated.

17-2420 held out. Wings extended at right angles to the body.

17-2430 lethal over Curly. Balanced for a recessive lethal and the dominant lethal with curly wings; "permanent heterozygote."

17-2440 Lobe. Reduced eye.

17-2460 vestigial. Wings and halteres reduced.

17-2465 vestigial brown.

Chromosome 3 Mutants

17-2470 Antennapedia. Striking phenotype in which antennae have been replaced by fully developed legs.

aristapedia. See **spineless-aristapedia.**

17-2475 bar-3. Bar-shaped eyes.

17-2477 bar-3 radius incompletus.

17-2480 claret. Eyes ruby; slightly narrow body and pointed wings.

17-2500 ebony. Body gradually turns black in adults. Allow flies to age several hours or a day before classifying.

17-2550 radius incompletus. Wing vein L2 interrupted.

17-2560 rosy. Eyes with brownish cast, darkening with age. Useful in chromatographic comparisons because isoxanthopterin is absent.

17-2570 scarlet. Eyes bright red; ocelli colorless.

17-2575 sepia. Eyes change from brownish red to black with age.

17-2580 sepia ebony.

17-2582 sepia lpo/aldox.

17-2595 spineless-aristapedia. Ends of antennae enlarged and leg-like.

17-2600 Wrinkled. Wrinkled wings.

Chromosome 4 Mutants

17-2620 eyeless. Eyes reduced in size, variable even on same fly, eyes more nearly round than oval; temperature influenced, overlapping wild at low temperature (18°C).

17-2640 shaven. Bristles, especially of abdomen, reduced often to a wisp.

Multichromosomal Mutants

17-2690 Bar white singed(I);ebony(3).

17-2692 white singed(I);ebony(3).

17-2700 vermilion(I);brown(2). Eyes pale apricot.

17-2708 white(1);apterous(2).

17-2720 white(I);vestigial(2).

17-2725 white(I);sepia(3). Eyes white.

17-2726 white(I);sepia ebony(3).

17-2728 yellow forked attached(1) and white(I);apterous(2).

17-2735 yellow(I);dumpy(2);spineless-aristapedia(3);eyeless(4). Excellent for demonstrating random segregation.

17-2740 brown(2);scarlet(3). Eyes white.

17-2750 Curly over Plum dp b(2);Dichaete over Stubble(3). For determining linkage groups; wings curly; eyes purplish; wings divergent; bristles short and thick.

17-2753 apterous(2);sepia(3).

17-2755 dumpy(2);sepia(3).

17-2760 vestigial(2);ebony(3).

17-2765 vestigial(2);sepia(3).

Drosophila Cultures and Supplies

Orders must be received at least two weeks in advance of the desired shipping date, unless otherwise noted.

Sets and Kits

17-1900 Introductory *Drosophila* Set
Two cultures: winged and wingless (apterous). Marker characteristics are tied to sex, so students need no magnification for separating males and females. Winged and wingless segregate 3:1 in the F_2. With instructions.

17-1904 Basic *Drosophila* Set (wild and apterous)
Includes two cultures: wild (red eyes, normal wings) and apterous (wingless). Ideal for studying an autosomal recessive gene (not sex-linked).

17-1905 Basic *Drosophila* Set (wild and vestigial)
Includes two cultures: wild (red eyes, normal wings) and vestigial (reduced wings), which may be bred for study of a nonsex-linked recessive gene.

17-1910 Sex-Linked Set
Two cultures, wild (red eyes) and white (white eyes), for studying inheritance of a sex-linked recessive gene.

17-1915 Independent Assortment Set
Two cultures: vestigial and ebony.

17-1920 Independent Assortment Set
Two cultures: dumpy and sepia.

17-1925 Gene Interaction Set
Two cultures: brown and scarlet. The expected F_2 ratio is 9 wild:3 brown:3 scarlet:1 white (brown; scarlet).

17-1932 Dominant Genes Set
Three cultures: Bar, Lobe, and Wrinkled.

17-1935 Recessive Genes Set
Seven cultures: wild, white, brown, vestigial, vestigial brown, dumpy, and sepia.

17-1938 Linkage Determination Set
Five cultures: one Curly/Plum;Dichaete/Stubble and one culture of each of four mutants: white(1), Lobe(2), sepia(3), and eyeless(4).

17-1958 *Drosophila* Student Kit 1
For use by one or two students. Includes card for requesting prepaid delivery of two *Drosophila* cultures of your choice, and the following materials: FlyNap® Kit, 12 culture vials, 12 vial plugs, 12 labels, Instant *Drosophila*, Medium, measuring cup, *Drosophila* sorting brush, Carolina™ D*rosophila* Manual.

17-1959 *Drosophila* Student Kit 2
For 3 to 5 students. May also be used for AP® Biology Lab 11, Part B. Includes card for requesting prepaid delivery of two *Drosophila* cultures of your choice, and the following materials: FlyNap® Kit, 36 culture vials, 36 vial plugs, Instant *Drosophila* Medium, 72 labels, 2 *Drosophila* sorting brushes, 100 *Drosophila* sorting cards, Carolina™ D*rosophila* Manual.

17-1960 *Drosophila* BioKit®
With living fruit flies. For a class of 30. Offers firsthand experience with Mendelian inheritance. Includes card for requesting prepaid delivery of two *Drosophila* cultures of your choice, and the following materials: 3 FlyNap® Kits, 7 Carolina™ *Drosophila* Manuals, 72 culture vials, 72 vial plugs, 72 labels, Instant *Drosophila* Medium, 6 *Drosophila* sorting brushes, 100 *Drosophila* sorting cards, 30 Student Guides, Teacher's Manual. Reusable with 17-1961 Replacement Student Guide Set.

17-1961 Replacement Student Guide Set
Replacement set of 30 Student Guides for use with 17-1960 *Drosophila* BioKit®.

17-1964 Chromosome Mapping BioKit®
For a class of 30. Students study crossing-over and distance between genes on chromosomes. Three chromosome 1 mutants (white, miniature, and forked) are used. Kit includes the same materials as the 17-1960 *Drosophila* BioKit®. A card is included for the prepaid delivery of one wild culture and one white miniature forked *Drosophila* culture.

17-1969 Alcohol Tolerance in *Drosophila* Kit
With this hands-on inquiry activity, students compare alcohol tolerance in fruit flies with and without the enzyme alcohol dehydrogenase (Adh). Adh-negative mutants cannot break down the ethanol they consume, leading to intoxication, and ultimately, death from alcohol poisoning. In a short, simple activity (two or three class periods), students study the relationship among a recessive gene, its enzyme product, and the resulting phenotype. Complete instructions include a list of extensions in genetics, adaptation, biotechnology, health, and ethics. Students make observations over a 24-hr period, requiring them to take flies home overnight. For eight groups of up to 4 students each. *Kit contains coupon for prepaid delivery of one Adh+ culture and one Adh– culture; live materials can be delivered with the kit upon request.* **Note:** *Approximately 100 cotton balls and about 160 mL of ethanol are needed, but not supplied.*

17-1970 Adh– and Adh+ *Drosophila* Set
Two cultures: alcohol dehydrogenase negative (Adh–) and wild type (Adh+). Adh– flies cannot break down alcohol.

17-3010 FlyNap® Kit

- Not explosive like ether—can be shipped via USPS.
- Easy to use—no bulky equipment needed.
- *Drosophila* remain anesthetized for 50 minutes to several hours.

Kit contains a 10-mL vial of FlyNap® and 12 anesthetic wands (patent No. 4,224,898). No need for separate anesthetizer. FlyNap® is safer than ether and is easier to use. Kit contains 100 doses. Shipped via surface mail only. *Sold only to schools and businesses.*

Note: FlyNap® is a registered trademark of Carolina Biological Supply Company.

17-3015 FlyNap®/Anesthetizer Kit
Contains 100 mL of FlyNap®, 3 *Drosophila* Anesthetizers, and 2 dropping pipets. Enough anesthetic for 100 anesthetizer charges. With instructions. Shipped via surface mail only. *Sold only to schools and businesses.*

17-3050 *Drosophila* Culture Kit
Includes one 17-3010 FlyNap® Kit, 36 culture vials, 36 vial plugs, Instant *Drosophila* Medium, 72 vial labels for *Drosophila* crosses, 2 sorting brushes, 100 sorting cards, and instructions. *Drosophila* cultures not included. Shipped via surface mail only. *Sold only to schools and businesses.*

17-3052 Carolina™ *Drosophila* Kit
Includes one 17-3010 FlyNap® Kit, 72 culture vials, 72 vial plugs, Instant *Drosophila* Medium, 72 vial labels for *Drosophila* crosses, *Drosophila* Record Sheets, two sorting brushes, 100 sorting cards, and instructions. *Drosophila* cultures not included. Shipped via surface mail only. *Sold only to schools and businesses.*

17-3060 *Drosophila* Laboratory Kit

Includes one 17-3015 FlyNap®/Anesthetizer Kit, 144 culture vials, 144 vial plugs, 12 *Drosophila* sorting brushes, 100 *Drosophila* sorting cards, 4 L of Instant *Drosophila* Medium, a roll of vial labels for *Drosophila* crosses, 100 *Drosophila* Record Sheets, and 12 Carolina™ D*rosophila* Manuals. Shipped via surface mail only. *Sold only to schools and businesses.*

Drosophila Cross Kits

Our cross kits contain the materials necessary for studying monohybrid, dihybrid, sex-linked, and autosomal-linked inheritance in *Drosophila*. Each kit contains a card for prepaid delivery of one culture of 2 strains of *Drosophila* and one F_1 culture from a cross between the two strains. In addition to the *Drosophila* cultures, each kit includes one 17-3050 *Drosophila* Culture Kit, which is shipped via surface mail only.

17-1984 *Drosophila* Monohybrid Cross Kit
Students study monohybrid inheritance using the *Drosophila* strains apterous (wingless) and wild type (winged).

17-1987 *Drosophila* Sex-Linked Cross Kit
Demonstrates sex-linked inheritance using white-eyed and red-eyed (wild type) *Drosophila*.

17-1990 *Drosophila* Dihybrid Cross Kit
Students study dihybrid inheritance using the two *Drosophila* mutants apterous (wingless) and sepia (sepia-eyed).

Drosophila Crosses

Each vial contains larvae and pupae of the F_1 generation of a cross between two different strains of *Drosophila*. The parent generation is removed before shipment.

17-2000 F_1 apterous × wild. A vial of F_1 fruit flies from a cross between wingless (apterous) females and winged (wild type) males. The expected F_2 ratio is 3 wild type:1 apterous.

17-2010 F_1 sepia × wild. A vial of F_1 fruit flies from a cross between sepia-eyed females and red-eyed (wild type) males. The expected F_2 ratio is 3 wild type:1 sepia. One of the crosses used in AP® Biology Lab 7.

17-2020 F_1 white × wild. Sex-linked. A vial of F_1 fruit flies from a cross between white-eyed (white) females and red-eyed (wild type) males. The F_1 males have white eyes. The expected F_2 ratio is 1 wild type:1 white. One of the crosses used in AP® Biology Lab 7.

17-2030 F_1 apterous × sepia. A vial of F_1 fruit flies from a cross between wingless red-eyed (apterous) females and winged sepia-eyed (sepia) males. The expected F_2 ratio is 9 wild type:3 apterous:3 sepia:1 apterous sepia.

17-2032 F_1 vestigial × sepia. A vial of F_1 fruit flies from a cross between vestigial-winged, red-eyed females and winged, sepia-eyed males. The expected F_2 ratio is 9 wild type:3 vestigial:3 sepia:1 vestigial sepia. One of the crosses used in AP® Biology Lab 7.

17-2035 F_1 vestigial × ebony. A vial of F_1 fruit flies from a cross between vestigial-winged; red-eyed female flies and male winged flies with ebony-colored bodies. The F_2 ratio is 9 wild type:3 vestigial:3 ebony:1 vestigial ebony.

17-2055 F_1 Custom *Drosophila* Cross
A vial of F_1 fruit flies. Select the parents from our general listing of *Drosophila* strains. *Order must be received **6 weeks** before the desired delivery date.*

Linkage Determination Crosses

17-2050 Two-Point Linkage
A vial of F_1 fruit flies from a cross between females having yellow bodies and white eyes (*yw*/*yw*) and wild males (++/*Y*). The 1:1 F_2 ratio is altered by a 1.5% crossover rate.

17-2051 Three-Point Linkage
A vial of testcross fruit flies from a cross between females heterozygous for three chromosome 2 mutants (+++/*b vg bw*) and males homozygous for the mutants (*b vg bw*/*b vg bw*). The positions of the mutants on chromosome 2 are: black (*b*), 48.5; vestigial (*vg*), 67.0; and brown (*bw*), 104.5.

Drosophila Supplies

17-1510 Genetic Data Sheets
For recording phenotypic classes, actual counts, and expected counts in monohybrid and dihybrid crosses. Includes the formula for computing chi-square values and a chi-square table. Set of 100 (4 × 6) forms.

17-3025 FlyNap® Anesthetic
Carolina's safe, easy-to-use anesthetic for *Drosophila* can be used with anesthetic wands (1,000 charges) or with the 17-3040 *Drosophila* Anesthetizer (100 charges). Shipped via surface mail only. *Sold only to schools and businesses.*

17-3040 *Drosophila* Anesthetizer
For use with FlyNap® or ether. Funnel-shaped end fits various culture vessels. Flies cannot come into contact with and be killed by liquid anesthetic. Slow release of anesthetic reduces risk of killing or sterilizing flies. Instructions included.

17-3120 *Drosophila* Culture Vials
Shatterproof plastic vials 1¼ diam. × 4 H. Plugs and caps not included.

17-3150 Insect Culture Incubator
Protects *Drosophila* and other insect cultures from cold temperatures. Styrofoam® incubator has hinged top with observation windows for checking thermometer and cultures. Complete with thermometer, adjustable heat control,

and instructions. One incubator holds up to 100 *Drosophila* cultures in our 17-3120 *Drosophila* Culture Vials. Inside dimensions: 16½ × 16½ × 9½ H. Insects not included. A Carolina™ exclusive product.

17-3190 Vial Labels for *Drosophila* Crosses
Pressure-sensitive labels for recording parents of *Drosophila* crosses. Also includes spaces for date and student's name. Roll of 500 (5 × 2.5-cm) labels.

17-3192 *Drosophila* Record Sheets
For recording *Drosophila* crosses and F_2 progeny. Set of 100 (4 × 6) forms.

Formula 4-24® Instant *Drosophila* Medium
Mold-resistant medium needs no cooking or sterilizing—just add water for a complete medium. Each liter will prepare 75 or more of our *Drosophila* culture vials (17-3120). Yeast, measuring cups, and instructions are included. Formula 4-24® Blue contains a coloring agent added to facilitate observation of larvae.

17-3200 Plain, 1-L bag
17-3202 Plain, 4-L bag
17-3204 Plain, Case of four 4-L bags
17-3210 Blue, 1-L bag
17-3212 Blue, 1-L bag
17-3214 Blue, Case of four 4-L bags

Other *Drosophila* Species

17-2870 *D. mojavensis*
17-2880 *D. pseudoobscura*
17-2890 *D. virilis*
17-2895 *D. hydei sturtevant* (flightless)

Nongenetic Uses of Fruit Flies

17-2900 Fruit Fly Culture Kit
For life cycle studies or feeding small animals. Includes one 17-2910 fruit fly culture, 6 culture vials with plugs and netting, Instant *Drosophila* Medium for about 72 cultures, and instructions.

17-2910 Fruit Flies
Flies are specially selected for wing variations which restrict flying, making them convenient to use for life cycle studies or feeding small animals. About 100 flies.