DROSOPHILA GENETICS 3.
Dihybrid Cross

P₁
vestigial  sepia
gg/vg    se/se

F₁
wild heterozygotes
+vg; +/se

F₁ gametes → +; +  +; se  vg; +  vg; se

F₂
+; +
+; se
vg; +
vg; se

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Carolina Drosophila Manual

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Carolina Biological Supply Company

Introduction ................................................. 4
Culturing ..................................................... 4
  Culture Vessels
  Media
  Bacterial Infection
  Controlling Mites
Life Cycle .................................................. 8
  Virgin Flies
Anesthetizing .............................................. 9
  FlyNap
  Ether
  Sorting and Selecting
Sexing ..................................................... 11
  Sexing Pupae
  Sex Markers
Mating and Counting .................................... 14
Genetic Notation and Phenotypes ..................... 14
Experimental Crosses .................................. 18
  Monohybrid Crosses
  Dihybrid Crosses
  Linkage Groups
Drosophila F₁ Crosses ................................ 24
Phenocopies .............................................. 25
Records and Analysis .................................. 25
Salivary-Gland Chromosomes ......................... 26
Further Reading ....................................... 26
Available Stocks ....................................... 27
Drosophila Cultures and Supplies ................... 30

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.

Carolina Biological Supply Company
Burlington, NC/Gladstone, OR

45-2620 Carolina Drosophila Manual
Introduction

Basic genetic mechanisms arose early enough in primitive organisms or were so superior to alternatives that they are shared by most organisms. It is possible to study the principles of genetics in one or a few organisms, and to gain understanding of the mode of inheritance in many.

The animal most widely used for genetic studies is the common fruit fly, Drosophila melanogaster. The fly is easily cultured, and its generation time is only two weeks at 21° C. One female may lay as many as 500 eggs in 10 days. Because Drosophila is small, cultures occupy little space; however, the fly is large enough for rapid notation of mutant characters.

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. While the life cycle of Drosophila may be shorter at higher temperatures, it is generally better to carry the cultures at 20° to 21° C. Higher temperatures are conducive to the growth of bacteria, fungi, and mites; lower temperatures greatly slow development of the flies.

The minimal equipment (Fig. 1) for raising fruit flies and for making crosses includes culture vessels, plugs, and medium; marking pencil or labels; anesthetic; white background (card) for examining flies; fine brush; bright, cool light; and magnifying glass, or (preferably) a widefield stereomicroscope.

Figure 1  Minimal equipment for studying fruit flies.
Culture Vessels

Transparent vials or bottles of glass or plastic can be used as culture vessels for Drosophila. For most classrooms and research, the optimal size of the vessels is 50 to 100 cm³. Vessels should be clean, but they need not be sterilized when the medium is properly prepared. Plastic vials should not be autoclaved, and may be used as they come from our shipping carton.

Plastic (polyurethane) foam or nonabsorbent cotton is used for plugs. Plastic plugs are neater, easier to handle, and last longer than cotton plugs. Plastic plugs may be used directly from the shipping package. Both cotton and plastic plugs should be autoclaved before reuse.

Continued use and pressure may compress the 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 for controlling moisture and for securing 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 greatly simplified the culture of fruit flies.

INSTANT DROSOPIALA MEDIUM: The ultimate development in fruit fly culture is Formula 4-24® Instant Drosophila Medium which needs neither cooking nor sterilizing. Each unit of about 1 liter is sufficient for preparing 50 to 70 cultures. Equal volumes of Instant Drosophila Medium and cool tap water are dumped into a vial and a few grains of dry viable yeast are sprinkled on top (Fig. 2). The metabolic byproducts of yeast include CO₂. Large amounts of yeast in a culture can produce enough CO₂ to render Drosophila sterile or even cause death. 6 to 10 grains 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.
Early in the history of Drosophila culture, it became a 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. The larvae pupate on the sides of the culture vessel just as well as on paper, and often the paper collapses into the medium, drowning the pupae. If paper is used in Drosophila cultures, potential problems with molding 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 with Formula 4-24*. The cultures of fruit flies that we ship contain plastic inserts to hold the medium in place during shipment.

COOKING MEDIUM: Mold inhibitors (ours is methyl p-hydroxybenzoate, Tegosept-M) 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 powder to boiling water.

Dissolve 15 g of agar and 1 to 2 g of Mold Inhibitor (87-6161) in 500 ml of boiling water. Add 130 ml of sulfur-free molasses and again bring to boiling. 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.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (tap)</td>
<td>750 ml</td>
</tr>
<tr>
<td>Tegosept</td>
<td>1 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>Molasses (sulfur-free)</td>
<td>130 ml</td>
</tr>
<tr>
<td>Cornmeal (yellow)</td>
<td>100 g</td>
</tr>
<tr>
<td>Cream of wheat</td>
<td>100 g</td>
</tr>
<tr>
<td>Oatmeal (not instant)</td>
<td>16 g</td>
</tr>
<tr>
<td>Brewer’s yeast</td>
<td>60 g</td>
</tr>
<tr>
<td>Banana pulp</td>
<td>10 g</td>
</tr>
</tbody>
</table>

Cooked Drosophila Media (Five alternate formulae)
**Bacterial Infection**

Bacterial contaminants sometimes infect *Drosophila* cultures, causing reduction in fly hardiness, sterility, and often death. The infections appear as a gray or drab yellow-green viscous sheen on the medium surface. Periodic cleansing of laboratory workspace and utensils and the use of filtered or distilled water will aid in preventing accidental introduction of bacteria into the cultures. If infected cultures occur, daily applications of an aqueous solution of 0.5 percent penicillin G or 0.1 percent 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**

*Drosophila* cultures sometimes become infested with mites (Fig. 3). Any infested culture should be removed immediately from the laboratory and sterilized. The best prevention against an infestation of mites is cleanliness. All utensils and working areas should be kept clean. A culture should not be kept longer than one month.

Lindane-treated shelf paper will kill mites which walk across it. If culture vessels of *Drosophila* are set on such paper, mites cannot cross the paper and the fruit flies in the culture vials are in no way injured. Mites can also be controlled by treating work surfaces with 1 part benzyl benzoate in 5 parts isopropanol.

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**Figure 3** A typical long-haired mite.

**Figure 4** Fruit fly egg.

**Figure 5** Fruit fly larva.
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 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. The adult fruit flies may live for several weeks.

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.

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.

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 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 12 hours old. The virginity of the flies can be tested by keeping the females by themselves in a culture vial for 3 to 4 days before transferring them to another vial with the males. If larvae appear in the vial that contained only the females, then the females were not all virgin and the cross will not be meaningful.
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.

Anesthetizing

Investigators have used many means to immobilize fruit flies for examination. They have crowded them into constricted glass tubes. They have chilled them. They have knocked them out with ether and methoxyflurane, and they have kept them in a continuous flow of carbon dioxide. When bulky or expensive apparatus is not available, the novice kills or loses many flies with these antiquated approaches to immobilizing fruit flies. Now, even elementary-grade students can easily, safely, and inexpensively anesthetize fruit flies with our FlyNap Kit (patent No. 4,224,898).

FlyNap®

With FlyNap (Fig. 8) fruit flies can be anesthetized in an empty vial (Fig. 8 & 9), in an anesthetizer, or while they are still in their culture vial. FlyNap is safer than ether for both the students and 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.

The anesthetic wand is a primary component of the 17-3010 FlyNap Kit. Dip the absorbent end of the wand into the FlyNap. Tap the bottom of the culture vial on the tabletop to knock the flies to the bottom of the vial. With one finger, push the plug slightly to one side. Remove the wand from the FlyNap and quickly stick the anesthetic end into the culture vial beside the plug so the anesthetic tip is below the plug. Keep the culture vial upright with the wand in place. Watch the flies closely and remove the plug and wand when the flies are anesthetized (2 minutes in an empty vial, 4 minutes in a vial with medium). Immediately spill the flies onto a card for study. When replugging the culture vial, be careful not to insert a part of the plug that may have FlyNap residue on it.
The length of time the flies remain anesthetized depends on the amount of FlyNap on the wand, and on the number and age of the flies in the culture vial. The FlyNap wand meters the correct amount of anesthetic for our standard Drosophila Culture Vial. Less anesthetic is needed for smaller vessels; more anesthetic is needed for larger ones. Use two wands with FlyNap for 250-ml vessels. Flies can be anesthetized in 2 minutes in a Carolina Anesthetizer charged with 1 ml of FlyNap.

In the amount transferred on the wand, FlyNap has no ill effect on Drosophila eggs, larvae, pupae, or young adults. Under moist culture conditions anesthetized flies might stick to the culture medium or become sticky from contact with the medium. If you think that could happen, the flies can be transferred to an empty culture vial and then anesthetized within 2 minutes. Some flies may exhibit light trembling of the legs and/or wings immediately after being anesthetized with FlyNap; this is acceptable and will stop after 1 or 2 minutes.

**Ether**

To use the Carolina Anesthetizer (Fig. 10), remove the hollow stopper from the top and remove the cap from the bottom. Fill the hollow stopper one-third full with ether for anesthesia. 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.*

If you have not etherized flies before, read this paragraph and 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 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 stereomicroscope.

When the novice etherizes fruit flies, he often has a tendency to over-etherize them; therefore, the Carolina Anesthetizer is made to release the ether slowly.

![Figure 10 Drosophila Anesthetizer.](image)
Individuals experienced in handling fruit flies may wish to etherize them rapidly. It is a simple matter to speed up the release of ether into the inner etherizing chamber by adding holes with the end of a red-hot teasing needle (with 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. The flies are killed or sterilized if they are re-etherized too many times in a short period.

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.

**Sorting and Selecting**

The anesthetized flies should be placed in a row on a white card. The flies are moved about with a teasing needle, a fine brush, or any suitable tool. The flies should be examined with a stereomicroscope at a magnification of at least 12X to 15X 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

![Figure 11](image)

**Figure 11** Ventral posterior view of female and male fruit flies.
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 (puparium).

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.

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.

**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 100X 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

**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.
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.

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 sex-linked traits, such as white, directly from their fathers. Our stocks of attached-X are homozygous for yellow and forked: 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. 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.

![Diagram of Attached-X inheritance](image)

**Figure 15** Attached-X inheritance.
Daughters result from fertilization of attached-X eggs by Y-carrying sperm. Investigations of various unusual chromosomal combinations have shown that the Y chromosome is insignificant in determining the sex of a fruit fly—sexual development is directed by the relative amount of X chromosome present.

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 eggs and sperm produces a viable superfemale (Fig. 15).

**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 F1. The progeny of the first cross is the first filial generation, the F2. The next generation is the F3 and so on.

The virgins selected for a cross should be swept onto a card that has been folded down the center. About six virgins are tapped into a culture vial with about the same number of males of another strain. For reciprocal crosses, additional cultures are set up with the sex of each strain reversed.

About 7 to 10 days after a cross is started, the parents should be removed. This is to preclude breeding between generations and to avoid confusion when counts are made.

About six pairs of flies, which need not be virgin, are chosen from the F1 and placed in a fresh culture vial to produce an F2. After 7 to 10 days the F1 flies should be removed from the culture.

A count of the F2 progeny should start the day after emergence of the new generation. Generally on the first day of emergence, a culture will produce more females than males. On successive days the proportion of males tends to increase so that the sex ratio balances.

The progeny should be anesthetized and counted every other day for about 10 days. Counts made during less than 10 days may omit individuals with slow developmental rates because of their sex or some mutation. Counts beyond 10 days run the risk of including flies of the next generation. Once counted, the flies should not be returned 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 a
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.
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.

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.

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), shape, and size (Fig. 24).

**Experimental Crosses**

**Monohybrid Crosses**

**INTRODUCTORY DROSOPHILA SET:** This set (17-1900) consists of two fruit fly cultures, one winged and the other wingless. 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 which is abbreviated ap.

When the winged flies (+/+) are mated with the apterous flies (ap/ap), the offspring (the F1) all have the genotype +/ap and are winged. Because all
the F1 flies are winged, the wild gene $+$ is termed dominant and the mutant gene $ap$ is called recessive.

Matings between the F1 flies produce offspring (the F2) 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.
RECESSIVE GENE: Set up reciprocal crosses between wild and a stock of aperous, or vestigial, or sepia, or ebony. Following the techniques described in the Mating and Counting Section, record the phenotype of the F₁, and cross the F₁ flies among themselves to produce an F₂ (3:1).

TESTCROSS: Backcross any of the F₁ 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 F₁ (Fig. 31).

DOMINANT GENE: Make reciprocal crosses between wild and either Lobe or Wrinkled and carry through to the F₂ (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 F₂ 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 involving a recessive autosomal mutation, except that expression of the mutation is limited to half of the males in the F₂. On the other hand, when a white-eyed female is mated to a wild male, all the males in the F₁ have white eyes and all the females have red eyes; the F₂ has a 1:1 ratio with half of each sex having white eyes (Fig. 33).

SEX-LINKED DOMINANT GENE: Set up and carry through the F₂ reciprocal crosses between wild and Bar. When a wild female is mated with a Bar-eyed male, all the females in the F₁ show Bar and all the males are wild; the F₂ ratio is 1:1 with half of each sex showing Bar. When a homozygous Bar female is mated

---

**Figure 31** Testcross.

**Figure 32** Thomas Hunt Morgan. Photograph courtesy of the Division of Biology, California Institute of Technology.
with a wild male, all of the F₁ show Bar and the F₂ 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 F2 crosses between vestigial (chromosome 2) and ebony (chromosome 3), or between aperous(2) and sepia(3). The expected F2 ratio is 9:3:3:1 (see inside front cover).

GENE INTERACTION: Produce an F2 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 F2 from a cross between female white(1);vestigial(2) and male wild. The F1 females are wild and the males are white (1:1). The expected F2 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 spineless(3). Back-cross virgin F1 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.

TWO SEX-LINKED GENES: Obtain an F2 from a cross between yellow miniature or eosin miniature females and wild males. If very tight linkage is desired, use yellow white females.

\[
\begin{array}{c|c|c}
P_1 & \varnothing & \varnothing \\
& +/+ & B/Y \\
& wild & Bar \\
F_1 & \varnothing & \varnothing \\
& +/B & +/Y \\
& Bar & wild \\
\end{array}
\]

\[
\begin{array}{c|c|c}
P_1 & \varnothing & \varnothing \\
& B/B & +/+ \\
& Bar & Y \\
F_1 & \varnothing & \varnothing \\
& +/B & B/Y \\
& Bar & Bar \\
\end{array}
\]

**Figure 34** Reciprocal crosses for sex-linked dominant gene.
DOUBLE CROSSOVERS: If the student is to work with three or more genes in the same linkage group, he should 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 sepia spineless kidney ebony rough(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 F1 males show the mutation, it is a sex-linked recessive. If all the F1 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 F1 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 F1 males of one of the four phenotypes produced in Step 1: Cy;D, Cy;Sb, Pm;D, or

<table>
<thead>
<tr>
<th>Chromosome 1</th>
<th>Chromosome 2</th>
<th>Chromosome 3</th>
<th>Chromosome 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>yellow</td>
<td>6.1 Curly</td>
<td>26.0 sepia</td>
<td>0.0+ shaven</td>
</tr>
<tr>
<td>0.0 + scute</td>
<td>13.0 dumpy</td>
<td>40.7 Dichaete</td>
<td>0.0+ cubitus</td>
</tr>
<tr>
<td>0.8 prune</td>
<td>48.5 black</td>
<td>44.0 scarlet</td>
<td>interruptus</td>
</tr>
<tr>
<td>1.5 white</td>
<td>54.5 purple</td>
<td>46.0 Wrinkled</td>
<td>0.0+ grooveless</td>
</tr>
<tr>
<td>13.7 crossveinless</td>
<td>54.8 Bristle</td>
<td>47.0 radius</td>
<td>0.0+ sparkling-polished</td>
</tr>
<tr>
<td>20.0 cut</td>
<td>55.2 aperous</td>
<td>incompletus</td>
<td>0.2 eyeless</td>
</tr>
<tr>
<td>21.0 singed</td>
<td>57.5 cinnabar</td>
<td>52.0 rosy</td>
<td></td>
</tr>
<tr>
<td>27.7 lozenge</td>
<td>67.0 vestigial</td>
<td>58.2 Stubble</td>
<td></td>
</tr>
<tr>
<td>33.0 vermillion</td>
<td>72.0 Lobe</td>
<td>58.5 spineless</td>
<td></td>
</tr>
<tr>
<td>36.1 miniature</td>
<td>75.5 curved</td>
<td>64.0 kidney</td>
<td></td>
</tr>
<tr>
<td>51.5 scalloped</td>
<td>100.5 plexus</td>
<td>69.5 Hairless</td>
<td></td>
</tr>
<tr>
<td>56.7 forked</td>
<td>104.5 brown</td>
<td>70.7 ebony</td>
<td></td>
</tr>
<tr>
<td>57.0 Bar</td>
<td>107.0 speck</td>
<td>79.1 bar-3</td>
<td></td>
</tr>
<tr>
<td>64.8 maroonlike</td>
<td></td>
<td>91.1 rough</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.7 claret</td>
<td></td>
</tr>
</tbody>
</table>
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 F1 Crosses**

The F1 crosses of *Drosophila* that we ship contain only F1 offspring. The date on the label is when the parent (P1) flies were crossed in the vial. The F1 flies were removed from the vial before shipment unless otherwise noted on your shipment. F1 flies should begin emerging 12 to 14 days after the date on the label.

Except in cases of sex linkage or dominance, all the F1 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 F1 males.

To produce an F2, transfer 12 to 15 F1 flies (there is no need to select virgins) to a vial with fresh medium. After 7 to 10 days, remove the F1 flies from the F2 culture.

At 21° C the F2 progeny should begin emerging 12 to 14 days after the F2 vial is set up. A count of the F2 progeny should start the day after emergence of the new generation. Generally on the first day of emergence, a culture will produce more females than males. On successive days the proportion of males tends to increase so that the sex ratio balances.

The progeny should be anesthetized and counted every other day for about 10 days. Counts made during less than 10 days may omit individuals with slow developmental rates because of their sex or some mutation. Counts beyond 10 days run the risk of including flies of the next generation. Once counted, the flies should not be returned to the same culture vessel.

**Figure 35** Drosophila Record Sheet (17-9340). Actual size is 4 x 6 inches.

**Figure 36** Genetic Data Sheet (17-9380). Actual size is 4 x 6 inches.
Phenocopies

A phenocopy is an environmentally induced modification which is not inherited, but resembles the phenotype of a known mutant gene. Our 17-1940 Phenotype Control Set is used to show that the expressed character (in this case, reduced eye) may be under control of a dominant or recessive gene, or under environmental influence. The set includes medium, sodium metabolate, and three cultures: wild, Lobe(2), and eyeless(4).

Dissolve the contents (0.7 g) of the packet of sodium metabolate in 1 liter of warm tap water to make 1 liter of 0.07% solution. Stir well.

Use the solution with Formula 4-24® Instant Drosophila Medium in place of the tap water called for in the instructions on the container. Prepare and label 12 or more vials of medium with sodium metabolate, and several vials of medium with tap water for controls.

Divide each of the two kinds of medium into three groups: (1) Parents added to each vial are 12 mated pairs of wild; (2) parents added to each vial are 12 virgin wild females and 12 eyeless males, or the reciprocal cross; and (3) parents added to each vial are 12 virgin wild females and 12 Lobe males, or the reciprocal cross.

Remove the parents from the vials after five days. In the cases of groups (2) and (3), subcultures of the parents on control medium can be examined later to determine whether or not the females were virgin.

Score the emerging flies from the treatments and controls for the frequency of eyeless and Lobe phenotypes. Chi-squares may be calculated and compared with chi-square tables to determine the “significance” of the observed frequencies.

Records and Analysis

It is important to keep clear and concise records. We suggest a form such as that shown in 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 F2 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. It should be kept in mind that the chi-square method does not alone prove that the inferred ratio is the correct ratio. The chi-square method 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 percent 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). They should be well-fed, uncrowded, and somewhat more moist than cultures used for stocks and crosses. The larvae of larger fruit flies, such as *D. mojavensis*, have larger salivary glands than *D. melanogaster* and give better results for the beginner.

Third-instar larvae should be selected about the time they crawl out of the medium onto the sides of the vessel and evert the anterior ends of the trachial system, but before the cuticle hardens.

For removal of the salivary glands, a larva is placed in a drop of 2% aceto-orcein (in 45% acetic acid) or 0.7% aqueous sodium chloride solution on a microscope slide. A stereomicroscope and a pair of dissecting needles are needed for the operation. One needle is used to hold the larva in place. The second needle is placed directly behind the mouthparts and used to detach the head and pull out the salivary glands. If the dissection is done 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. The glands should not be allowed 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 your 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. The slide should not be allowed to dry; the edges of the coverslip can be temporarily sealed with fingernail polish or melted wax. The slide is then ready for examination with a compound microscope.

Figure 37  Salivary-gland chromosomes.
Further Reading


Available Stocks

Large, vigorous cultures of D. melanogaster are shipped in shatterproof 4” x 11/4” diameter vials. Order cultures by catalog number and name. See our current Biological Materials Catalog for prices.

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

17-2100 Scute. (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.
17-2126 Inversion delta-49, yellow white forked.
17-2130 lozenge. Eyes reduced, almond shaped, glossy.
17-2140 maroonlike. Eyes brownish purple.
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 cross-overs; 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; cross-veins 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.
17-2245 white-eosin miniature.
17-2255 white cut. Eyes white; wings cut to point and scalloped.
17-2260 white miniature forked. Eyes white; wings miniature; bristles reduced and bent or split.
17-2270 yellow. Yellow body, wing hairs, and veins.
17-2275 yellow forked attached. Yellow forked female, wild male; female has two X chromosomes attached to each other and one Y chromosome (XXY); and YY and the superfemale (XXX) usually die, daughters have a Y from the father while sons have a Y from the mother and an X from the father—reverse of normal.
17-2277 yellow forked attached & white. Females yellow with forked bristles; males with white eyes.
17-2278 yellow forked attached & white Bar.
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 aperous. No wings.
17-2330 black. Black on body, tarsi, wing veins; darkens with age.
17-2335 black curved.
17-2337 black purple curved plexus speck. The purple eye is ruby at hatching, darkening to purplish ruby with age; plexus has extra veins in the wings, especially toward tips and margins; speck causes black specks on the axils of the wings and a dark body color.
17-2340 black vestigial.
17-2345 black vestigial brown.
17-2350 Bristle Lobe over Curly. Balanced lethal; bristles short and thick; eyes reduced; wings curly.
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.
17-2477 bar-3 radius incompletus.
17-2480 claret. Eyes ruby. Slightly narrow body and pointed wing.
17-2490 Dichaete Hairless over Payne inversion. Balanced lethal stock; wings divergent and raised; several bristles absent.
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 going from brownish red to black with aging.
17-2580 sepia ebony.
17-2585 sepia spineless.
17-2586 sepia spineless kidney ebony rough. The expression of kidney is variable and tends to overlap wild type; front of eye indented and tuft of vibrissae and hairs below eye. Eyes of rough are round and slightly smaller and narrower than wild type.
17-2590 spineless. Bristles size of hair.
17-2595 spineless-aristapedia. Ends of antennae enlarged and leg-like.
17-2600 Wrinkled. Wrinkled wings.
Chromosome 4 Mutants

17-2610 cubitus interruptus grooveless eyeless shaven. At lower temperatures (19°C) cubitus interruptus shows one or more gaps in the cubital vein; increasing overlap with wild type from 25°C up. The sharp transverse groove between scutellum and thorax is nearly eliminated by grooveless.

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.

17-2650 sparkling-polished. Eyes glossy and darker than wild.

Multichromosomal Mutants

17-2690 Bar white singed(1):ebony(3).

17-2692 white singed(1):ebony(3).

17-2700 vermilion(1):brown(2). Eyes pale apricot.

17-2705 vermilion(1):sepio(3). Eyes light at eclosing, darkening with age.

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

17-2710 white(1):brown(2). Eyes white.

17-2720 white(1):vestigial(2).

17-2725 white(1):sepio(3). Eyes white.

17-2726 white(1):sepio ebony(3).

17-2728 yellow forked attached (1) & white(1):apterous(2).


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

17-2750 Curly over Plum d pb(2):Dichaete over Stubble(3). For determining linkage group; wings curly; eyes purplish; wings divergent; bristles short and thick.

17-2753 aterous(2):sepio(3).

17-2755 dumpy(2):sepio(3).

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

17-2765 vestigial(2):sepio(3).
Drosophila Cultures and Supplies

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 F2. With instructions.

17-1904 Basic Drosophila Set. Two cultures: wild and aperous (wingless).

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

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

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

17-1920 Independent Assortment Set. One culture each of dumpy and sepia.

17-1925 Gene Interaction Set. Two cultures, brown and red. The expected F2 ratio is 9 wild:3 brown:3 red. 1 white.

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

17-1935 Recessive Genes Set. For Yellow Version of BSCS: wild, white, brown, vestigial, brown, dumpy, and sepia.

17-1940 Phenotype Control Set. For showing that the expressed character (in this case, reduced eye) may be under control of a dominant or recessive gene, or under environmental influence. Set includes instructions, sodium carbonate, 1 unit of Formula 4-24® Instant Drosophila Medium, and three cultures: wild, Lobe(2), and eyeless (4).

17-1945 Comprehensive Drosophila Set. Consists of 17-1940, plus six additional cultures: Bar(1), white miniature forked(1), dumpy(2), aristata (3), sepia (3), and Curly over Plum; Dichaete over Stubble.

17-1950 Drosophila Breeding Kit. Two cultures (wild and vestigial), two 1-liter units Instant Drosophila Medium, 17-3010 FlyNap® Kit, 100 plugs, and instructions. Purchaser provides culture bottles.

17-1951 Drosophila Breeding Kit. Same as 17-1950, but with wild and white.

17-1958 Drosophila Student Kit 1. Designed for use by one or two students. Includes card for requesting prepaid delivery of two Drosophila cultures and: 12 culture vials, 12 vial plugs, 12 labels, 12 pkts. instant medium, measuring cup, FlyNap® kit, Drosophila sorting brush, Drosophila manual, and instructions.

17-1959 Drosophila Student Kit 2. For use by three to five students. Includes card for requesting prepaid delivery of two Drosophila cultures and FlyNap® kit, Carolina Drosophila manual, 36 culture vials, 36 vial plugs, unit instant Drosophila medium, 72 labels, 2 Drosophila sorting brushes, and 100 Drosophila sorting cards.


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 in our 17-1960 Drosophila BioKit®. A card is included for the prepaid delivery of one wild and one white miniature forked Drosophila culture.

17-1966 Inheritance of White Eye Color BioKit®. For 30 students to study independent assortment, epistasis, linkage, and crossing-over using various strains of white-eyed Drosophila. A card for the prepaid delivery of one wild and five different white-eyed Drosophila.

17-1970 Drosophila Linkage Kit. Students study linkage using two chromosome 3 mutants (bar on chromosome 3 and radius incomplectus). Each kit includes our 17-3050 Drosophila Culture Kit and a card for prepaid delivery of the two Drosophila cultures (wild and bar on chromosome 3 radius incomplectus).

Drosophila Crosses

17-2000 F1. aperous x wild. A vial of F1 fruit flies from a cross between wingless (apterous) and winged (wild) parents. The expected F2 ratio is 3 wild:1 aperous.

17-2020 F1 white x wild. Sex-linked. A vial of F1 fruit flies from a cross between white-eyed (white) females and red-eyed (wild) males. The F1 males have white eyes. The expected F2 ratio is 1 wild:1 white.
17-2030 F1: aperous x sepia. A vial of F1 fruit flies from a cross between wingless red-eyed (aperous) flies and winged sepia-eyed (sepia) flies. The expected F2 ratio is 9 wild:3 aperous:3 sepia:1 aperous sepia.

17-2035 F2: vestigial x ebony. A vial of F2 fruit flies from a cross between vestigial-winged female flies and male flies with ebony-colored bodies. The F2 ratio is 9 wild:3 vestigial:3 ebony:1 vestigial ebony.

17-2055 F2: Custom Drosophila Cross. A vial of F2 fruit flies. Select the parents from our general listing of Drosophila strains and notify us 6 weeks before the desired delivery date.

Culture Kits

17-3050 Drosophila Culture Kit. Includes 36 culture vials, 36 vial plugs, unit of Instant Drosophila Medium, 72 vial labels for Drosophila crosses, FlyNap® Kit, 2 sorting brushes, 100 sorting cards, and instructions. Drosophila cultures not included.

17-3052 Carolina Drosophila Kit. Includes 72 culture vials, 72 vial plugs, unit of Instant Drosophila Medium, 72 vial labels for Drosophila crosses, pad of Drosophila Record Sheets, FlyNap® Kit, 2 sorting brushes, 100 sorting cards, and instructions. Drosophila cultures not included.

Insect Culture Incubator

17-3150 Insect Culture Incubator. Protects Drosophila and other insect cultures from cold temperatures. Styrofoam incubator has top (hinged) 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 17-3085 Drosophila Culture Vials. Inside dimensions: 38 x 38 x 20 cm high. (Flies not included.)

Formula 4-24® Instant Medium

Formula 4-24® Instant Drosophila Medium with mold resistance, needs no cooking or sterilizing—just add water for a complete medium. Yeast, measuring cups and instructions are added for each order.

Formula 4-24® Plain
17-3200—1-Liter Bag
17-3202—4-Liter Bag
17-3204—Case (four 4-liter bags)

Formula 4-24® Blue
17-3210—1-Liter Bag
17-3212—4-Liter Bag
17-3214—Case (four 4-liter bags)

See our current catalog for prices.

Carolina
FlyNap®

Drosophila remain anesthetized for 50 minutes or longer without being killed or sterilized. Not explosive like ether. Inexpensive—each kit contains 100 doses.

17-3010 FlyNap® Kit. For anesthetizing Drosophila in their own culture vessels. Kit contains vials of FlyNap®, 12 anesthetic wands, and morgue. FlyNap® (patent No. 4,224,898).

17-3015 FlyNap®/Anesthetizer Kit. Contains 100-ml unit of FlyNap® (17-3025), three Drosophila Anesthetizers (17-3040), and two FlyNap® dropping pipets. Enough for at least 100 charges of the anesthetizer. Complete with instructions. Shipped via surface mail only.

17-3025 FlyNap®. Carolina's safe, easy-to-use anesthetic for Drosophila. Can be used with anesthetic wands or with 17-3040 Drosophila Anesthetizer. Enough for 100 charges with the Drosophila Anesthetizer or 1000 doses with our anesthetic wands. 100-ml bottle. Shipped via surface mail only.
Drosophila Life Cycle

Egg → Larva → Pupa → Larva → Egg