Carolina Drosophila Manual

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Carolina Biological Supply Company

Acknowledgement

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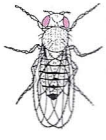
DROSOPHILA GENETICS 3.
Dihybrid Cross

P_1

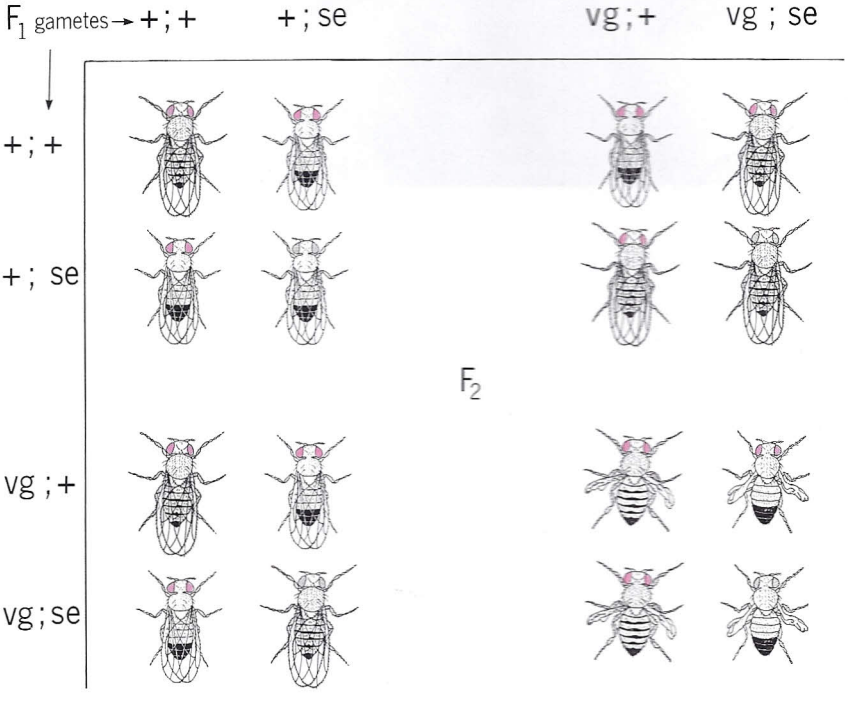
vestigial
vg/vg

sepia
se/se



F_1

wild heterozygotes
+/vg ; +/se



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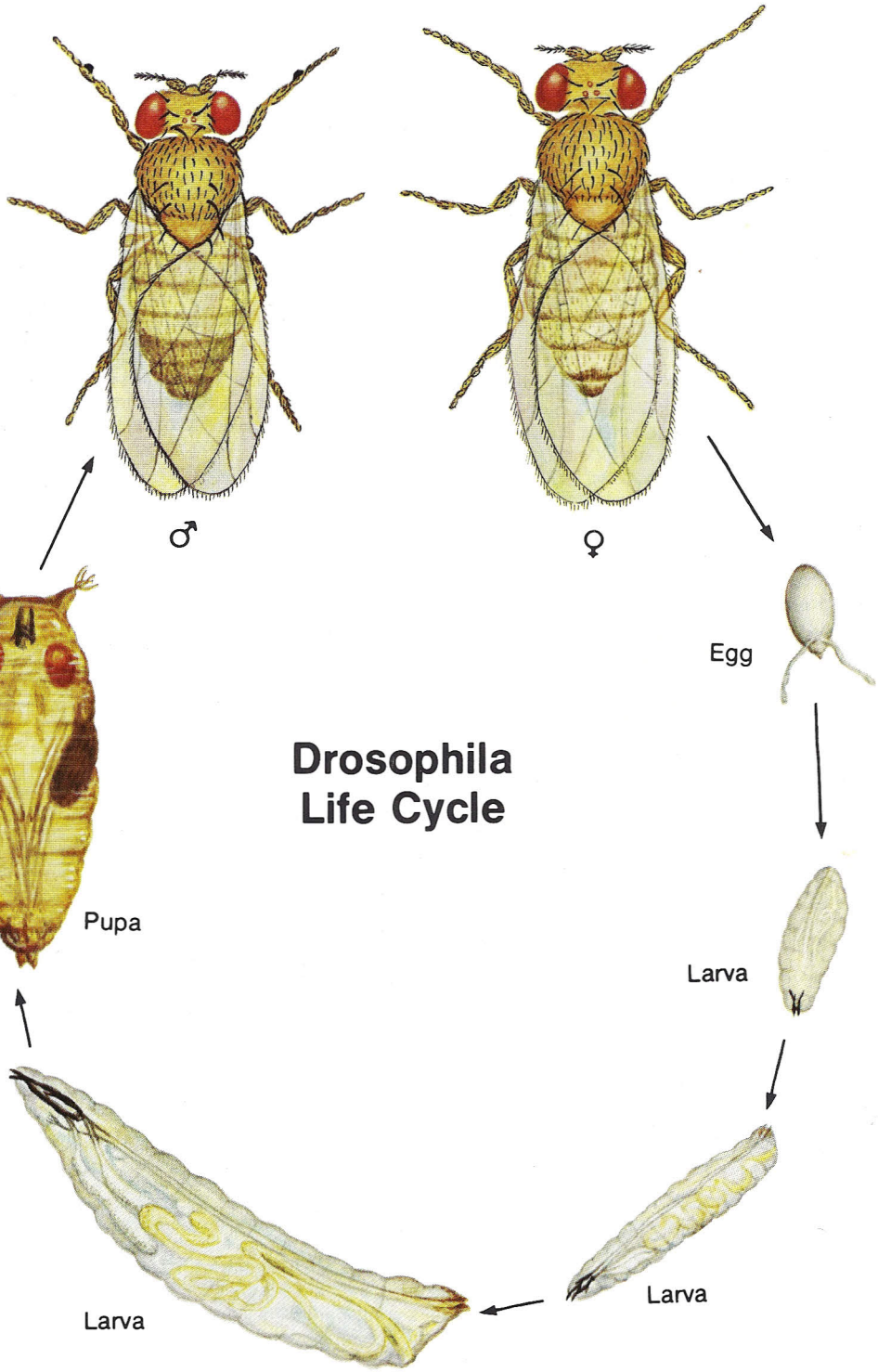

Drosophila Life Cycle

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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.

Figure 6
Fruit fly pupa.

Figure 7
Fruit fly adult.

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.

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

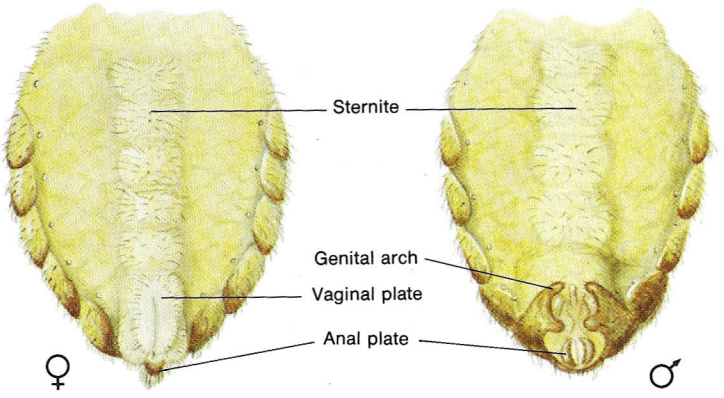

Figure 11 Ventral posterior view of female and male fruit flies.

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).

Figure 12 Sex combs on front legs of male fly.

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 miscroscope 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

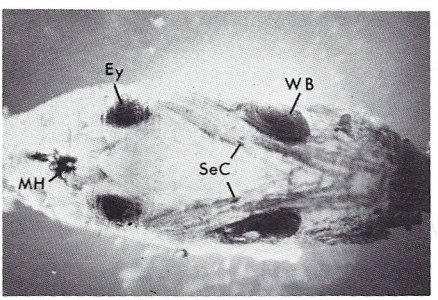

Figure 13 Ventral view of male *Drosophila* pupa. Ey, Eye; MH, Mouth Hook, SeC, Sex Comb; WB, Wing Bud. Photography: J. Hadden and J. A. Cunningham.

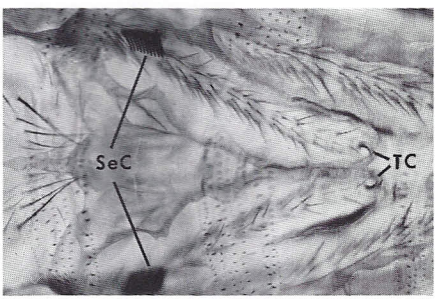

Figure 14 Mid-ventral view of male *Drosophila* pupa. SeC, Sex Comb; TC, Tarsal Claw.

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.

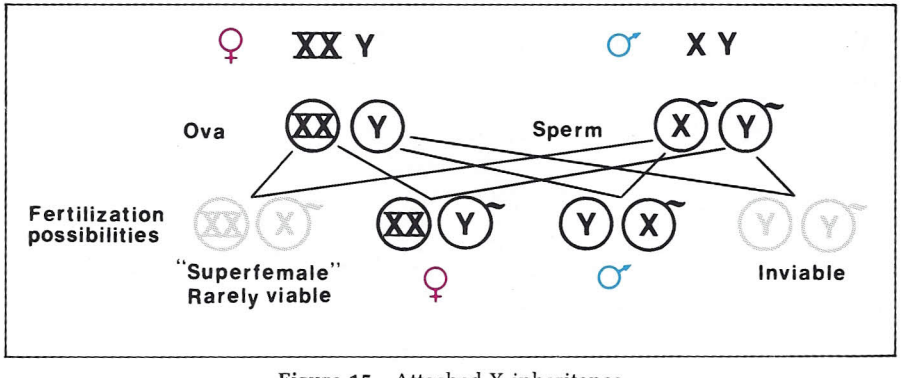

Figure 15 Attached-X inheritance.

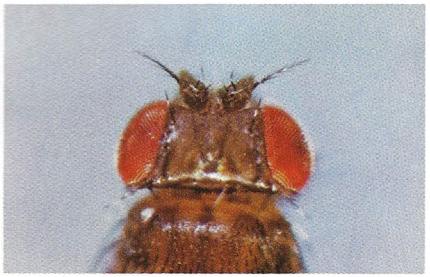

Figure 16 Wild-type *Drosophila*.

Figure 17 Homozygous aristapedia.

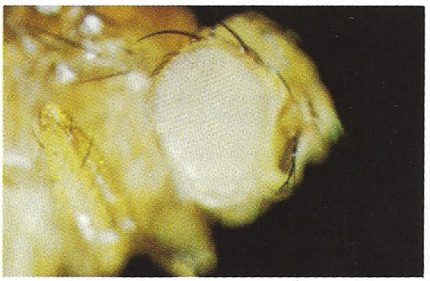

Figure 18 Recessive sex-linked white.

Figure 19 Recessive autosomal eyeless.

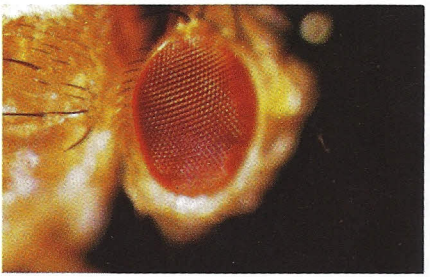

Figure 20 Red eye of wild type.

Figure 21 Recessive autosomal sepia.

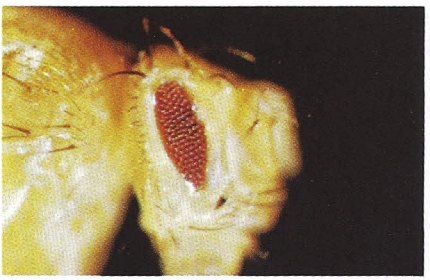

Figure 22 Dominant sex-linked Bar.

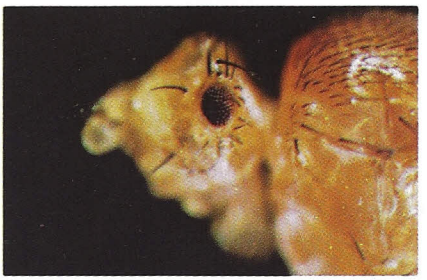

Figure 23 Dominant autosomal Lobe.

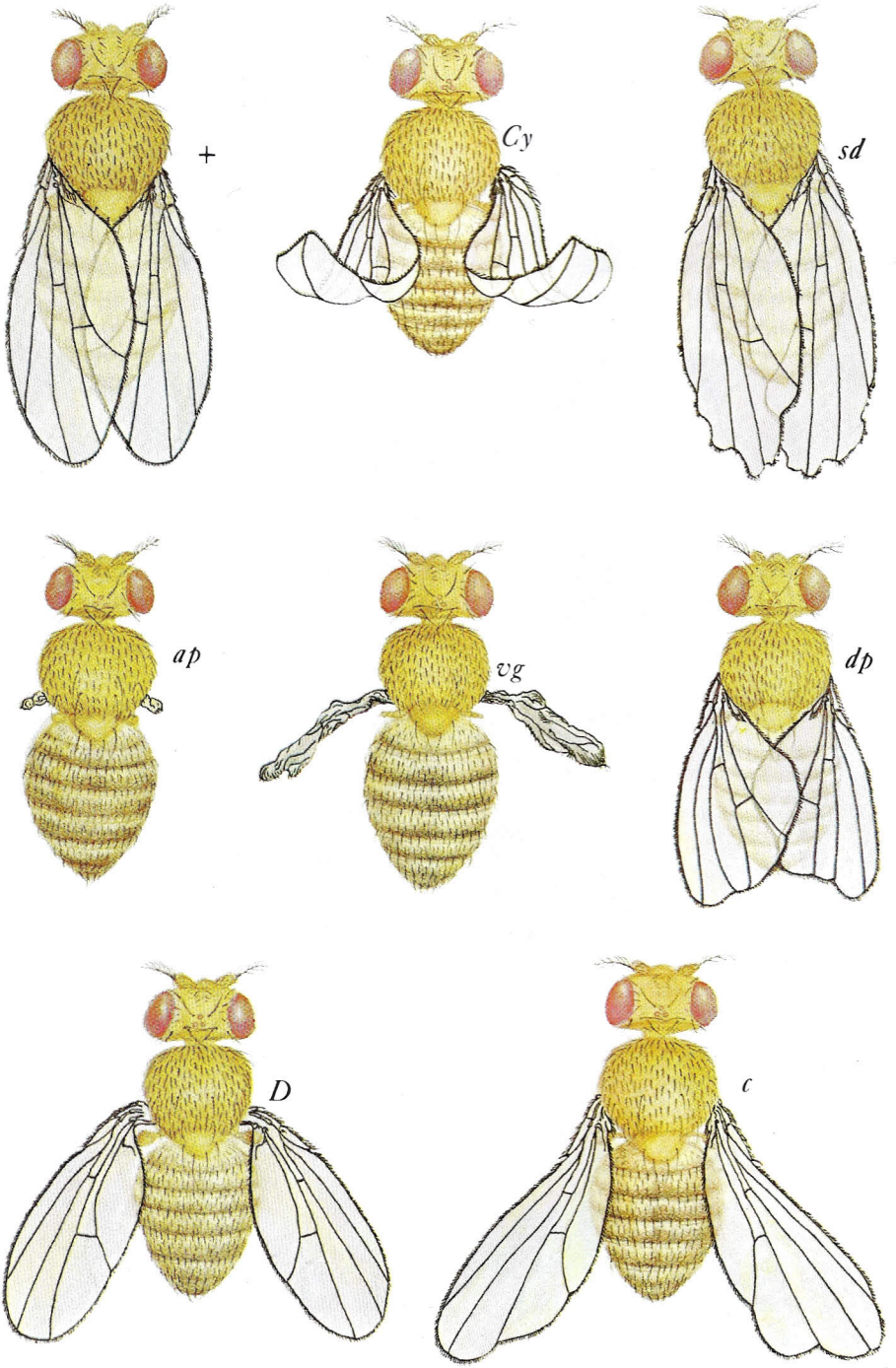

Figure 24 Wing mutations: +, wild; *Cy*, Curly; *sd*, scalloped; *ap*, apterous; *vg*, vestigial; *dp*, dumpy; *D*, Dichaete; *c*, curved.

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	52.0 rosy	0.2 eyeless
21.0 singed	57.5 cinnabar	58.2 Stubble	
27.7 lozenge	67.0 vestigial	58.5 spineless	
33.0 vermilion	72.0 Lobe	64.0 kidney	
36.1 miniature	75.5 curved	69.5 Hairless	
51.5 scalloped	100.5 plexus	70.7 ebony	
56.7 forked	104.5 brown	79.1 bar-3	
57.0 Bar	107.0 speck	91.1 rough	
64.8 maroonlike		100.7 claret	