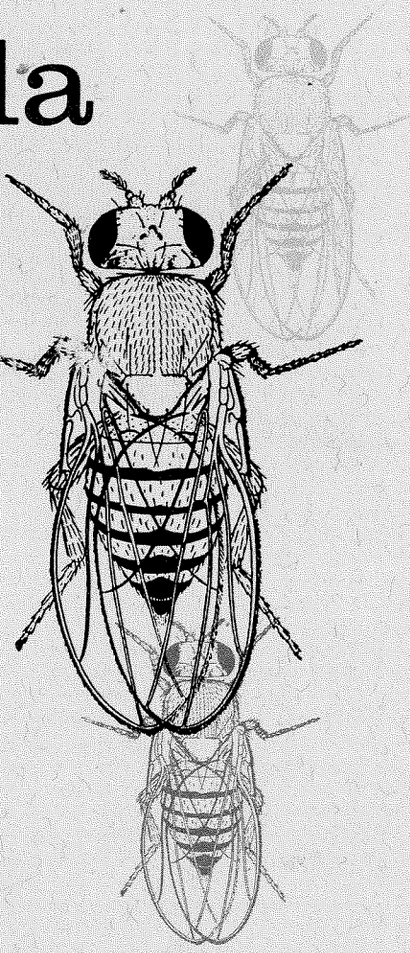
Orders for additional copies of *Drosophila Guide* should be mailed to Publications Office, Carnegie Institution of Washington, 1530 P Street, N.W., Washington, D.C. 20005. (Ordering information can be obtained by mail or telephone, 202-387-6411.)

Flies and other supplies for the experiments, in kits or separately, can be purchased from Ward's Natural Science Establishment, Inc., Post Office Box 92912, Rochester, New York 14692 (telephone 1-800-962-2660); and from Carolina Biological Supply Company, Burlington, North Carolina 27215 (telephone 1-800-334-5551) and Gladstone, Oregon 97027 (telephone 1-800-547-1733).

Drosophila Guide



Demerec and Kaufmann

Published by
CARNEGIE INSTITUTION OF WASHINGTON

Drosophila Guide

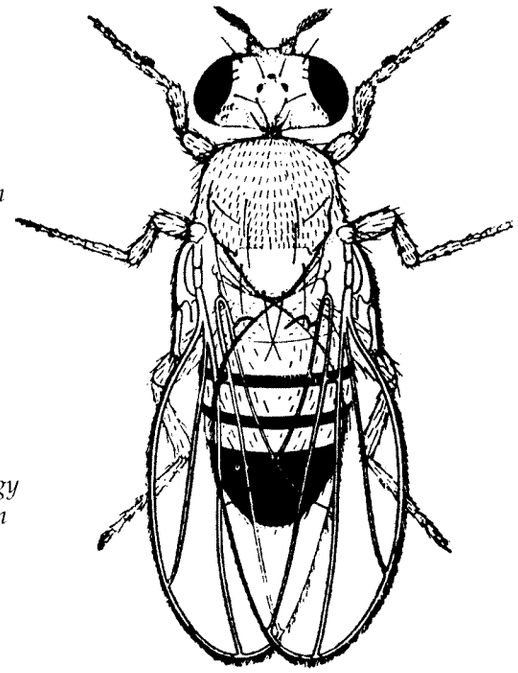
Introduction to the Genetics and Cytology of
Drosophila melanogaster

M. Demerec
Director, 1943–1960
Department of Genetics
Carnegie Institution of Washington

B. P. Kaufman
Department of Zoology
University of Michigan

With revisions by

Allan Spradling
Director, Department of Embryology
Carnegie Institution of Washington



Carnegie Institution of Washington
Washington, D.C.

Tenth Edition, 1996
(First published in 1940)

CONTENTS

First edition August 1940
Second edition (revised) April 1941
Third edition (revised, Appendix added) October 1943
Fourth edition (revised) September 1945
Fifth edition (revised) February 1950
Sixth edition (revised) March 1957
Seventh edition (revised) March 1961
By Berwind P. Kaufmann in collaboration with Jennie S. Buchanan (curator), Agnes C. Fisher (editor), and Henry H. Jones (photographer)
Four printings
Eighth edition (revised reading list) July 1967
Reprinted 1969, 1972, 1973, 1974, 1978
Ninth edition (revised reading list and appendix) June 1986
Reprinted 1989, 1992
Tenth Edition (revised) August 1996

INTRODUCTION

Questions that arise in everyday life are often best answered by experimentation. Among them are questions about the operation of the laws of heredity. People often ask why a blond child is born to dark-haired parents, or a blue-eyed child to brown-eyed parents; why a pet dog or cat may have several different types among its offspring; why seeds from a purple-flowered plant may yield plants that bloom in a variety of colors. Questions like these are encountered every day and are of interest to most of us. The answers are known; they can be found in books. But many books dealing with genetics (as the science of heredity is called) are written in such a way that the facts they contain are accessible to relatively few people. Nevertheless, the fundamental laws that constitute the basis of our knowledge about genetics, known as Mendel's Laws of Inheritance, are so simple that they can easily be grasped by anyone eager to get the information. They are general, applying equally well to plants and to animals, including man. Many relatively simple experiments illustrating these laws are within easy reach of all. Nature itself is constantly performing such experiments for us, and in many flower gardens an observer familiar with the laws can find them demonstrated.

In a simple study of the laws of heredity, the first step is to cross individuals that differ from each other in some one well defined characteristic. The next step is to obtain offspring from the hybrids, and then determine in subsequent generations the proportion showing the characters brought into the cross by each of the original parents. From these proportions, the mechanism responsible for transmission of the determiners (genes) is deduced. Thus in genetic studies it is important to observe several generations and also to include in the observations a large enough number of individuals to furnish a reliable basis for conclusions. Consequently, organisms that breed rapidly, are easy to raise, and produce many offspring make suitable material for such studies. Among the best known is the little "fruit fly" with the scientific name *Drosophila melanogaster*. This fly requires only ten to twenty days to complete a generation; one pair of parents may produce several hundred offspring; and the flies can be raised on a variety of simple culture media. These are the main reasons why Drosophila is one of the most important organisms for genetic studies. It would not be an exaggeration to say that we have learned more about the basic laws of heredity from the study of this fly than from work on all other organisms combined.

About 1909, Professor T. H. Morgan, who was then at Columbia University, began genetic studies with Drosophila. Since then the work with this fly has been greatly extended, and is now being carried on in more than two hundred research laboratories scattered over all six continents. Through the initial efforts of Professor

Morgan and his group, and more recent work by investigators in other laboratories, a large collection of lines of Drosophila that show various heritable characters has been accumulated, and has served as invaluable material for genetic research.

This pamphlet presents a brief outline of the biology of Drosophila and of methods employed in genetic work and in the study of chromosomes. Its purpose is to furnish a background for breeding experiments with Drosophila. A series of experiments designed to illustrate some of the fundamental principles of genetics is included. Since the booklet does not attempt to give an outline of the principles of heredity, it should be used in conjunction with one of the many textbooks or popular books on genetics. Some of these are listed in the section Sources of Additional Information, page 28.

When the first edition of *Drosphila Guide* was prepared, the authors expected it to be serviceable primarily to beginning students of biology. In practice, it proved useful also in college genetics courses, and was adopted as a handbook. Before preparing the third edition, therefore, the authors consulted a number of instructors whose students had worked with the *Guide*, and as a result several valuable recommendations, based on practical observation and classroom experience, were incorporated and the Experiments section was added. The next three editions included only a few minor revisions. In the seventh edition, some of the older illustrations were replaced and additional descriptive material was inserted in the text. In the eighth edition, minor changes and rearrangements were made. In the ninth edition, Professor Elof Axel Carlson made several additional changes and provided a new reading list. In this tenth edition, Allan Spradling has revised the reading list and added a new set of experiments related to transposition.

For many years, the Carnegie Institution supplied flies and other materials for Drosophila laboratory work through the Curator of Drosophila Stocks, Genetics Research Unit, Cold Spring Harbor, New York. This service has been closed. Flies and other supplies can be purchased commercially (see back cover).

THE LIFE CYCLE OF DROSOPHILA MELANOGASTER

Stages and Duration

Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membranes. The egg produces a larva, which eats and grows and at length becomes a pupa. The pupa, in turn, develops into an imago, or adult. The duration of these stages varies with the temperature. At 20°C (68°F) the average length of the egg-larval period is 8 days; at 25°C (77°F) it is reduced to 5 days. The pupal life at 20°C is about 6.3 days, whereas at 25°C it is about 4.2 days. Thus at 25° the life cycle may be completed in about 10 days, but at 20° about 15 days are required. Drosophila cultures ought to be kept in a room where the temperature does not range below 20° or above 25°C. Continued exposure to temperatures above 30°C may result in sterilization or death, and at low temperatures the viability of flies is impaired and the life cycle prolonged (for example, at 10°C the larval stage requires about 57 days, at 15°C about 18 days). It should be kept in mind that the temperature within a culture bottle may slightly exceed that of the surrounding air, because of heat developed by yeast fermentation.

The Egg

The egg of *Drosophila melanogaster,* which is represented in figures 1A and 1B, is about 0.5 of a millimeter long. As can be seen in the lateral view (fig. 1B), the dorsal side is flatter than the somewhat rounded ventral surface. An outer investing membrane, the chorion, is opaque and shows a pattern of hexagonal markings. A pair of filaments, extending from the anterodorsal surface, keeps the egg from sinking into soft food on which it may be laid. A skilled technician can strip the chorion from the egg to expose the shiny, transparent, chitinous coat, or vitelline membrane. Penetration of spermatozoa into the egg occurs through a small opening, or micropyle, in the conical protrusion at the anterior end, as the egg passes through the uterus. Many sperms may enter an egg, though normally only one functions in fertilization. These spermatozoa have been stored by the female since the time of mating. Immediately after entrance of the sperm, the reduction (meiotic) divisions are completed and the egg nucleus (female pronucleus) is formed. The sperm nucleus and the egg nucleus then come into position side by side to produce the zygote nucleus, which divides to form the first two cleavage nuclei, the initial stage of development of the embryo. Eggs may be laid by the mother shortly after they are penetrated by the sperm, or they may be retained in the uterus during the early stages of embryonic development.

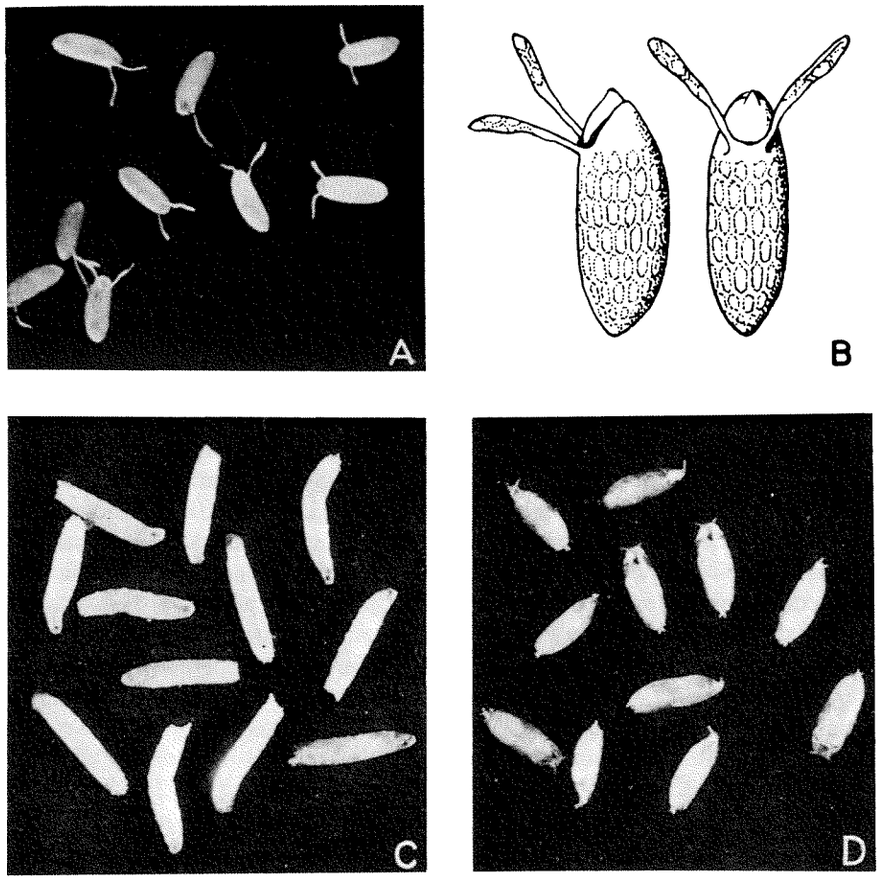


Fig. 1. Eggs, larvae, and pupae of *Drosophila melanogaster*. A: Photomicrograph of eggs. B: Drawings of lateral (at left) and dorsal (at right) views of egg, to show chorion. The contrast between the flatter, or dorsal, surface and the more rounded ventral surface is apparent in the lateral view. C: Photomicrograph of third-instar larvae. D: Photomicrograph of pupae.

The Larval Stages

The larva, after hatching from the egg, undergoes two molts, so that the larval period consists of three stages (instars). In the final stage, or third instar (fig. 1C), the larva may attain a length of about 4.5 millimeters. The larvae are such intensely active and voracious feeders that the culture medium in which they are crawling becomes heavily channeled and furrowed. This "working" of the larvae is the simplest criterion for deciding at a glance, a few days after the start of egg laying, whether or not the expected generation is developing successfully.

A general description of the structure of the larva is beyond the scope of this booklet. Attention is directed (figs. 2A and 2B), however, to the approximate locations of the gonads (testes or ovaries), the ganglion (brain), and the salivary glands. The gonads (see fig. 2B) are located in the "fat bodies" that lie along the sides in the posterior part of the larva. Because the testes of a male larva are so much larger than the ovaries of a female larva of corresponding or even greater size, there is no difficulty in determining the sex of a given individual. With a little experience the worker can make the identification without dissection, because he can see through the transparent body wall whether a relatively large gonad (testis) or an inconspicuous one (ovary) is present. The gonad of a male larva represented in figure 2A is drawn approximately to scale; the ovary of a female, drawn to about the same scale, is shown in figure 2B.

Ability to distinguish males and females in the larval stage permits the student of Drosophila chromosomes to obtain tissue from an animal of known sex. This is important in the event that cells of only one sex are desired, as, for example,

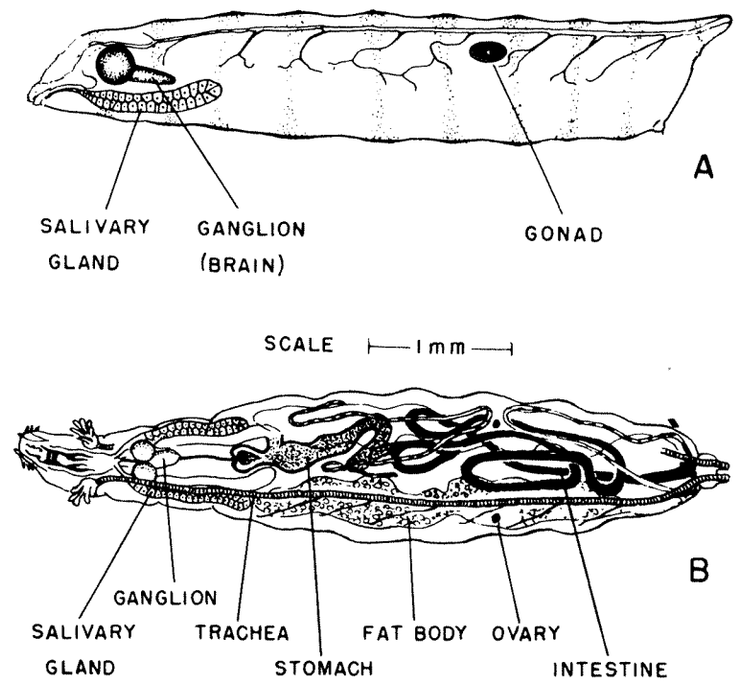


Fig. 2. Diagrams of third-instar larvae of *Drosophila melanogaster*. A: Lateral view, showing approximate locations of salivary glands, ganglion, and gonads. The gonad or testis of the male, here represented, is larger than the gonad or ovary of the female, shown in B. (After unpublished drawing by C. B. Bridges.) B: Dorsal view of female larva, with additional detail. (Adapted from E. Strasburger.)

in experiments that attempt to measure changes produced by some previous treatment in the X chromosome contributed by the sperm. Only female larvae would be useful in such experiments, because an individual of this sex normally receives an X chromosome from her father. It may be asked why we cannot get tissue for chromosome studies from the adult males and females after they have emerged. In answer it should be pointed out that the best cells for the study of dividing chromosomes come from the ganglion of the larva, and that the salivary glands of the third-instar larva contribute the enormous chromosomes in which various chromosomal rearrangements or alterations can be recognized. Of course the experienced worker can distinguish microscopically between cells from males and cells from females by the differences in their sex chromosomes, but that involves a considerable amount of unnecessary labor if cells of only one sex are desired.

A description of the salivary-gland chromosomes is reserved for a later section. Attention should be focused at present on the location of the paired salivary glands, which open into the digestive tube through a single duct a short distance behind the mouth parts.

Pupation

When larvae are preparing to pupate, they creep from the culture medium and adhere to some relatively dry surface, such as the side of the bottle or a piece of paper toweling that has been inserted in the food. Drosophila pupates within the last larval skin, which is at first soft and white but slowly hardens and darkens. The transformation undergone during the pupal period need not be described in detail here, but it culminates in the development of an individual having the body form and organs of the imago. When the changes have been completed, the adult emerges by forcing its way through the anterior end of the pupal case. At first the fly is very long, with unexpanded wings. Within a short time, however, the wings have expanded; and the body gradually attains the more rotund form shown in figure 3. Upon emergence, flies are relatively light in color, but they darken during the first few hours. It is possible by this criterion to distinguish recently emerged flies from older ones present in the same culture bottle.

Recognizing the Sex of the Adult Fly

Male and female flies can be distinguished from each other in several ways. The tip of the abdomen is elongated in the female, and somewhat more rounded in the male (figs. 3, 5). As the female ages and the abdomen becomes distended with maturing eggs, flies of this sex are recognizable at a glance. In many stocks, including the wild type, the pattern of darker markings on the abdominal segments (fig. 3) is sufficiently distinctive in the two sexes to permit their separation on this basis without recourse to the microscope. The abdomen of the female has seven segments that are readily visible with low-power magnifiers, whereas that of the male has five. (Details discernible with higher magnification are diagramed in fig. 5.) Males have the so-called sex comb, a fringe of about ten stout black

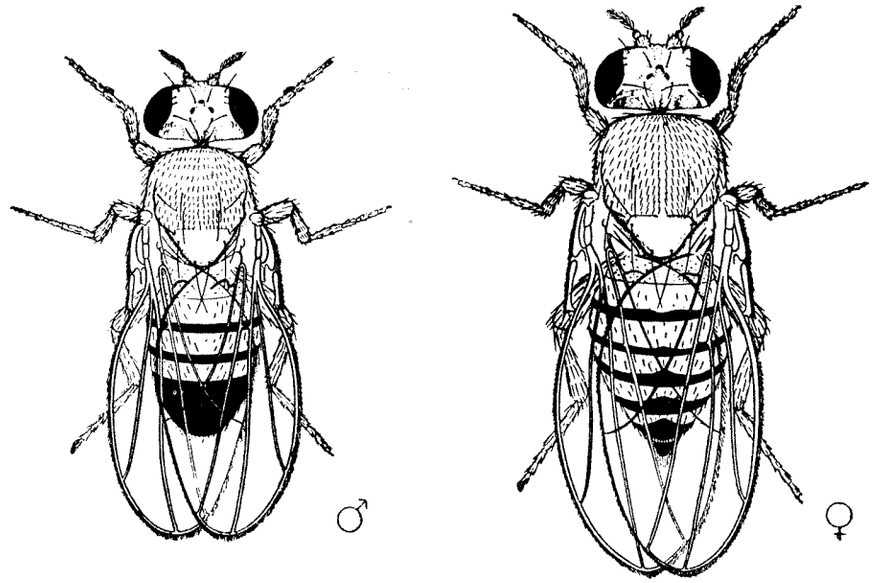


Fig. 3. Adult flies of *Drosophila melanogaster:* male at left, female at right. (From T. H. Morgan.)

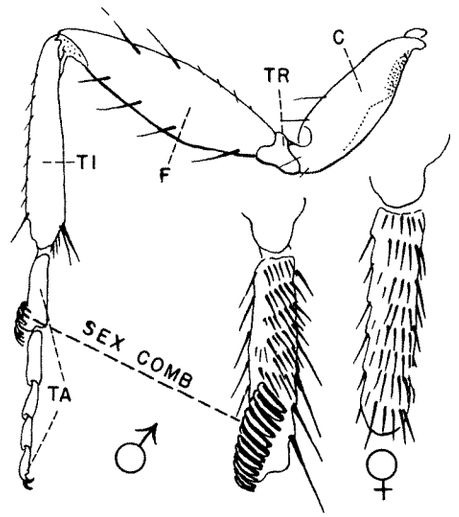


Fig. 4. Diagram of left foreleg of male *Drosophila melanogaster.* C, coxa; TR, trochanter; F, femur; TI, tibia; TA, tarsus. Detail at lower right shows metatarsus of male with metatarsal comb (sex comb) and metatarsus of female, which lacks the sex comb. (After drawings by G. F. Ferris, in *Biology of Drosophila,* edited by M. Demerec, chap. 5, p. 406; see "Suggestions for Reading.")

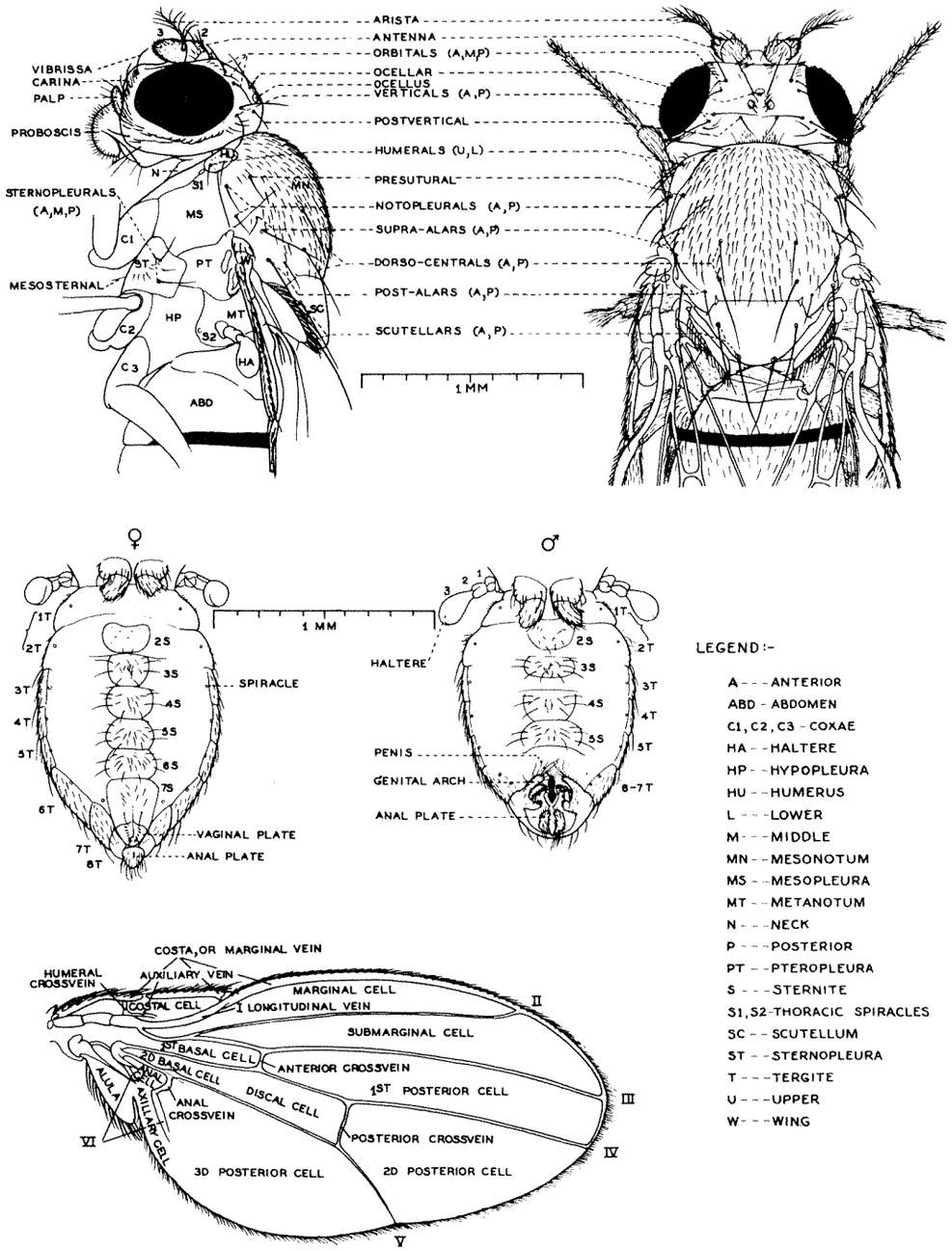


Fig. 5. External structure of Drosophila. Above: at left, lateral view, and at right, dorsal view, of head, thorax, and part of abdomen. Center: ventral views of abdomens of female (left) and male (right). Below: wing venation. (Original drawings by C. B. Bridges, in Drosophila Information Service, no. 3, 1935, as Worksheet 1; and in *Tabulae Biologicae,* vol. 14, p. 343, 1937.)

bristles on the distal surface of the basal (uppermost) tarsal joint of the foreleg (fig. 4). Such bristles are lacking in the female. By these various criteria it is possible to tell the adult male from the female either by naked-eye inspection or, in questionable individuals, with the aid of low-power magnification. It is apparent that the ability to make this distinction rapidly and accurately is essential in all breeding experiments.

METHODS OF BREEDING DROSOPHILA

Drosophila melanogaster is found in abundance on soft fruits like grapes, bananas, and plums, especially if they are overripe and have begun to ferment. Adult flies as well as larvae feed on fruit juices; and since yeast is present wherever fermentation is in progress, it is believed that yeast constitutes an important part of their diet. Therefore Drosophila may be raised on any fermenting medium.

Banana Food

The simplest culture medium is a piece of well ripened banana, dipped into a suspension of yeast-in-water and placed in a small glass container such as a shell vial, quarter-pint milk bottle, or small mayonnaise jar. Push a strip of paper toweling into the banana to hold it in place. Put the parent flies into the container, and close it with a cotton plug. Flies grow well in cultures so prepared, but this method is very little used at present, because by the time the flies begin to hatch the medium has become so soft that it is difficult to remove the flies from the bottle. The method is a useful expedient for occasional experiments, however. Narrow containers, such as vials, hold the banana food in place better than wide bottles.

Other Culture Media

When flies are transferred or examined, as will be seen in the next section, they must be shaken out of the culture bottle. For this reason, the culture medium has to be firm enough so that it does not drop out in the process of shaking. A medium of suitable consistency can easily be obtained by including agar among the ingredients, although some media (see below) are prepared without agar.

The principal requirements of a good culture medium are (1) a sufficient amount of sugar to feed the larvae and promote the growth of yeast, and (2) a proper consistency. The first element may be supplied by any one of a variety of fruits, such as bananas, raisins, or prunes, by molasses or Karo syrup, or by cane sugar; the necessary "body" is achieved by cooking with cereals or agar, or both. The foods most widely used are banana medium, cornmeal medium, cornmeal–molasses–rolled oats medium, and Cream of Wheat medium. The formulas for all four are

given below, in parts per 100 grams or cubic centimeters (1 g = 0.035 oz; 1 cc = 0.034 oz). About 60 cc of medium is required for a half-pint milk bottle, and half that amount for a quarter-pint bottle.

	Banana	*Cornmeal*	*Oatmeal*	*Wheat*	
Water	47.8	74.3	72.7	77.5	cc
Agar	1.5	1.5	. . .	. . .	g
Banana pulp	50.0	. . .	. . .	. . .	g
Molasses or Karo	. . .	13.5	11.0	11.5	cc
Cornmeal	. . .	10.0	14.0	. . .	g
Rolled oats (not quick-cooking)	. . .	. . .	1.6	. . .	g
Cream of Wheat	. . .	. . .	. . .	10.3	g
Tegosept M (10% in 95% grain alcohol)	0.7	0.7	0.7	0.7	cc

Banana medium: Dissolve the agar in the water by boiling. Add the banana pulp and Tegosept, and reheat until close to boiling. The pulp should be prepared from peeled overripe bananas by mashing them with a fork or by putting them through a meat chopper, potato ricer, or strainer.

Cornmeal medium: Dissolve the agar by boiling in about two-thirds of the water. Add molasses or Karo, and bring again to boiling point. In the meantime, combine the cornmeal with the remaining one-third cold water; pour this mixture into the boiling agar-molasses solution. Next, add Tegosept and cook for a few minutes, stirring constantly, until the medium, while still thin enough to pour easily, is ready to jell firmly upon cooling.

Cornmeal–molasses–rolled oats medium: Add the molasses and rolled oats to about two-thirds of the water, and bring to a boil. Mix the cornmeal with the remaining cold water, and pour into the boiling solution. Cook the mixture until it reaches a consistency that will pour easily but jell firmly as it cools. Stir while adding the Tegosept. This food sometimes becomes soft as the flies start to emerge. A small amount of agar, dissolved in boiling water before the addition of the molasses and rolled oats (see formula for cornmeal medium), will guard against loss of firmness.

Cream of Wheat medium: This medium was devised by B. Spassky, of Columbia University, primarily to avoid the use of sugar. Add about two-thirds of the water to the molasses and Tegosept, and bring to a boil. Mix the Cream of Wheat with the remaining cold water, and pour into the boiling solution. From this point on, stir constantly to prevent burning. After the mixture comes to a second boil, cook until it has the proper consistency (see previous formulas). The Cream of Wheat medium tends to become soft in humid summer weather.

Any of the above-described media, when ready, should be poured immediately into containers that have been sterilized by boiling. The most common containers are half-pint or quarter-pint milk bottles. While pouring the food, avoid spilling it on the tops or sides of the bottles. In each bottle place a strip of paper toweling, with one end in the food, to provide additional surface onto which the larvae can crawl to pupate. Cover the bottles with cheesecloth, and let stand, preferably in the refrigerator, until the food has cooled and the bottles have dried out. If

necessary, wipe excess moisture from the insides of the bottles before plugging them. Plug with unwaxed milk-bottle caps; if only waxed caps are available, perforate them with a needle to admit air. As an alternative to caps, plug the bottles with cotton batting, which will be easier to handle if it is wrapped in cheesecloth.

Culture bottles containing medium prepared with Tegosept may be stored for a few days, but medium prepared without Tegosept should be used as soon as it is cool. Just before placing flies in the bottles, seed the food with yeast by adding a pinch of powdered dry yeast or a drop of a thick suspension of fresh yeast cake in water.

Control of Molds

The purpose of "Tegosept M" (a trade name for methyl-*p*-hydroxybenzoate) is to reduce the growth of molds, which are likely to contaminate cultures and will retard the development of the flies if not controlled. Other mold inhibitors are available; we have chosen Tegosept M on the basis of its effectiveness during years of experience in the laboratory. It is important not to add more Tegosept than is required to control the mold, for it also hinders the growth of yeast and of flies. Tegosept is available from commercial sources such as those given on back cover.

TECHNIQUES FOR GENETIC STUDIES

Handling of Flies

Flies are etherized in order to keep them quiet while they are being examined and when they are transferred to culture bottles for matings. A few drops of ether will anesthetize a batch of flies, and they can be kept asleep for half an hour or longer by re-etherizing at intervals. Several types of etherizers have been devised in various Drosophila laboratories. The simplest ones can be constructed with little effort from materials readily available.

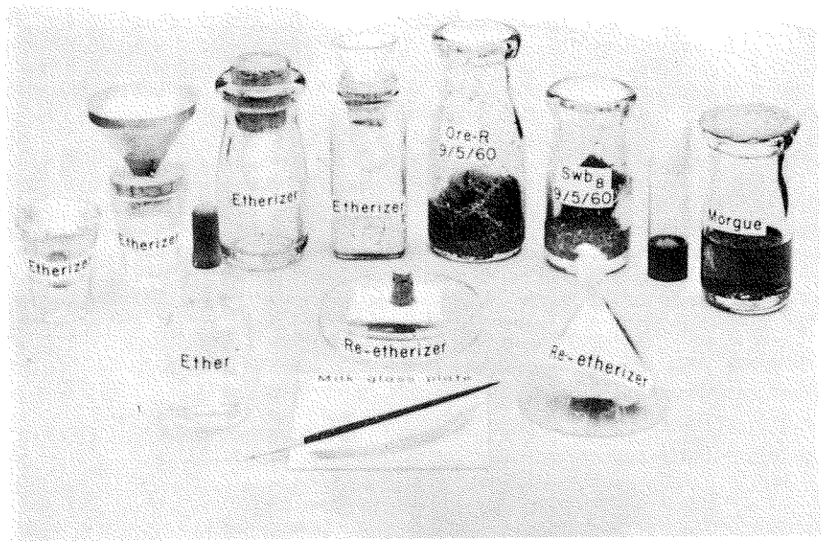
An adequate etherizer can be made from a small container with an opening the same size as that of the culture bottles (fig. 6, third from left). For example, if cultures are kept in milk bottles, a quarter-pint milk bottle will make a good etherizer; and if cultures are kept in vials, a vial will be satisfactory. Into a cork that fits the container, drive a nail part way, so that it will protrude a short distance into the container when the container is corked. Around the nail wind some absorbent cotton, and tie with a thread. When flies are to be anesthetized, put

a few drops of ether on the cotton, shake the flies from the culture bottle into the etherizing bottle (as in fig. 7), and immediately cork it so that the nail with the ether-soaked cotton protrudes into the container. In a few seconds the flies become immobilized. They are shaken out of the etherizer for examination (fig. 7, bottom picture).

Another simple type, which has been described by Dr. W. F. Hollander (see *Turtox News* for January 1958), can be made from a small aluminum funnel and a small "shot glass" from the dime store (fig. 6, extreme left). The top part of the funnel should be cut down to suitable size. To do this, make guide marks about ¼ inch apart around the rim, and cut in from the edge along these marks with sturdy scissors or tinsnips. Bend the resulting strip outward and trim off, but leave enough "stripping" to bend down around the rim of the glass, so that the funnel fits the glass like a lid. Then make similar slits, about ⅛ inch apart, in the tube end and bend them up toward the funnel. Cotton for the ether can be wrapped around the stem and held firmly in place by means of these prongs. In a modification of this etherizer, also shown in figure 6 (second from left), the funnel is not trimmed or bent. Its stem fits into a hole bored in a large cork, and the cotton is held in place by tying it securely around small nails or pins driven into the bottom of the cork.

The kind of etherizer shown in figure 7, top and bottom, has some special advantages. It consists of a bottle about the size of a half-pint milk bottle, a small plastic funnel (obtainable in many hardware stores), a no. 11 cork (*not* a rubber stopper), and a no. 10 gelatin veterinary capsule. Since these capsules are not always easy to acquire, one is included with each initial shipment of flies, as a shipping container for a small quantity of the mold inhibitor Tegosept. To make the etherizer, pour a little plaster of Paris into the bottle and tamp in some cheesecloth or cotton to receive the ether. Cut down the wider opening of the funnel until it will fit loosely into the inner rim of the milk bottle in which flies are cultured. Choose the longer or shorter part of the capsule, depending on the size of your bottle, and in its rounded end pierce about a dozen holes with a heated needle. The holes should be large enough to admit ether fumes but not large enough to allow flies to fall through. The stem of the funnel goes through a hole bored in the cork, and the cork fits into the opening of the capsule. Flies are shaken through the funnel into the capsule as it rests in the mouth of the ether bottle. They do not tend to crawl up the stem of the funnel, although they would escape from an open-mouthed bottle or capsule. It is easy to determine when the flies are anesthetized, by looking through the walls of the bottle and capsule, and then to uncork the capsule and shake them out.

It is best to examine the flies on a white surface: either white cardboard, a milk-glass or opaque-glass plate, or a piece of clear glass placed over stiff white paper. Flies are moved around with a "pusher," which may be a small camel's-hair brush, a small strip of copper, or any pointed object. If the flies should begin to wake up before examination is completed, they can be re-etherized without being returned to the etherizing bottle. The re-etherizer usually consists of a flat dish (section of a small Petri dish) with a strip of absorbent paper fastened



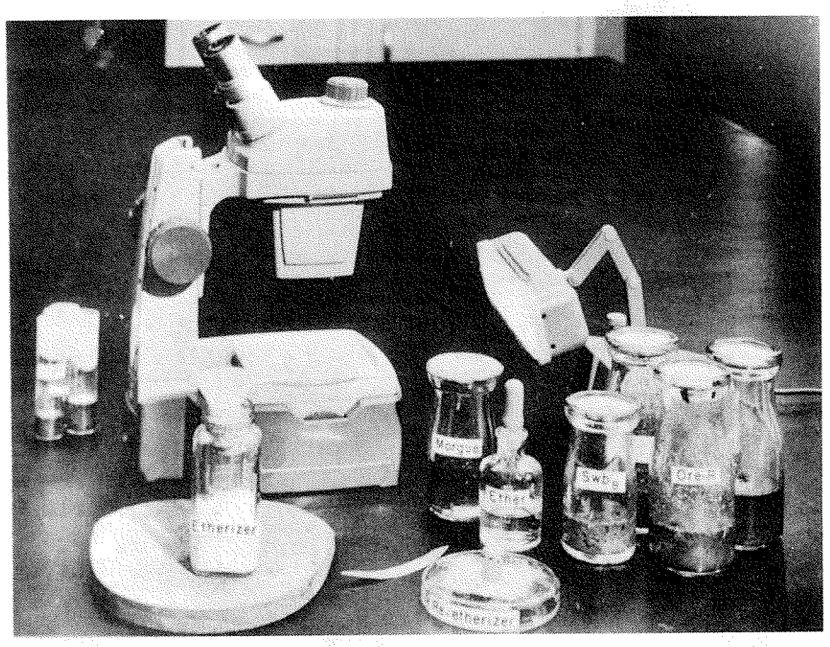


Fig. 6. Utensils used in breeding work with Drosophila. Upper picture: (back row) four simple types of etherizer; stock cultures in half-pint and quarter-pint milk bottles (note that each culture has a label); vial with cornmeal food; morgue; (in front) anesthetizing ether; two types of re-etherizer; milk-glass plate; and two "pushers," one a camel's-hair brush and the other a strip cut from copper sheeting. Lower picture: desk in a Drosophila laboratory, with culture vials, binocular microscope and light, etherizer, re-etherizer, morgue, and culture bottles.

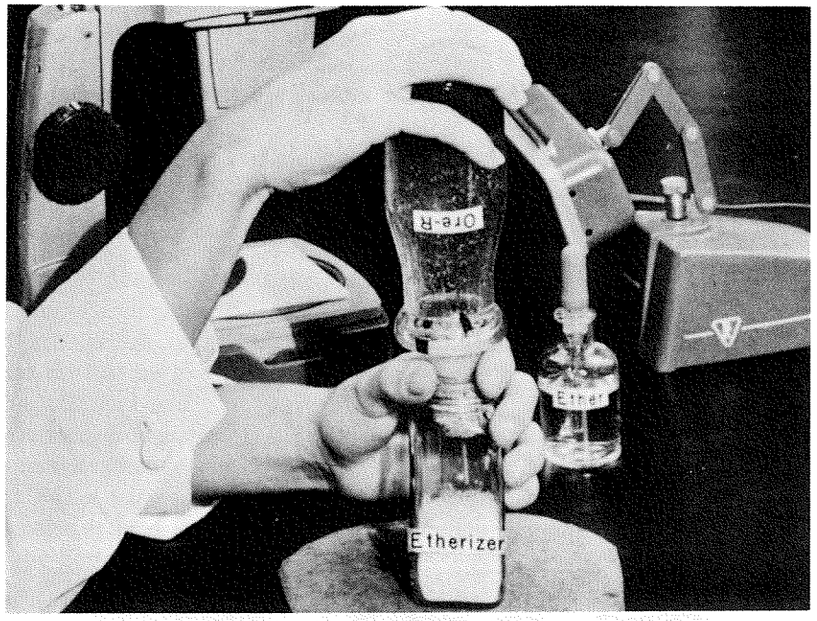


Fig. 7. Two steps in handling Drosophila. Upper picture: flies are shaken from a culture bottle into an etherizer. Lower picture: etherized flies have been placed on a milk-glass plate to be examined with the binocular microscope.

to the inside (fig. 6 center). A few drops of ether are placed on the paper, and the flies are covered with the re-etherizer until they go to sleep again. The cone type of re-etherizer shown in figure 6 (right) is made from a glass funnel with the stem broken off or shortened. A small wad of cotton is forced through the hole so that it sticks out at the top, and folds of cheesecloth are fastened inside the cone with Scotch tape. The cone is placed over the flies as soon as they show signs of waking; then ether is dropped onto the protruding cotton and penetrates to the cheesecloth. The advantage of this re-etherizer is that it keeps the flies from moving out of range before the ether can be applied. A disadvantage is that the experimenter may be exposed to more ether fumes than he would be while using the flat type of re-etherizer.

Care should be taken not to overetherize flies, since that can prevent their further use or interfere with diagnosis. In flies killed by overetherizing, the wings are extended at a 45° angle. Flies that have been examined and recorded, and are not required for further matings, can be disposed of in a jar containing light mineral oil or alcohol ("morgue" in fig. 6).

Stock Cultures

When stocks are kept by an inexperienced person, it is well to examine each fly carefully whenever new cultures are prepared, so that any contamination (by "foreign" flies from other cultures or from nature) may be detected in time to prevent loss of the original stock. In preparing a new culture, from 5 to 10 pairs of parent flies are put into a bottle or vial. Etherized flies should not be dropped onto the food, because they will get bogged down in it before they revive. Flies may be placed in a small cone made of paper toweling, which is gently lowered into the bottle. If the cultures are kept in milk bottles with cardboard stoppers, etherized flies may be put on the under side of the stopper and the culture bottle may be inverted over it, remaining upside down until the flies come out of anesthesia; or the bottle may be kept on its side until the flies wake up.

Stocks should be changed every three or four weeks, depending on the temperature. At least two cultures of each stock should be kept, in case one should be unsuccessful. It is important not to keep cultures too long, and to clean old culture bottles as soon as they are discarded. Old bottles may become infested with mites (minute parasites that live on flies), which can cause a great deal of trouble and interfere with viability of the flies, especially of mutant types.

Every stock bottle should have a label, showing the names of the stock's mutant characters (in symbols) and the date of transfer (see fig. 6).

Experimental Matings

As was mentioned in an earlier section, the sperm received by a female fly during a mating is retained, and serves to fertilize a large number of eggs. Therefore, in an experimental cross between flies belonging to two different lines, it is essential to have virgin females.

As a rule, females do not mate within 12 hours after hatching; and so if the old flies are shaken out of a culture bottle, all females collected during the next 12-hour period are likely to be virgin. When it is not feasible to collect females at frequent intervals, virgin females may be obtained by selecting third-instar female larvae (distinguished by size of the gonad; see fig. 2) and keeping them in food-containing vials until the flies emerge. Another method is to isolate single pupae in individual vials, each containing a small piece of moist blotting paper. These pupae will probably include both males and females, but all females emerging from the pupal cases will be virgin. Since both these methods are very laborious, they are used only in special circumstances.

When a cross is made between a virgin female and a male, the flies may be put directly into a culture bottle, or they may be placed in a vial containing food and transferred to a culture bottle after 2 days. Females do not lay eggs until they are 2 days old, and the culture medium will be in better condition for young larvae if females are not placed in the bottle until they are ready to lay eggs.

Every bottle must have a label showing the characteristics of each parent.

Before the progeny begin to emerge, approximately 7 to 9 days after the cultures are started, the parents should be shaken out of the experimental bottles so that they will not be confused with the offspring when counts are made. Another precaution that should be taken is to stop using flies for counts and for matings before the next generation begins to emerge. In cultures raised at ordinary room temperature (20–25°C), this will not occur sooner than 20 days after the culture is started.

When good culture conditions are maintained, flies live for several weeks. They are fertile as long as they live.

Recording

Needless to say, careful records should be kept of all experiments. After flies have emerged they should be shaken from the culture bottle into an etherizer and, when asleep, placed on a white surface, as described on page 12. There they are examined and classified into groups according to the characters they display. The number of individuals found in each group should be recorded. This procedure should be repeated every second or third day until the culture becomes old. In Drosophila laboratories, flies are examined under a binocular microscope, at a magnification of at least 12 to 15×. Satisfactory work can be done, however, with an ordinary magnifying glass that magnifies 2 to 8 times. Some characters can readily be distinguished with the naked eye.

THE CHROMOSOMES OF DROSOPHILA

The student beginning genetic work with Drosophila will not find it necessary to have a firsthand knowledge of the chromosomes of this species. As the work progresses, however, problems may arise that can best be approached by an anal-

ysis of the structural changes that have occurred within the chromosome complex. It is for advanced students meeting such problems that the material of this section is presented. Our attention is focused primarily on the large salivary-gland chromosomes, because their clearly perceptible banding provides excellent material for the demonstration of linear differentiation of the chromosomes and for the identification of any changes that may be induced therein.

Somatic Mitoses

It has been definitely established that, in Drosophila as in other organisms, the genes or determiners, whose expression is manifested in measurable characters such as eye color or bristle length, are located in the chromosomes. Through combined genetic and cytological studies, individual chromosomes of *Drosophila melanogaster* have been identified, and they have been labeled X, Y, 2, 3, and 4. They are shown in figure 8. It will be noticed that the chromosome complex of the female has two rod-shaped X's and therein differs from that of the male, which has one rod-shaped X and one J-shaped Y chromosome. The X and Y chromosomes, therefore, are called the sex chromosomes. The more or less equal-armed, V-shaped second and third chromosomes and the dot-shaped fourth chromosomes are known as the autosomes. As represented in figure 8, the second and third chromosomes resemble each other closely, but it is possible for the experienced student of cells (cytologist) to distinguish them from each other by certain structural peculiarities. The chromosomes shown in figure 8 are in metaphase of somatic mitosis, and are split longitudinally in preparation for separation of their halves in the anaphase stages of the same mitotic division. Such chromosomes are prepared for study from neuroblast cells of the ganglion of the larva. Because of their small size (evidenced by the 10-micron scale in the figure) they must be studied with a microscope equipped with an oil-immersion objective.

If slides of the somatic mitoses are desired, sections of the ganglion may be

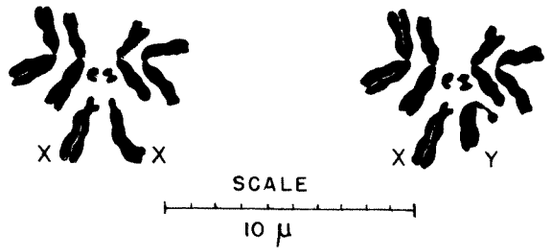


Fig. 8. Metaphase chromosomes from dividing cells of larval ganglion (brain) of *Drosophila melanogaster*. Chromosomes of female, shown at left, consist of three pairs of autosomes and one pair of rod-shaped X chromosomes. Chromosomes of male, shown at right, consist of three pairs of autosomes, one X chromosome, and one Y chromosome.

prepared by the paraffin technique, which is described in detail in textbooks of histological method. A much quicker procedure, in which the ganglion is merely flattened between slide and cover slip in a drop of acetic acid and carmine (aceto-carmine) or acetic acid and orcein (acetic-orcein), will be discussed in the following section in connection with the preparation of salivary-gland chromosomes for study. The chromosomes in figure 8 were drawn from preparations made by the aceto-carmine smear method.

The Salivary-Gland Chromosomes

The salivary-gland chromosomes of flies have been known for the best part of a century, but the constancy that they show in the arrangement and relative density of their different bands or discs, and the significance of this detail in analyzing chromosomal rearrangements, were not recognized until 1933. A photomicrograph (a photograph taken with the aid of a microscope) of salivary-gland chromosomes of female *Drosophila melanogaster* is shown in figure 9. It will be noticed that the five long arms radiate as if from a common center. This region, called the chromocenter, is formed by the mutual attraction and close association of those parts of all the chromosomes that immediately adjoin their spindle-attachment regions. We must keep in mind, however, that the salivary-gland chromosomes are no longer capable of division, and that the spindle-attachment regions cannot function, as they do in actively dividing cells, to help distribute the chromosomes to the poles of a spindle. In figure 9 the chromosomes are labeled X (the X or first chromosomes), 2L and 2R (the left and right limbs of the second chromosomes), 3L and 3R (the left and right limbs of the third chromosomes), and 4 (the fourth chromosomes). The word "chromosomes" is plural in this description because each of the arms seen in the photograph consists of two closely appressed strands: one maternal in origin, provided by the nucleus of the egg; the other of paternal origin, provided by the sperm at the time of fertilization. When the paired homologues are identical in constitution (as in fig. 9) there is no way of determining which is derived from the female parent and which from the male, but if the two differ appreciably in gene sequences the differences will be perceptible in the giant chromosomes of the salivary-gland cells. As an illustration, let us suppose that the sequence of banding in a chromosome of a wild-type fly is *abcdefghij,* and that in another fly a part of this chromosome, the section *cdefgh,* has become inverted so that the sequence is *ab hgfedc ij.* If one parent in a mating provides one of these sequences, and the second parent the other, we may see in the salivary-gland chromosomes of their offspring an inversion configuration similar to that shown in figure 10A. Identical regions of paternal and maternal chromosomes are paired throughout their entire length; *a* pairs with *a, b* with *b, h* with *h, g* with *g,* and so forth. Therefore, in the example just cited, a loop configuration is produced. Likewise, two different chromosomes of a set may be involved in an exchange. For example, chromosome 2, having the sequence *klmnop,* might exchange a part with chromosome 3, having the sequence *uvwxyz,* to make two new chromosomes constituted as follows: *klm vwxyz* and

u nop. In this hypothetical case, section *klm* has been transferred from one chromosome to another and section *u* has been transferred in the reciprocal manner; this type of rearrangement is known as a reciprocal translocation. Now let us suppose that these chromosomes have been introduced by the sperm at the time of fertilization into an egg in which the two chromosomes in question carry the normal sequences *klmnop* and *uvwxyz*. In the salivary-gland chromosomes of larvae of the progeny of this mating we should see a cross-shaped configuration similar to that shown in figure 10B, since *u* pairs with *u, m* with *m, n* with *n, v* with *v*, and so on. These illustrations will indicate the method employed in studying the salivary-gland chromosomes, and their value in determining the nature of changes induced by such experimental procedures as X-raying of males. The changes produced by the treatment in chromosomes of the sperms can be recognized, and the positions of breaks localized, by comparison with the unchanged chromosomes of the female to which an irradiated male is mated. Among

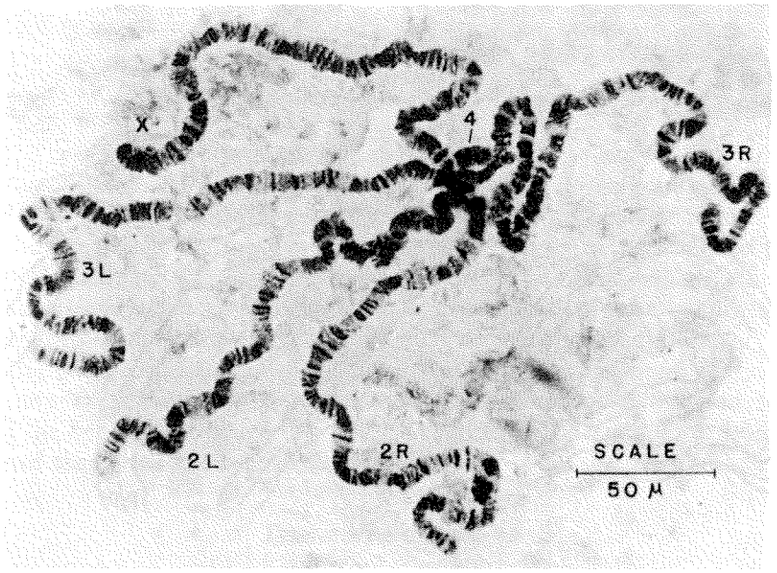


Fig. 9. Photomicrograph of spread and stained salivary-gland chromosomes of female larva of *Drosophila melanogaster*. X, the X chromosomes, closely paired, side by side; 2L and 2R, the left and right limbs of the paired second chromosomes; 3L and 3R, the third chromosomes; 4, the fourth chromosomes. (From B. P. Kaufmann, in *Journal of Heredity,* vol. 30, 1939.) A fuller description is given in the text. In salivary glands of the male, the Y chromosome is so reduced in size as to be almost indistinguishable, and the unpaired X chromosome appears somewhat more slender than the paired X's of the female and stains less intensely.

the various stocks of Drosophila kept for experimental purposes, there are many containing chromosomal inversions and translocations, and they will be made available to the advanced student who wishes to familiarize himself with methods of salivary-gland-chromosome analysis.

Salivary-gland chromosomes can be demonstrated with the aid of a compound microscope having a 16-mm 10× (low-power) objective and a 10× ocular, but

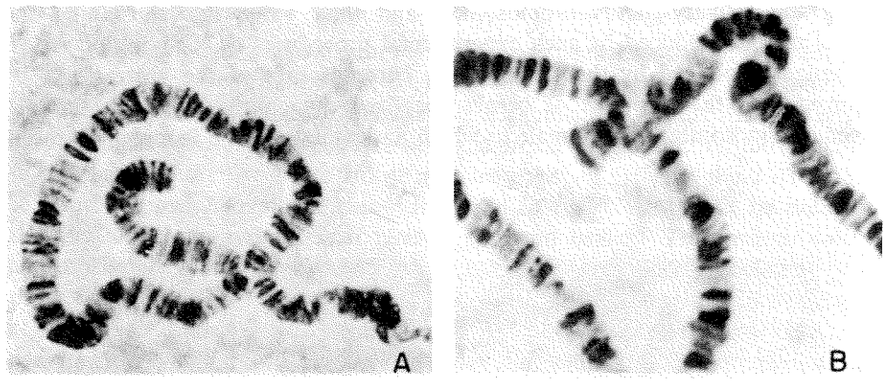


Fig. 10. Photomicrographs of salivary-gland chromosomes of *Drosophila melanogaster*, showing inversion and reciprocal translocation. A: Pairing between two X chromosomes, one of which contains an inverted section. In the diagram below, the chromosome carrying the wild-type sequence of genes has been labeled *abcdefghij*, and the chromosome bearing the inverted section has the sequence *abhgfedcij*. A more complete description of this loop configuration is given in the text. B: Pairing between two normal chromosomes and two that have exchanged sections. The second and third chromosomes are involved (2L and 3R). In the diagram below, 2L bears the letters *klmnop* and 3R the letters *uvwxyz*. The two chromosomes that have exchanged parts carry the sequences *klmvwxyz* and *unop*. Pairing between these chromosomes and those carrying the unaltered sequence of parts produces the cross-shaped configuration. Fuller description in text.

are seen more easily if a 4-mm, 43× (high-power) objective is available. Recognition of the details of banding and accurate identification of the chromosomes require an oil-immersion objective. Some improvement of the degree of contrast in banding may be achieved by placing a green filter between the microscope and the light source. An inexpensive filter can be made by cementing a piece of green cellophane between two glass slides with a transparent mounting medium such as Canada balsam.

PREPARING SALIVARY-GLAND CHROMOSOMES FOR STUDY

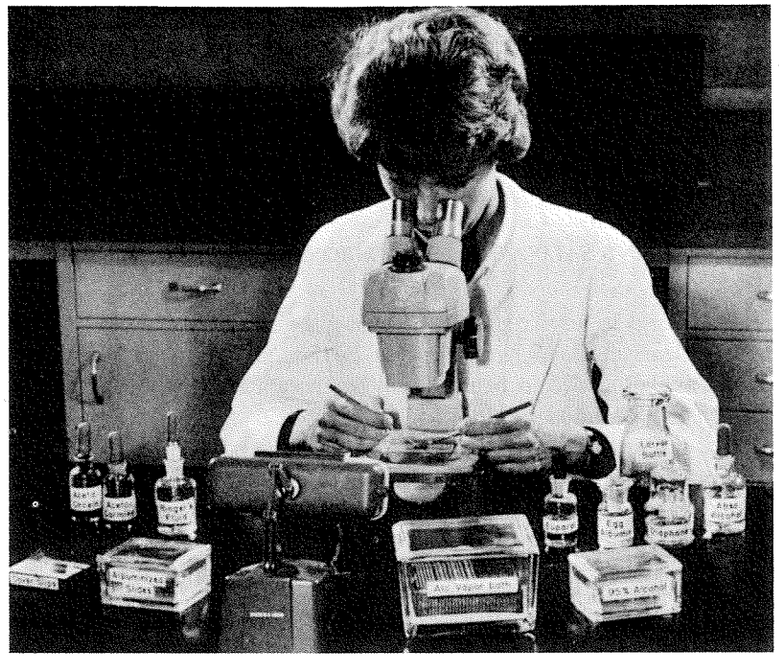
A prime requisite in any study of chromosomes is the preparation of slides of good quality, clearly stained. It is not to be hoped that preparations quite as diagrammatic as that shown in figure 9 will be encountered frequently. Configurations in which the chromosomes are so evenly spread and so free of overlapping strands are a rare event, even in laboratories where such material is prepared daily. It is possible, however, to consistently turn out preparations in which the salivary-gland chromosomes can be recognized by their characteristic banding if the following instructions are observed.

Growing the Larvae

Special care is necessary in growing the larvae whose salivary glands are to be used. One of the chief factors in obtaining large, firm chromosomes is the selection of well fed larvae raised at a low temperature. Crowded culture bottles should be avoided; it is best to transfer the parents every second day, so that no single bottle will contain too many eggs. After removal of the parents the surface of the food is sprinkled lightly with fresh or dried yeast, and the culture bottle is transferred to a cool place (about 16° to 18°C is recommended). Salivary glands should be taken from fully grown larvae. If no paper has been placed in the bottles, the larvae preparing to pupate will usually creep out of the food onto the sides of the bottle, from which they can be collected. The method of determining the sex of the larvae has been described.

Temporary Preparations

In general, the procedure consists in dissecting the glands from the larva, fixing and staining them in aceto-carmine or acetic-orcein, then pressing them in a small drop of the stain between a slide and a cover slip (figs. 11–14). For removal of the glands (fig. 11), the larva is usually placed in a drop of a salt solution, which may be prepared by dissolving 7 parts of sodium chloride in 1000 parts of distilled water. Students who have become familiar with the anatomy of the larva often prefer to dissect directly in a drop of the staining solution. For this work it is essential to have either a dissecting microscope or a dissecting lens mounted in a suitable holder. In removing the glands we use a pair of dissecting



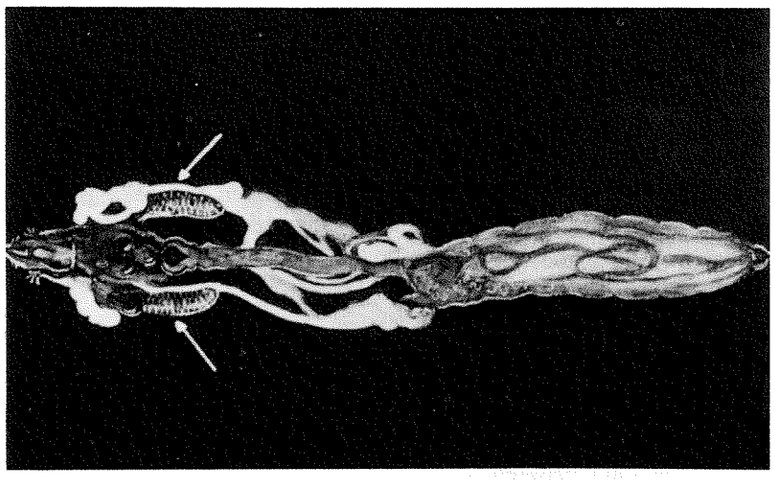


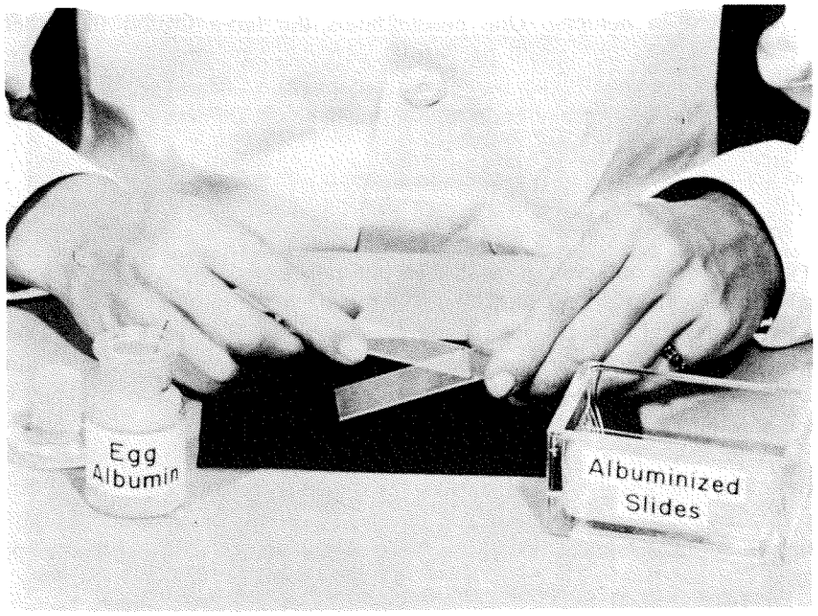
Fig. 11. Upper picture: materials used in preparing salivary-gland chromosomes for study. Glands are dissected from the larva with clean, rust-free needles, on a slide placed on the stage of a dissecting microscope. Equipment includes cover slips, bottles of acetic-orcein, aceto-carmine, Ringer's fluid, Euparal, egg albumin, Diaphane, and absolute alcohol, and dishes containing albuminized slides, slides in alcohol vapor, and slides in 95 per cent alcohol. Lower picture: from a drawing of a larva the anterior portion of which was dissected to show the salivary glands (see arrows).

needles, mounted in holders. One needle holds the larva firmly; the other is placed directly behind the mouth parts to detach the head (fig. 11). The glands are usually pulled out with the motion of decapitation. They are then covered with the stain, either on a slide or in a small dish. It is important that the glands never be permitted to dry out.

Aceto-carmine stain is a saturated solution of carmine (obtained from any biological or chemical supply house) in 45 per cent acetic acid (made from glacial acetic). To prepare it, add the carmine to the acid and boil vigorously for about 2 hours. A reflux condenser is desirable but not essential. Allow undissolved material to settle, then decant and filter. If the stain is found to be too concentrated, as indicated by black-looking chromosomes that fail to spread properly, it may be diluted with 45 per cent acetic acid. In this laboratory we have found that aceto-carmine prepared with the reflux condenser needs to be diluted with an equal amount of 45 per cent acetic acid. One precaution should be observed in aceto-carmine staining. If iron or steel dissecting needles remain in contact with the stain, the dissolved iron darkens the stain considerably. This difficulty can be minimized by employing clean, rust-free needles, or avoided entirely by dissecting with stainless-steel needles or with cactus spines or other plant spines if procurable.

Although aceto-carmine colors the nuclear contents most vividly, it may also stain the cytoplasm more intensely than acetic-orcein, which is a more specific dye for chromatin. Orcein, originally obtained from lichens that grow in the tropics, is now prepared by synthetic means and may be purchased from drug and biological supply houses. A 2 per cent concentration of the dye in 45 or 60 per cent acetic acid makes a satisfactory stock solution. Preparation with a reflux condenser is recommended. If the stock solution is too concentrated, dilution with 45 or 60 per cent acetic acid may be necessary before good results are obtained.

Stain the glands about 3 minutes; experience will show whether a shorter or longer time is needed. Chromosomes that have been fixed and stained for too long a period may be brittle, with poor differentiation between bands of different thickness, whereas chromosomes that are understained may spread well but have indistinct banding. Next, transfer the glands to a clean glass slide in a small drop of the stain (fig. 12) and apply a cover slip (fig. 13). Spreading is the next step. First, lay a piece of paper toweling or blotting paper over the cover; then press firmly with the ball of the thumb or with some blunt instrument. The toweling will absorb the excess stain as it is forced from the space between slide and cover. Apply the final pressure by tapping and stroking the cover with the blunt instrument directly over the glands (fig. 13). No description can indicate the amount of pressure to be applied in this step. The chromosomes should be spread but not broken. Several trials may be necessary before the worker feels that he has mastered the procedure. The slides are now ready for observation under the microscope, but it is evident that evaporation of the fluid under the cover will soon cause the material to dry out. Melted paraffin applied to the edges of the cover will provide a seal that should protect the preparation for a few days. Some-



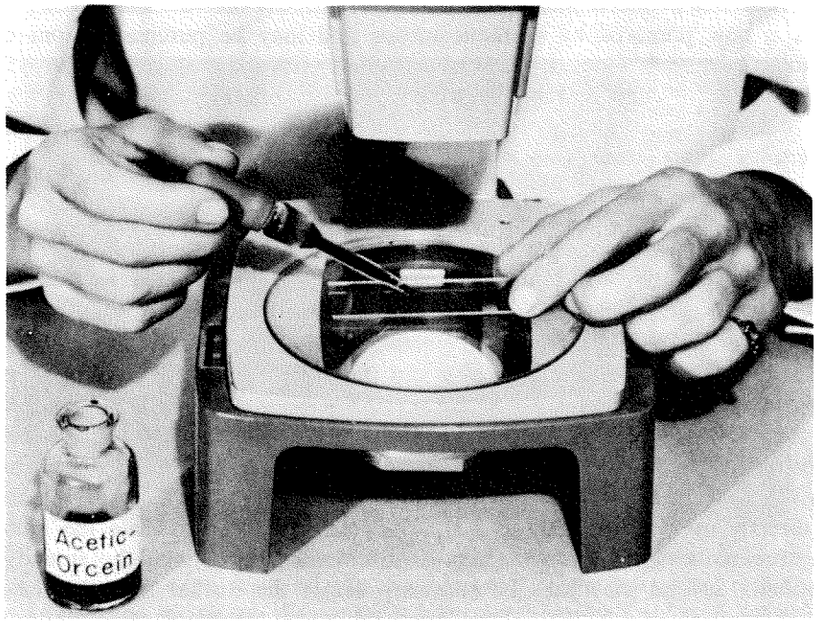
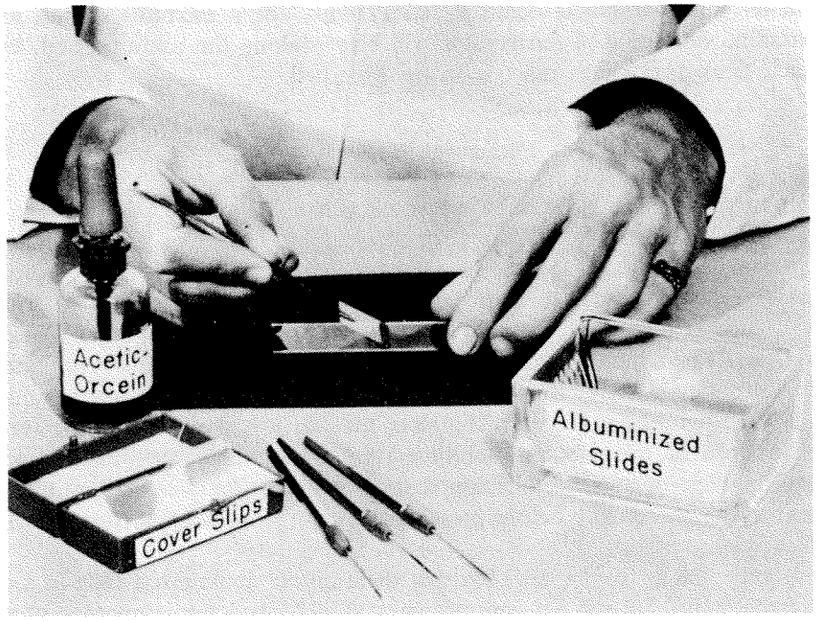


Fig. 12. Upper picture shows method of albuminizing slides. The salivary glands, after removal from the larva, are placed in a drop of aceto-carmine or acetic-orcein on the dried film of albumin, as shown in the lower picture.



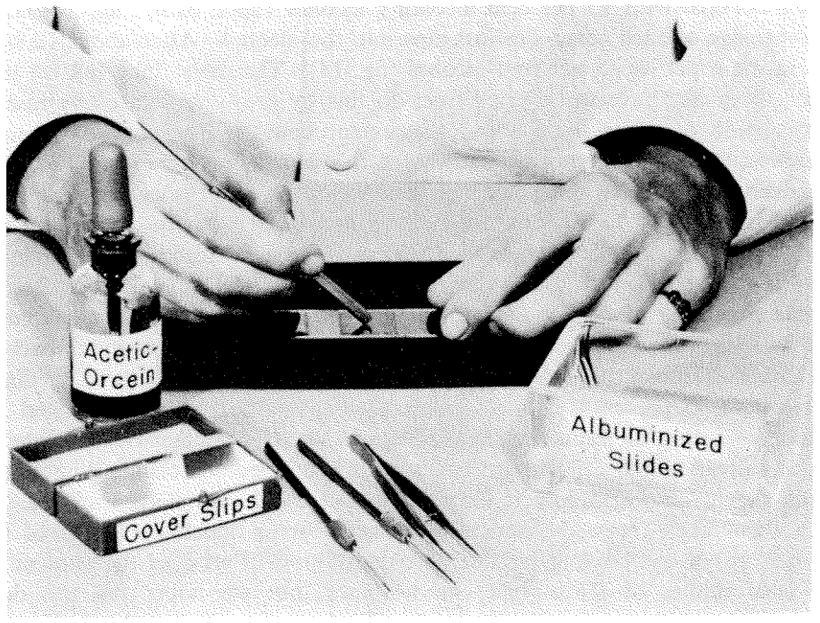


Fig. 13. Application of the temporary cover (upper picture) is followed by tapping with a blunt instrument to flatten the glands (lower picture).

what better than paraffin is dentists' "sticky wax," or a mixture of gum mastic and paraffin. Storage in a refrigerator will help prolong the useful period, but at best such temporary slides must soon be discarded.

Permanent Preparations

Slides for permanent use can be prepared with a few modifications of the procedure outlined above. In making permanent slides the cover must be removed after pressing the glands, and therefore some steps must be taken to anchor the chromosomes to the slide. For this purpose the slides are first treated with an albumin solution prepared by mixing together 100 cc of distilled water, 25 grams of powdered egg albumin, and 0.5 gram of thymol (the last is a preservative). The mixture should stand for several days, preferably in a cool place, until the undissolved albumin has settled. The clear part on top is then decanted for use. Spread a drop of this albumin solution evenly and thinly over each slide, by drawing the edge of another, unchipped slide across it (fig. 12). Allow the albumin film to dry thoroughly before pressing the glands; meanwhile, store the slides in a dust-free receptacle.

Dissect the glands from a larva and put them on an albuminized slide in a small drop of the stain. Spread the chromosomes as described for temporary preparations. Place slides prepared in this way in a dish containing a layer of paper toweling covered with 95 per cent alcohol ("alcohol vapor bath," fig. 14) so that the lower edge of each cover slip just dips into the alcohol. After about 12 hours, immerse the slides in 95 per cent alcohol (fig. 14). The cover may fall off in the alcohol, or it may have to be pried free; do this by gently and carefully inserting a razor blade or other sharp-edged instrument beneath one corner. Then the preparations, which must not be allowed to dry out at any time, may be passed successively through absolute alcohol and xylol or other clearing agent, and mounted in balsam. This procedure is described in detail in textbooks of histological technique. In our laboratory, however, the dehydration in 95 per cent alcohol is followed directly by mounting with Euparal or Diaphane (commercially available mounting media) or with a similar type of solution that we prepare by dissolving gum sandarac in a mixture of 2 parts oil of eucalyptus and 1 part paraldehyde until it has the consistency of thick molasses. These three media are miscible with 95 per cent alcohol. Immediately after the slide is removed from the alcohol, a drop of the mounting medium is placed on the part containing the flattened salivary-gland cells, and a cover glass is applied. The mounting medium hardens slowly—more rapidly if the slide is placed near a warm radiator. The slide is then ready for study, and may be kept for an indefinite length of time.

We realize, of course, that innumerable questions will arise as the student proceeds with studies of Drosophila chromosomes; but we hope that the details furnished here will supply the fundamental information he needs to begin his investigations.

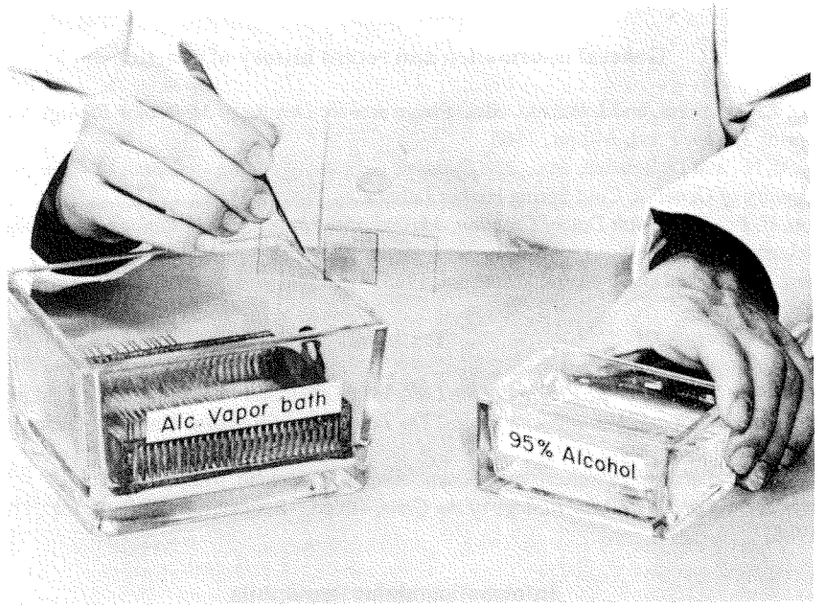


Fig. 14. Slides are passed successively through alcohol vapor and 95 per cent alcohol (upper picture). The temporary cover is then removed, if it has not already dropped off, and a permanent mount is made with Euparal or Diaphane (lower picture).

Sources of Additional Information

General information and recent history of genetics

Cairns, J., G. I. Stent, and J. Watson, eds., *Phage and the Origins of Molecular Biology,* Cold Spring Harbor Press, 340 pp., 1966.

Fedoroff, N., and D. Botstein, eds., *The Dynamic Genome, Barbara McClintock's Ideas* in the *Century of Genetics,* Cold Spring Harbor Laboratory Press, 415 pp., 1992.

Judson, H. F., *The Eighth Day of Creation: Makers of the Revolution in Biology,* Simon and Schuster, 686 pp., 1979.

Watson, J. D., *The Double Helix,* Atheneum, 1968.

Textbooks

Alberts, B. *et al.*, *Molecular Biology of the Cell*, 3rd ed., Garland Publishing, Inc., 1294 pp., 1994.

Berg, P., and M. Singer, *Dealing with Genes: The Language of Heredity*, University Science Books, 269 pp., 1992.

Suzuki, D. T. *et al.*, *An Introduction to Genetic Analysis,* Freeman, 612 pp., 1986.

Watson, J. D. *et al.*, *Molecular Biology of the Gene*, 4th ed., Benjamin Cummings, Inc., 1163 pp., 1988.

Information about Drosophila

Ashburner, M. L., *Drosophila: A Laboratory Handbook,* Cold Spring Harbor Press, 1331 pp., 1989.

Ashburner, M. L., and E. Novitski, eds., *The Genetics and Biology of Drosophila,* Vols. la–1c, Academic Press, 1426 pp., 1976.

Bate, M., and A. Martinez-Arias, eds., *The Development of Drosophila melanogaster,* 2 vols., Cold Spring Harbor Press, 1558 pp., 1993.

Lawrence, P. A., *The Making of a Fly: The Genetics of Animal Design,* Blackwell Publications: London, 228 pp., 1992.

Drosophila techniques

Ashburner, M., *Drosophila: A Laboratory Manual,* Cold Spring Harbor Press, 434 pp., 1989.

Information directories of Drosophila genes

Lindsley, D. L., and E. Grell, *Genetic Variations of Drosophila melanogaster,* Carnegie Institution of Washington Publication No. 627, 1968.

Lindsley, D. L., and D. Zimm, *Genetic Variations in Drosophila melanogaster,* Academic Press, 1992.

Flybase (an electronic Drosophila genetic database): http://morgan.harvard.edu

The Drosophila Genome Project: http://fly2.berkeley.edu

Other useful Web sites

http://www.virtual library:Drosophila

http://www-leland.stanford.edu/-ger/drosophila.html

http://www.ciwemb.edu (Carnegie Institution of Washington)

EXPERIMENTS

Living flies of the types required for the experiments described here can be purchased commercially (see back cover).

General Instructions

Starting the cultures: Flies are shipped from the stock center in small containers or vials. As soon as possible after you receive them, prepare stock bottles of each type sent you. Make the food according to instructions furnished earlier in this *Guide*. Before transferring the flies from the vials, be sure there is no excess moisture covering the food or the sides of the bottles, in which flies might stick or drown.

Flies can be shaken from vial to bottle without etherizing if care is taken. To be able to remove the cotton stopper from the vial without losing the flies, turn the base of the vial toward some strong source of light: flies usually move toward the light and will therefore leave the mouth of the vial. Or flies may be shaken to the bottom by tapping the vial rapidly on the desk or table. Then quickly remove the cotton stopper, shake the flies into the bottle, and cover at once. Do not discard the vial, but attach it to the culture bottle with adhesive tape or an elastic band. Eggs that may have been laid in the vial will thus be saved to continue their development. Label each bottle as the transfer is made, using the symbols indicated in the following paragraphs. The label should include the date of transfer.

Symbols: A general description of the wild-type fly appears in the first pages of this manual. *Mutant types* are inherited departures from the standard phenotype, and *aberrations* are reorganizations of the chromosomal carriers of the genotype. Each mutant type is given a name, suggesting the main diagnostic feature. The name is usually a simple descriptive adjective, such as "black," or a noun, such as "Bar." For convenience in listing, a representative symbol is assigned to each mutant type. It is essentially an abbreviation, and may consist of the

initial letter of the mutant name (for example, *e* for ebony) or of this letter plus one or more additional letters from the rest of the name (for example, *ey* for eyeless, *dp* for dumpy).

Names and symbols of recessive mutant types, such as ebony and dumpy, begin with small letters. Names and symbols of dominant mutant types begin with capitals, for example, Bar (*B*) and Notch (*N*). However, the wild-type allele of a dominant or recessive mutant is represented not by the alternative-sized letter but by the symbol +. Thus, the dominant wild-type allele of *b* (black) is not *B*, but b^+, and the recessive wild-type allele of *B* (Bar) is not *b*, but B^+. One reason for this system is clearly illustrated by the examples just given. The symbol + is read "wild type" in any connection, even as an alternative to a multiple mutant, for example, *y w m*/+.

Several mutants have been found that occupy similar positions on the genetic map. Such mutants constitute a series of multiple alleles. Names given them are based on the locus name; for example, the eye-color genes apricot and eosin are located at the white locus, and their symbols are w^a for apricot and w^e for eosin.

A list of mutants and aberrations of *Drosophila melanogaster*, by D. L. Lindsley and E. H. Grell, was published in 1968. (See Suggestions for Reading, page 29.)

The mutants and aberrations that are useful for these preliminary experiments are listed below in alphabetical order of their symbols. The first number in the parentheses indicates the chromosome where the gene is located; the second number, its position on a linkage map. ♂ = male, ♀ = female; ♂♂ = males, ♀♀ = females.

(Please note that this is not a stock list, but merely a description of available mutants and aberrations. Several mutant characteristics are often combined in one stock, for example, *y bw e ey*, or *Cy/Pm; H/Sb*. See the instructions for experiments, pages 33–45, for names of the stocks required.)

B: Bar (1–57.0) Eye restricted to a narrow vertical bar in ♂ and in homozygous ♀. Homozygous ♀ fully viable; heterozygous ♀ has number of facets intermediate between homozygous ♀ and wild type. The character may be regarded, therefore, as semidominant. Viability good.

bw: brown (2–104.5) Eye color light brownish on emergence, darkening to garnet; testes and vasa colorless; Malpighian tubes somewhat paler than the wild type. Eyes of the double recessive combinations *v/v;bw/bw, cn/cn;bw/bw,* and *bw/bw;st/st* are white.

C: A dominant crossover reducer or suppressor; usually an inversion or translocation. An inversion in the right limb of the third chromosome that suppresses crossing over might be symbolized In(3R)C.

cn: cinnabar (2–57.5) Eye color bright red, becoming dull with age; ocelli colorless. The combination *cn/cn;bw/bw* has white eye color.

Cy: Curly (2) Associated with second-chromosome inversion. Wings curled upward strongly. Usually lethal when homozygous.

dp: dumpy (2–13.0) Wings truncated and reduced to about two-thirds the length of those of the wild type. Certain of the bristles and hairs on the thorax also are affected by this mutation. Viability good.

e: ebony (3–70.7) Body color shiny black, much darker than that of the wild type. Newly emerged flies are lighter in color than older flies. To avoid confusing young ebony flies with wild type, allow flies to age several hours or a day before classifying. Viability about 0.8 that of wild type.

ey: eyeless (4–0.2) Eyes usually reduced to about one-fourth to one-half the area of the wild-type eye, although various degrees of reduction are found, sometimes differing in the two eyes.

f: forked (1–56.7) Bristles all shortened, gnarled, and bent, with ends split or sharply bent; effect on hairs similar, but detectable only with high-power magnifiers.

H: Hairless (3–69.5) Acts mainly on bristles, removing postverticals and abdominals, also anterior dorsocentrals and others. Lethal when homozygous.

l: General term for lethal gene. The various lethals produce the same effect, namely, death of the individual at an early stage of development, although differences can easily be found in their mode and time of action. The designation *ClB* indicates, for example, that the X chromosome, in which Bar is located, also carries a lethal gene (*l*) and a crossover suppressor (in this case an inversion).

L: Lobe (2–72.0) Eye reduced in size, with nick in anterior edge. Several alleles of Lobe are known. $Lobe^2$ is recommended for these experiments, since in the heterozygous condition it does not overlap wild type. Homozygote has very tiny eyes and lowered viability. In some strains is completely lethal when homozygous.

m: miniature (1–36.1) Wings small or miniature, only slightly longer than abdomen; surface dark gray from crowded hairs on small cells.

Pm: Plum (2) Dominant eye color, resembling brown, but dotted or flecked with darker spots. Associated with a second-chromosome inversion. Homozygous *Pm* is lethal; viability of heterozygote is good.

Sb: Stubble (3–58.2) Bristles less than half normal length, the blunt stubble being somewhat thicker than the base of the wild-type bristle. Homozygous *Sb* is lethal.

sc^8: $scute^8$ (1–0+) Affects form and number of certain bristles. A slight scute, modifying somewhat the supra-alars, sternopleurals, and occasional bristles in other regions. Extra bristles present. Inseparable from *$In(1)sc^8$*, which encompasses most of the length of the X chromosome (from 1B to 20B).

se: sepia (3–26.0) Eye color on emergence transparent brownish red, darkening to sepia, and finally black. Ocelli remain wild type in color.

ss: spineless (3–58.5) All bristles reduced to little larger than hairs, dorsocentrals least reduced; postscutellars erect.

st: scarlet (3–44.0) Eye color very bright yellowish red, distinguishable from wild type even with the naked eye, darkening somewhat with age. Ocelli colorless, even in old flies. Eyes of the double recessive combination *st/st;bw/bw* are white.

v: vermilion (1–33.0) Eye color very bright red, lighter than wild type; ocelli colorless. The combination *v/v;bw/bw* has white eye color. Viability good.

w: white (1–1.5) Eyes nearly snow white; ocelli, Malpighian tubes, and testis sheath colorless. Reoccurs frequently (about once among 300,000 flies) and has numerous alleles intermediate between *w* and wild type.

w^a: white-apricot (1-1.5) Eye color of ♂ yellowish pink; ♀ eye color somewhat yellower. Larval Malpighian tubes colorless. $w^a/w^a;bw/bw$ eye color is slightly lighter than w^a, darkening with age; $w^a/w^a;st/st$ is very light pinkish yellow, slightly darker in ♂.

w^e: white-eosin (1–1.5) Eye color of female yellowish pink; that of male light pinkish yellow.

y: yellow (1–0) Body color rich yellow; hairs and bristles brown with yellow tips; wing hairs and veins yellow; larval setae and mouth parts yellow to brown; hence *y* can be distinguished in larval stage.

y: Underscoring the symbol indicates that the mutant is carried in X chromosomes which are united at the spindle-fiber end (attached X's). Therefore, *y* represents attached-X chromosomes carrying yellow in each component; *y v f* refers to attached X's carrying *y, v,* and *f* in each component; *y w/sc* represents attached X's, one carrying *y* and *w*, the other *sc*, the phenotype being wild-type ♀.

Monohybrid Crosses

In these experiments we determine the method of inheritance when a single contrasting pair of characters is involved, that is, when the offspring are hybrids for a single character pair. From the material supplied we may measure inheritance in crosses involving dumpy and wild type, sepia and wild type, or ebony and wild type. (A cross involving ebony and dumpy simultaneously will be described later, under the heading Dihybrid Crosses.) Let us consider a plan for making a monohybrid cross with dumpy and wild type.

1. Mate *virgin* dumpy (*dp*) females (♀♀) with wild-type (+) males (♂♂). Single pairs may be mated, but for a cross of this type we suggest that 5 or 6 pairs of flies be placed in a bottle. (One bottle should supply enough progeny, but extra bottles may be prepared if desired in order to avoid delays arising from mishaps.) These parent flies are known as the first parental generation, or P_1. It is important that the females be virgin. The method for collecting virgin females was described

in the section Experimental Matings. Label the bottles *dp* ♀ × + ♂, and add the date.

2. After 7 to 8 days, remove the parents. Study carefully the differences between the two types, so that you can distinguish dumpy from wild type at a glance. The parents may be transferred to a fresh culture bottle, as a reserve in case the first culture is lost.

3. The new generation of flies resulting from this cross is known as the first filial (F_1) generation. Examine the F_1 flies with respect to wing length. What conclusions can you draw from these observations?

4. Place 5 or 6 pairs of F_1 flies in a fresh culture bottle. For this mating the females need not be virgin, because all the flies in this generation are alike with respect to wing length.

5. After several days remove the F_1 flies (they may be transferred to a fresh culture bottle and kept in reserve).

6. When the second filial generation (F_2) flies emerge, determine how many are wild type and how many are dumpy. Continue counting for 7 to 8 days after the flies begin to emerge. As flies are counted they are disposed of in the "morgue" (see fig. 6), unless it is indicated that they should be saved for a further experiment.

We suggest that the data be tabulated in some such form as the following:

Experiment no. _____ Date of beginning _____

P_1 dumpy (*dp*) ♀ × wild-type (+) ♂

F_1

Date of counting	No. of wild-type (+) flies

F_2

Date of counting	No. of flies	
	+	*dp*
Total.....		
Per cent.....		

From your total count, calculate the proportions of flies that were dumpy and wild type. Compare with what you would expect on theoretical grounds. You may determine the goodness of fit by either of two statistical methods, the standard error (S.E.) or the chi square (χ^2). If the number of F_2 flies is fairly large (about 100), your ratios should not depart widely from the expected values.

Note that, although the character dumpy was not apparent in the F_1 flies, it was not lost or contaminated by association with the wild type, for it reappeared in the F_2 offspring. Explain, with diagrams, the mechanism by which this simple Mendelian inheritance takes place, including the separation of genes in the production of the sperm and the egg, and their reunion at fertilization in the zygote from which the fly develops.

If desired, the reciprocal cross, + ♀ × *dp* ♂, may be made. The results should be similar to the preceding ones.

Sepia eye color is another good character for making monohybrid and dihybrid crosses. Its advantage over dumpy is that it has better viability, so that good ratios are usually obtained even under poor culture conditions. It has the disadvantage of not being so easily detectable as dumpy: whereas dumpy can be separated from non-dumpy without magnifiers, low-power magnification is necessary to classify sepia flies.

Backcross

A cross between an F_1 hybrid and an individual homozygous for one or more recessive genes that are present in heterozygous condition in the F_1 hybrid is known as a backcross. For example, if F_1 flies from the monohybrid cross described in the previous experiment were mated with *dp* flies, that would constitute backcrossing, and the offspring of such a cross would be called the backcross generation.

1. This experiment may be conducted together with the previous experiment. Virgin F_1 females from the cross *dp* × + may be mated with *dp* males from a stock culture, or else virgin *dp* females from a stock culture may be mated with F_1 males. In either case, 5 or 6 pairs of flies are placed in each of three bottles.

If this experiment is started independently, repeat parts 1 and 2 of the previous experiment in order to obtain F_1 flies.

2. At the end of 7 to 8 days, remove the parents.

3. When the flies of the backcross generation emerge, determine how many are wild type and how many are dumpy. Continue counting for 7 to 8 days after the flies begin to emerge.

Data may be recorded on the form suggested in the previous experiment for tabulating F_2 flies.

Dihybrid Crosses

Demonstration of the method of inheritance when two pairs of contrasting characters are considered simultaneously:

1. Mate *dp* ♀♀, having normal (non-ebony) body color, with *e* ♂♂, having normal (non-dumpy) wings, or make the reciprocal cross, using virgin females.

2. Remove the parents after 7 to 8 days, and transfer them to a reserve culture.

3. Determine the types of F_1 flies with respect to wing length and body color. Are both dumpy and ebony recessive to the contrasting wild-type characters?

4. Mate several pairs of F_1 flies. Females need not be virgin.

5. Remove the F_1 after 7 to 8 days, and transfer them to provide reserve cultures.

6. Determine the number of the different types of F_2 flies, counting approximately 250 flies. They should be of four types: non-ebony, non-dumpy (wild-type); ebony, non-dumpy; non-ebony, dumpy; and ebony, dumpy.

Record your results in some such form as the following:

Experiment no. _____ Date of beginning _____

P_1 ebony (*e*) ♀ × dumpy (*dp*) ♂

F_1 _____

Date	No. of wild-type flies

F_2

Date	No. of flies			
	+	*e*	*dp*	*e dp*
Total.....				
Per cent.....				

7. Compare the frequencies of the four types with the theoretically expected ratio for two pairs of characters assorting at random. (Use the χ^2 method to determine the goodness of fit.)

8. Determine the dumpy:non-dumpy and the ebony:non-ebony ratios separately. Do they correspond to a 3:1 ratio individually? What is the relation of the dihybrid ratio to the two 3:1 ratios?

9. Explain your results, using diagrams to show, for each of the two generations,

(a) the independent assortment of factors in the formation of the gametes (germ cells), and (b) the random union of the gametes at fertilization.

A dihybrid cross can also be made in which one of the initial parents carries both of the recessives *dp* and *e*, and the other both of the wild-type alleles. Since *e* has a slightly lower viability than wild type, a deficiency in the relative size of the *e* class is expected.

Cross Involving a Dominant Mutant Character

1. Mate Lobe with wild-type flies, using virgin ♀♀ of either stock.
2. Remove parents after 7 to 8 days, and transfer to a fresh culture bottle.
3. Determine the types of F_1 flies with respect to eye shape. Is Lobe completely or partially dominant?
4. Mate several pairs of F_1 flies. The ♀♀ need not be virgin.
5. Remove the F_1 after 7 to 8 days, and transfer to a fresh culture bottle.
6. Classify the different types of F_2 flies. Compare the observed ratio between the types with that expected on theoretical grounds. Record and explain results.

Linkage between Two Autosomal Genes

Demonstration of the phenomenon of linkage between two genes located in the same chromosome—in this case, the third chromosome of *Drosophila melanogaster:*

1. Mate *se ss* with wild-type flies, using virgin ♀♀ of either stock.
2. Remove the parents after 7 to 8 days, and transfer to a fresh culture bottle.
3. Determine the type of F_1 flies. Are the mutants or their wild-type alleles dominant?
4. Mate virgin F_1 ♀♀ with *se ss* ♂♂, and make the reciprocal cross also.
5. Remove the F_1 ♀♀ and *se ss* ♂♂ after 7 to 8 days, and transfer to a fresh culture bottle.
6. Classify the different types of flies from the F_1 cross with respect to *se* and *ss*, and determine the percentage of each type. There are four possible types: the parental types se^+ ss^+ (wild-type eye and size of bristles) and *se ss*; and the recombination (or crossover) types *se* ss^+ and se^+ *ss*.

From which of the reciprocal crosses are solely parental combinations obtained in the progeny? In which sex of Drosophila does crossing over not occur between linked genes? Calculate the percentage of crossing over from the reciprocal cross, and compare it with the standard recombination value for sepia and spineless (about 26 per cent).

Record and explain results in full.

Sex Linkage

Demonstration of linkage between certain genes and sex:

A. With wild-type females.

1. Mate wild-type virgin ♀♀ with *w m f* ♂♂. (In the two sex-linkage crosses only one character need be followed, and white has been chosen for these instructions. Results of the same type can be obtained by following either of the other two genes.)
2. Remove parents after 7 to 8 days, and transfer to a fresh culture bottle.
3. Determine the types of F_1 flies. They should be wild type.
4. Mate several pairs of F_1 flies. ♀♀ need not be virgin.
5. Remove F_1 after 7 to 8 days, and transfer to a fresh culture bottle.
6. Examine the F_2 flies, and count the number of white-eyed flies and wild-type red-eyed flies by sexes. What is the proportion of w^+ to *w* flies? What is the sex of all *w* flies?

Record and explain results.

B. The reciprocal cross.

1. Mate virgin *w m f* ♀♀ with + ♂♂.
2. Remove parents after 7 to 8 days, and transfer.
3. Determine the types of F_1 flies by sexes.
4. Mate several pairs of F_1 flies. ♀♀ need not be virgin.
5. Remove F_1 after 7 to 8 days, and transfer, providing 4 bottles in all.
6. Examine the F_2 flies, and count the number of white-eyed flies and red-eyed flies by sexes. (Save the flies for the next experiment.) The proportion of w^+ to *w* flies should be quite different from that resulting from the reciprocal cross in A above.

Record and explain the results of these two crosses, making diagrams to show the transmission of the sex chromosomes.

Locating Three Sex-Linked Genes

Examine at least 500 F_2 flies from the B experiment on sex linkage for the characters white, miniature, and forked, and record the various combinations of those genes and the wild-type genes. The following types should be scored: the parental types (1) wild type (w^+ m^+ f^+) and (2) *w m f*; and the recombination or crossover types (3) *w m* f^+, (4) w^+ m^+ *f*, (5) *w* m^+ f^+, (6) w^+ *m f*, (7) *w* m^+ *f*, and (8) w^+ *m* f^+. Determine the percentage of occurrence of each of the eight classes.

Calculate the percentage of crossing over or recombination between each pair of genes independent of the other genes. Recombination classes 3, 4, 5, and 6 show crossing over between *w* and *f*; therefore, add the percentages of occurrence of the four classes to obtain the percentage of crossing over between *w* and *f*. Classes 3, 4, 7, and 8 show crossing between *m* and *f*; determine the percentage of crossing over as for *w* and *f*. Classes 5, 6, 7, and 8 show crossing over between *w* and *m;* find the percentage of crossing over.

The first two crossover values indicate the relative distances of *w* and *m* from *f* on a genetic map, but not the order of the three genes. Both *w* and *m* might be on the same side of *f,* or one might be on either side. The percentage of crossing over between *w* and *m,* however, will show the relative positions of the three genes, since this value should approximate either the sum or the difference of the first two crossover values.

By this technique all the known genes in a given chromosome can be located in relation to one another. A few hundred genes have thus been mapped in the X chromosome of *D. melanogaster.*

Draw a map of the X chromosome carrying these three mutant genes, making the distances between them correspond in scale to the crossing-over values. Compare with the standard map in your text.

Identify the two complementary classes that represent crossovers in region 1, the two classes that represent crossovers in region 2, and the classes of double crossovers. Compare the frequency of double crossovers with that expected on the assumption that crossing over in one region is independent of that in the other. This ratio

$$\frac{\%\ \text{doubles}}{\%\ \text{of all crossovers in region 1} \times \%\ \text{of all crossovers in region 2}}$$

is known as the coefficient of coincidence. If it is less than 1.0, crossing over in one region must interfere with that in the other.

Record and explain your results, preparing diagrams to show how the various types of crossover arise.

Testcross

To determine whether four pairs of genes show any linkage or whether all four assort independently:

1. Mate *y bw e ey* virgin ♀♀ with wild-type ♂♂.

2. Remove the parents after 7 to 8 days, transfer them to a fresh culture bottle, and discard them after another 7 to 8 days.

3. Determine the phenotypes of the F_1 flies. Are they all alike? The results should confirm the fact (already indicated by their symbols) that yellow, brown, ebony, and eyeless are all recessive genes. If the reciprocal cross had been made—

that is, *y bw e ey* ♂♂ × wild-type ♀♀—would you have expected the F_1 males to have a different phenotype? If so, explain.

4. Cross F_1 ♂♂ with *y bw e ey* virgin ♀♀, making up two or three culture bottles.

5. After 7 to 8 days, remove the F_1 ♂♂ and *y bw e ey* ♀♀; transfer them to fresh culture bottles, and discard them after another 7 to 8 days.

6. Classify 500 or more of the progeny according to the sixteen possible phenotypes.

7. Record and explain your results. How many kinds of gametes can the testcross parent supply? In a testcross the total variability in the progeny is attributable to the heterozygous parent. What is the relation between the ratio of the various kinds of gametes produced by this parent and the ratio of the phenotypes in the progeny? Test the goodness of fit of your data to the expected ratio by the χ^2 method.

8. Test the goodness of fit of the segregation of each of the four mutant genes separately to the expected ratio for each of them. Is there any indication of viability differences? Would they explain any significant deviations from expectancy in the entire ratio? Is there any evidence that combinations of recessive mutants lower the viability additively or geometrically?

ClB Cross

To determine the effect of an inversion, when heterozygous, on crossing over between linked genes, and the effect of a sex-linked lethal gene on the sex ratio:

1. Cross *ClB/w m f* ♀♀ with *w m f* ♂♂.

2. After 7 to 8 days transfer the parents to a fresh culture bottle, and discard them after another 7 to 8 days.

3. Classify about 100 offspring with respect to *B, w, m, f.*

4. Record and explain your results, using a diagram.

Attached-X Cross

To determine the effect of a permanent attachment of the two X chromosomes on the transmission of sex-linked characters:

1. Cross *y* ♀♀ with *w m f* ♂♂.

2. After 7 to 8 days transfer the parents to a fresh culture bottle, and discard them after another 7 to 8 days.

3. Classify 50 to 100 of the offspring with respect to *y, w, m, f.*

4. Record and explain your results, with diagrams to show the transmission of the sex chromosomes.

Determination of Linkage Group

To determine which of the four linkage groups of *D. melanogaster* a given mutant belongs to:

1. Examine flies of the mutant strain given you.

2. Cross virgin ♀♀ of that strain with *Cy/Pm;H/Sb* ♂♂.

3. After 7 to 8 days transfer the parents to a fresh culture bottle, and after another 7 to 8 days discard them.

4. Examine the F_1 flies; classify 50 to 100. Is your mutant character dominant? If it fails to show in this generation, what must be its mode of inheritance? If it shows up, but only in ♂♂, what must be its mode of inheritance and its linkage group?

5. (a) If your mutant is dominant, select *Cy H* (or *Cy Sb, Pm H,* or *Pm Sb*) ♂♂, also showing the character given you, and cross them with wild-type virgin ♀♀.

(b) If your mutant is recessive but not sex linked, testcross *Cy H* (or *Cy Sb, Pm H,* or *Pm Sb*) ♂♂ with virgin ♀♀ from the stock given you.

Note: Select as parents the type with the two dominant mutant genes that will least interfere with classification of the mutant given you.

6. Transfer the parents after 7 to 8 days to a fresh culture bottle, and after another 7 to 8 days discard them.

7. Classify the F_2 flies. If your mutant character is dominant and shows up only in the ♀♀, to what linkage group does it belong? If your mutant gene segregates from *Cy* (or *Pm*), to which linkage group does it belong? If it segregates from *H* (or *Sb*), to which linkage group does it belong? If it assorts independently of both *Cy* (or *Pm*) and *Sb* (or *H*), and is not sex linked, to what linkage group must it belong?

8. Record and explain your results. Prepare diagrams to show the segregation, assortment, and recombination of the chromosomes.

Selection Experiments

To compare the effectiveness of natural and artificial selection on mutant characters having different modes of inheritance:

You will be given a dominant autosomal mutant, a recessive autosomal mutant, and a recessive sex-linked mutant. Familiarize yourself with their appearance.

1. Cross ♂♂ of each of the mutants with wild-type virgin ♀♀.

2. Remove and discard the parents after 7 to 8 days.

3. From the F_1 start F_2 cultures for each of the three mutants.

4. Classify 200 flies from each F_2 culture 5 to 7 days after they begin to emerge. (In the sex-linked mutant it will be necessary to classify sex; in the others it will not.)

5. Start duplicate series for each mutant, with F_2 individuals as parents. For one series, Natural Selection, select 20 parents in the same ratio (to the nearest whole numbers) as the ratio in the entire sample of 200. The ♀♀ need not be virgin. In the sex-linked mutant culture the sexes of the mutant and wild type should also be in the proper ratio.

For the other series, Artificial Selection, take only wild-type F_2 flies as parents, and use only *virgin* ♀♀.

6. Repeat steps 4 and 5 for three additional generations.

7. Compare the percentage of each mutant type in successive generations within each series. Also compare the effectiveness of natural selection and artificial selection generation by generation for each mutant. Are the results similar or different for the three types of mutants?

Non-Mendelian Genetics: Gene Transposition

Transposable elements

All organisms contain transposable elements within their chromosomes. These elements were first discovered in Indian corn over 50 years ago by a geneticist named Barbara McClintock. About the size of a gene or small virus, transposable elements have the ability to move from one chromosomal site to another under certain conditions. These elements make up nearly 5% of the Drosophila genome and fall into about 50 recognized types, each present in one to several hundred copies scattered about the chromosomes. Many of the mutations used in earlier exercises to study patterns of inheritance, such as white-apricot, were caused when a transposable element inserted into the gene in question and inactivated it. Obviously, the behavior of transposable elements is a fundamentally important aspect of genetics.

Since about 1980, transposable elements have become major tools for Drosophila geneticists. A gene can be modified in the laboratory, "piggy-backed" onto a transposable element, and then re-inserted into the Drosophila genome. The transposable element used to ferry Drosophila genes is called the *P* element. It is possible to control the movement of *P* elements containing added genes because *P*-element transposition requires a gene product known as *P*-element transposase. *P*-element transposase is only produced by certain *P* elements. *P* elements that lack a transposase gene are completely stable unless another *P* element is present in the genome that produces transposase.

Because P-element movement can be genetically controlled, the elements can easily be used as mutagens to disrupt and "tag" new genes. This approach has many advantages over earlier methods of generating mutations with X-rays or chemical agents. Indeed, the great biological sophistication of transposable elements makes them an ideal tool for probing and analyzing the Drosophila genome.

Generating new P[w⁺] *insertions*

If a transposable element contains an easily scored gene, it is possible to visualize transposition quite easily. In this experiment we use a Drosophila strain in which a copy of the wild-type *white* gene, symbolized w^+, has been placed within a *P* transposable element on the X chromosome. This transposon, written *P[w⁺],* is not capable of producing transposase protein on its own. Normally, the X chromosome already contains a wild-type *white* gene, but in all the strains we will use the normal gene has been inactivated by mutation. Thus, the red pigmentation of the flies eyes depends on the functioning of the introduced w^+ copy on the *P[w⁺]* element. Nonetheless, since the w^+ gene still resides on the X chromosome, when males are crossed to *w* females one observes the same pattern of sex-linked inheritance of eye color as in experiment B on p. 38. Because the *P* element is stable, we cannot tell that the *white* gene is riding on a transposable element. Carry out such a control cross and record the sex and phenotype of the progeny.

The situation is very different when the same basic cross is carried out in the presence of *P*-element transposase. To create such a comparison cross we must combine the *P[w⁺]* element with a second *P* element called *P*[Δ2-3] that produces transposase, but is itself unable to jump due to damage at one of its ends. We cross the *P[w⁺]*-containing strain with flies bearing *P*[Δ2-3] on a third chromosome marked with the dominant marker Stubble (Sb), as shown below:

w P[w⁺]/ w P[w⁺] ; +/+ ♀ × *w* / Y ; Sb *P*[Δ2-3]/+ ♂ P1

w P[w⁺]/ Y ; Sb *P*[Δ2-3]/+ ♂ × *w* / *w* ; +/+ ♀ F1

w / Y ; +/+ (*P[w⁺]*) ♂ × *w* / *w* ; +/+ ♀ F2

Then, red-eyed, Stubble male progeny are selected that contain both *P* elements. The cells of these males contain both the *P[w⁺]* element and the transposase protein, so that a low level of transposition events will take place throughout their lives in the sperm-producing cells.

To reveal this, we now cross the males to *w* females as in the control cross. Normally, w^+ males crossed to *white* females should produce only red-eyed females and white-eyed male progeny because the male X chromosome is not inherited by male progeny. However, when the *P[w⁺]* element has transposed to a different chromosome, it can be inherited by a male, and these offspring will have red rather than white eyes. Calculate the number of transposition events that you observed in your experiment. See if you can estimate a "transposition frequency" for the *P[w⁺]* element.

The appearance of red-eyed males violates the "rules" of Mendelian inheritance. It was this type of observation that led McClintock to realize that certain genes could move from place to place within the genome. Imagine that you had done

the original cross to illustrate the principle of sex linkage and obtained these results. Would you have been able to figure out that a *white* gene must have moved from the X chromosome to an autosome?

To prepare for the next experiment, the red-eyed males containing new transpositions must be saved and each one crossed in a separate vial with 2–3 *white* females to establish new *P[w+]* insertion strains. To avoid further cycles of transposition, only males that also lack the *P*[Δ2-3] element are used to start new strains. These males can be recognized because their bristles will not show the effects of the Stubble mutation. A total of about 20 new lines should be generated. Each new line should receive an identifying name or number and be maintained by periodically transferring adults to fresh food.

There are two other unexpected results that you may observe while setting up these strains. Compare the eye color of males in several of your new transposition stocks. Flies in some stocks are likely to have eye colors that differ from other stocks, and may appear brownish, orange, or yellow in color. These differences are due to different amounts of the *white* gene product that are being produced in the different stocks. These differences illustrate what geneticists call "position effects." The same w^+ gene is producing more or less eye pigment depending on its exact location on a chromosome. This shows that neighboring sequences can influence a gene's ability to function efficiently. Finally, you may observe a stock whose eyes have flecks of different colors. This pattern of coloration within the eye is caused when the gene's activity during eye development changes randomly over time. This phenomenon is only observed when a gene resides within certain chromosome regions. Known as "position-effect variegation," its molecular mechanism is still a subject for debate.

Visualizing patterns of gene expression

Trapping gene "enhancers"

One of the most powerful uses of transposable elements is in the study of individual genes. Each of the estimated 12,000 Drosophila genes becomes active and produces its protein product only in specific cells and tissues and at precise times during the life cycle. Knowledge of these patterns of expression is extremely helpful in understanding what individual genes do. A specially constructed *P* element, *P[lacZ, w⁺]*, can relay information about the expression patterns of genes into which it has inserted. In addition to a wild-type *white* marker gene, the *P[lacZ, w⁺]* element carries the "lacZ" gene from the bacterium *E. coli.* The lacZ gene has been modified to make it sensitive to foreign gene regulatory elements known as enhancers. The lacZ gene encodes β-galactosidase, an enzyme that cleaves a particular bond joining molecules of the sugar galactose. The lacZ gene becomes active only if an enhancer is located very close to the position of the *P[lacZ, w⁺]* element on the chromosome. Therefore, when the *P[lacZ, w⁺]* transposable element inserts near or within a Drosophila gene, β-galactosidase will usually be produced according to that gene's normal pattern of activity.

Drosophila tissues that contain β-galactosidase can be recognized by simply incubating the tissues in the presence of the colorless molecule X-Gal. If the enzyme if present, the X-Gal is cleaved, generating a product that turns blue. In the absence of β-galactosidase, no color is produced. Thus, cells that turn blue following staining with X-Gal are those that normally express the gene located at the site of the *P[lacZ, w⁺]* insertion. This procedure of using the *P[lacZ, w⁺]* transposon to reveal detailed patterns of gene expression is known as "enhancer trapping." The basic idea underlying enhancer trapping was originally worked out in bacteria using certain bacterial viruses to ferry the lacZ gene to different sites. Subsequently it was applied in Drosophila using *P* elements, and variations have been used in a wide variety of genetically tractable organisms, including plants, mice, and nematodes.

The *P[w⁺]* transposon used in the transposition experiment also contained the lacZ gene. Therefore, it could have been written as *P[lacZ, w⁺]*. Thus, each of the lines generated in the previous experiment is likely to contain a different site of *P[lacZ, w⁺]* insertion, and to exhibit a different pattern of lacZ gene expression. To determine what the expression patterns are, it is only necessary to dissect tissues from larvae or adults and stain them with X-Gal. Because you are familiar with the dissection of third instar larvae, you may wish to compare expression patterns in larval tissues between these lines. Adult ovaries are also an excellent tissue to examine, since a large number of genes are expressed during the process of egg development (Fig. 1). The figure shows the pattern of expression of a specific gene that lies adjacent to the *P* element in one such line. It is expressed in a few follicle cells at each end of each developing egg (dark cells). These cells are known to participate in patterning the egg and its embryo along its anterior-posterior (head-tail) axis.

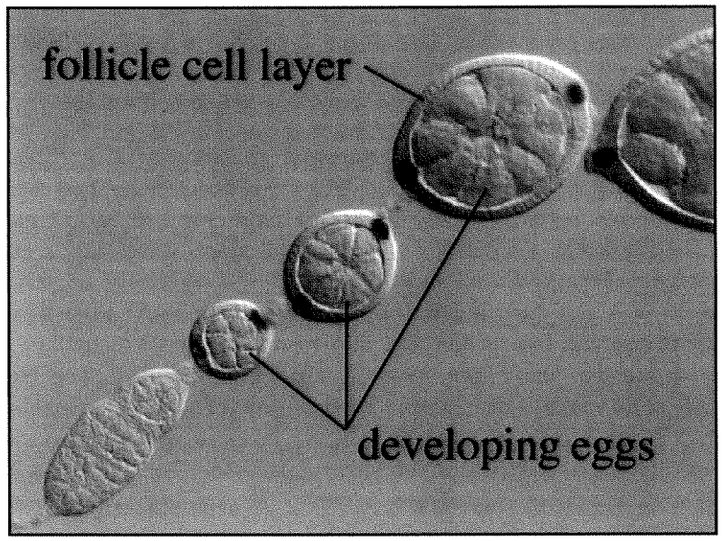


Fig. 1. Specific gene expression (dark cells) in subsets of developing follicle cells.

X-Gal staining

Gather all the materials you will need prior to beginning the dissection. The following protocol can be used.

1. Prepare a stock of tissue fixation solution by adding 4 parts of water to 1 part of concentrated buffer B and 1 part of fresh formaldehyde. Concentrated formaldehyde should be handled with gloves in a hood; your instructor may already have prepared this solution for you. Also your instructor will provide a source of X-Gal staining solution, which appears yellow in color.

2. Dissect the tissue or tissues to be stained in dissection buffer. Be as gentle as possible to avoid any damage to the tissues. When complete, transfer the tissues to a small volume of fixation buffer. If ovaries are to be dissected, adults should be transferred to fresh vials containing moist yeast 1–2 days prior to use, to increase the size of the ovaries.

3. Fix for 10 minutes at room temperature.

4. Remove the fixation buffer and discard. Immediately replace with phosphate-buffered saline (pBS). Do not let tissues dry out.

5. Wash for 10 minutes at room temperature. Repeat the wash once.

6. Remove the saline and add just enough X-Gal staining solution to cover the tissues. Do not allow to dry out.

7. Allow to stain for 60 minutes to overnight at 37°C. When staining is strong or complete, remove the staining solution and discard. Replace with a buffered saline solution that contains 50% glycerol. This will allow the tissue to be mounted and stored more easily.

8. Mount the tissues on microscope slides and cover with coverslips for easier viewing. Examine with a dissecting microscope or a compound microscope under low power. For long-term storage, seal the coverslips with nail polish and store at 4°C.

Do you see distinct patterns of gene expression in any of the strains containing *P[lacZ, w⁺]* insertions? Select two or more such lines and compare the patterns of staining in one or more tissues carefully. Some of the tissues may appear to be entirely stained, some entirely unstained, and others may have a specific "pattern" of staining where certain cells stain and others don't. Notice how the pattern of expression is very consistent within different individuals from a particular line. All these flies from a line should contain a *P[lacZ, w⁺]* at the same chromosomal site. Since the pattern observed is a reflection of the normal processes of gene regulation at that site, the highly accurate mechanisms that regulate expression cause this pattern to be so reproducible. In exceptional cases where you may see differences within a line, the following explanations may apply: 1) more than one insertion occured on different chromosomes, and different progeny inherited one or both of them; or 2) by mistake, the *P*[Δ2-3] element was inherited along with the *P[lacZ, w⁺]* element and it is causing continued movement of the transposon to new sites. Can you think of simple genetic tests for these possibilities?

Another thing to look for are patterns of gene expression within individual cells of a tissue. Although tissue cells often look very similar in a microscope, at the molecular level there may be very significant differences that are revealed in studying patterns of gene expression. For example, patterns within the layer of follicular cells that surround developing eggs frequently are related to specialized structural features these cells will produce in the eggshell. A close examination of these patterns can greatly increase your appreciation of how precisely genes are routinely regulated by multicellular organisms.

Buffers for X-Gal staining

Saline for dissection
- 130 mM NaCl
- 5 mM KCl
- 2 mM $CaCl_2$
- 10 mM Hepes (pH 6.9)

Store at -20°C.

Fixation Buffer
- 1 volume Buffer B
- 1 volume formaldehyde (37%)
- 4 volumes fresh H_2O

Make fresh every day.

Buffer B
- 100 mM KH_2PO_4/K_2HPO_4 (pH 6.8)
- 450 mM KCl
- 150 mM NaCl
- 20 mM $MgCl_2 \cdot 6H_2O$

PBS (one liter)
- 20.00 g NaCl
- 0.50 g KCl
- 0.50 g KH_2PO_4
- 1.47 g Na_2HPO_4 (anhydrous)

X-Gal staining solution
- 10.0 mM $NaH_2PO_4 \cdot H_2O/Na_2HPO_4 \cdot 2H_2O$ (pH 7.2)
- 150.0 mM NaCl
- 1.0 mM $MgCl_2 \cdot 6H_2O$
- 3.1 mM $K_4[FeII(CN)_6]$
- 3.1 mM $K_3[FeIII(CN)_6]$
- 0.3% Triton X-100

Just before use, preincubate at 37°C, then add 25 µl 8% X-Gal in DMSO (stored frozen) for each 1 ml staining solution.