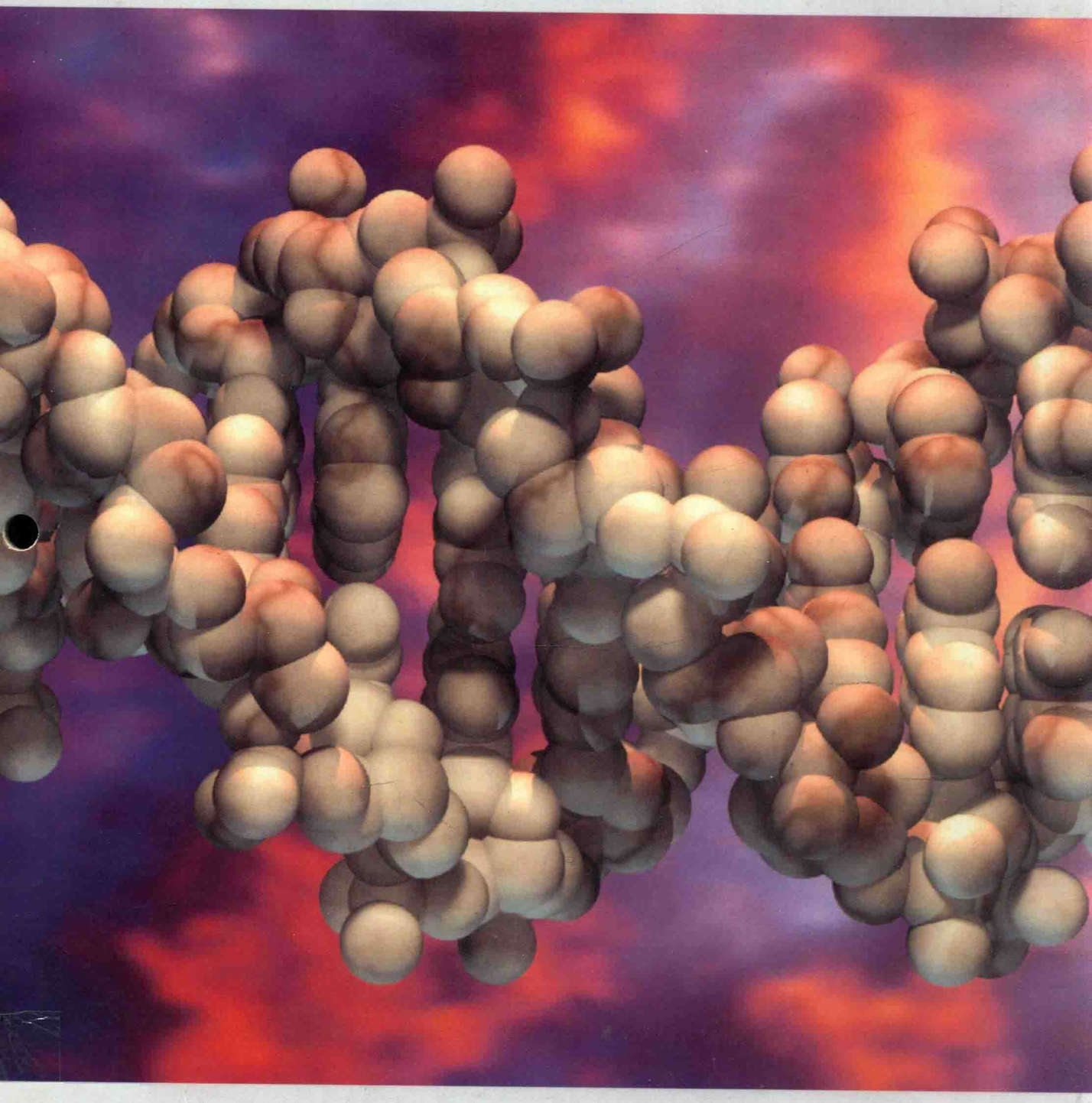
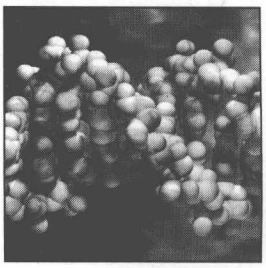
GENETICS

LABORATORY INVESTIGATIONS

ELEVENTH EDITION



Thomas R. Mertens • Robert L. Hammersmith



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GENETICS

Laboratory Investigations

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Prentice Hall
Upper Saddle River, New Jersey 07458

Library of Congress Cataloging-in-Publication Data

Mertens, Thomas Robert
Genetics : laboratory investigations / Thomas R. Mertens, Robert L. Hammersmith. — 11th ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-13-575986-2
1. Genetics—Laboratory manuals. I. Hammersmith, Robert L. II. Title.
QH440.5.M47 1998
574.5'078—dc21 97-315
CIP

Executive Editor: Sheri L. Snavely
Production Management: J. Carey Publishing Service
Art Director: Heather Scott
Interior Design: Anne Flanagan
Cover Art: Biografx—Kenneth Eward
Compositor: Preparé/Emilcomp
Manufacturing Manager: Trudy Pisciotti

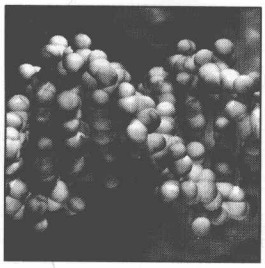
Simon & Schuster / A Viacom Company
Upper Saddle River, New Jersey 07458

Printed in the United States of America

10 9 8 7 6 5 4 3 2

ISBN 0-13-575986-2

Prentice-Hall International (UK) Limited, *London*
Prentice-Hall of Australia Pty. Limited, *Sydney*
Prentice-Hall of Canada, Inc., *Toronto*
Prentice-Hall Hispanoamericana, S. A., *Mexico*
Prentice-Hall of India Private Limited, *New Delhi*
Prentice-Hall of Japan, Inc., *Tokyo*
Simon & Schuster Asia Pte. Ltd., *Singapore*
Editora Prentice-Hall do Brasil, Ltda., *Rio de Janeiro*



GENETICS

Laboratory Investigations

PREFACE

Professional associations for geneticists in the United States increasingly recognize the importance of genetics education in their total program and commitment. For example, the Genetics Society of America and the American Society of Human Genetics have published a booklet, *Solving the Puzzle: Careers in Genetics* (1993), which has as its goal attracting potential students to the discipline and explaining what geneticists do. Speaking of his first course in genetics at Temple University, one of the contributors to the booklet, Hong Ma, now senior staff investigator at Cold Spring Harbor Laboratory, says, "I liked the experiments in which I could put my own hands to work, and the deductions which required my brain to do more than just memorize facts."

Dr. Ma's statement embodies what many of us have found attractive about genetics. The statement also emphasizes the importance of the laboratory in the instructional setting. We are firmly convinced that the student's laboratory experience in genetics is an essential part of the learning process and vitally important to fostering student interest in, and understanding of, the discipline. The laboratory experience can confirm and expand on what the student learns in the classroom and from the textbook, but its most important contribution is to give the student the opportunity to function as a scientist, using his or her "brain to do more than just memorize facts." To foster achieving this latter goal, a number of "open-ended" investigations have been included in this manual, the most obvious being Investigation 14. With appropriate adaptation, however, instructors can use the open-ended approach in any of the investigations in which experimental crosses are made or original (for the student) data are collected.

As in previous editions, we have included investigations to accommodate courses taught in a variety of academic settings. Some investigations provide two or more alternative ways of achieving the instructional objectives. More investigations are included than can be used in most situations. Instructors can select the alternatives that best suit their preferences and student needs. For example, doing only one of the meiosis investigations (6 or 7) may be appropriate.

Fourteen new illustrations have been added to this edition of the manual. For example, additional photographs of microsporogenesis in *Tradescantia* (Investigation 7) enhance our understanding of the meiotic process in angiosperms. New photographs for human karyotyping have also been provided for Investigation 11. Updating has been done throughout the book, and special attention has been given to selecting references for each investigation that are current and useful to both students and instructors. Each investigation is essentially self-contained, making it possible to use the 26 investigations in a variety of sequences and with any of the many genetics textbooks on the market.

Though the increasing cost of doing classroom laboratory work cannot be denied, we have included many investigations that are not expensive to use with sizable groups of students. Investigations 1, 2, 9, 14, and 25, involving matings with *Drosophila*, fall into this relatively inexpensive category, although they do require long-range planning to ensure their completion in the time frame of the course. Many other inexpensive investigations are also included (for example, Investigations 8, 13, and 23). Without question, most molecular/microbial genetics investigations are expensive to carry out. We have, however, designed several investigations in this area to be cost effective and to require a min-

imal amount of specialized equipment, yet to provide the student with appropriate experience and exposure to fundamental concepts of molecular/microbial genetics. To that end, a new investigation (17) on the polymerase chain reaction has been included for the first time in the eleventh edition.

To provide additional laboratory investigations without significantly increasing the length of the manual, we again chose to conclude the book with four supplemental laboratory topics. Each of the four contains enough background information (including appropriate literature citations) to allow instructors to develop the topic as a full-blown investigation or to permit individual students to pursue it as an individual project. No instructional objectives, student questions, or illustrations are provided for these four topics, however. We hope that this approach will increase the usefulness of our manual without adding significantly to its cost.

Most students using this manual either will be taking a general genetics course concurrently with the laboratory component or will have completed such a course before enrolling in the laboratory course. Consequently, they should have ready access to an appropriate general genetics textbook that will provide background and details on most topics included in this manual. Space limitations prevent the laboratory manual from including subject matter content that is more appropriately included in a textbook.

An *Instructor's manual* may be obtained from Prentice Hall by qualified instructional personnel. Answers to significant questions posed in the 26 investigations are provided in the *Instructor's manual*, along with supplementary information that is helpful in preparing and conducting the investigations.

The history of this laboratory manual dates to 1960, when the late Eldon J. Gardner of Utah State University published a slim manual that became the foundation for the present book. Beginning with the fifth edition (1970), T.R. Mertens assumed primary responsibility for developing, updating, and expanding the manual. R.L. Hammersmith was added as coauthor beginning with the eighth edition (1985). Over the years, many colleagues, graduate assistants, and students have contributed to the improvement of the investigations.

To all who have assisted, we give our thanks. Credit for illustrations and quotations in the present edition are given with appropriate investigations or according to the wishes of authors or publishers from whom permissions have been granted.

Investigations 13 (genetics of *Sordaria*), 23 (human fingerprint ridge counts), and 25 (genetic drift) were originally published in *The American Biology Teacher*, the first in May, 1968, the second in April, 1989, and the third in November, 1990. We are indebted to Dr. George A. Hudock of Indiana University for permission to modify two investigations included in his book, *Experiments in Modern Genetics* (John Wiley & Sons, 1967). These modifications of Hudock's work appear as our Investigations 19 and 22. We acknowledge with appreciation new photos of human chromosome spreads (Investigation 11) prepared by D.L. Van Dyke and J. Zabawski of Henry Ford Hospital, Detroit. Thanks are also due to Dr. Kenneth Weber of the University of Southern Maine for supplying the photograph of mitotic chromosomes showing sister chromatid exchanges (Investigation 5). We thank Carolina Biological Supply Company (Investigation 6), the Biological Sciences Curriculum Study (Investigation 23), and Helena Laboratories (Investigation 26) for illustrations they provided. The section of Investigation 26 that deals with electrophoresis of hemoglobin has been updated in this edition using material prepared by Helena Laboratories of Beaumont, Texas, and used with permission. Investigation 17 is based in part on material supplied by the Perkin Elmer Corporation and by Carolina Biological Supply Company, and thanks are due to both firms for their assistance. Kits for conducting this investigation may be purchased from either company. We wish to thank our editor, Sheri Snavely, and her staff for their support and efforts in the production of this manual. Their commitment to the project has made our task significantly easier. Finally, we wish to thank the instructors and students who have used the tenth edition of this manual. It is their commitment to the manual that has justified the production of the eleventh edition. We hope that the updating and additions found in the eleventh edition will continue to make the manual a useful instructional tool.

T. R. M.
R. L. H.

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INVESTIGATION 1

Drosophila and Maize Experiments in Genetics: Monohybrid Crosses

A number of organisms may be used for demonstrating the classical Mendelian laws. Two eukaryotic organisms that are exceptionally well understood genetically are *Drosophila* and maize (*Zea mays*). The vinegar or fruit fly, *Drosophila melanogaster*, is especially well suited to experimental crosses in the classroom laboratory. This small fly passes through a complete metamorphosis in 10 to 14 days at 25°C. In addition to a short life cycle, *Drosophila* possesses an abundance of genetic variability, is highly prolific, and is a convenient and inexpensive organism to study. Before performing genetic experiments with *Drosophila*, however, you must learn some basic facts about the biology and culture of this organism.

OBJECTIVES OF THE INVESTIGATION

Upon completion of this investigation, the student should be able to

1. **outline** the basic procedures for culturing and experimenting with ***Drosophila melanogaster*** and
2. **recognize** and **interpret** *Drosophila* and maize F_2 data that illustrate Mendel's law of segregation.

I. MEDIUM

Numerous media have been developed for the culture of *Drosophila*. Perhaps the easiest to use is "instant medium."[1] One need only add water to the concentrate to produce a medium that is immediately usable. No cooking is necessary; however, some workers suggest adding five drops of a 5% solution of propionic acid to assist in inhibiting mold. Although convenient, this medium may prove too expensive when large class enrollments require using a considerable amount.

Demerec and Kaufmann's *Drosophila guide* includes a number of recipes for different media.[2] The ingredients of another useful medium, which has been attributed to the late C.B. Bridges of the California Institute of Technology, are as follows.

20 g of agar
200 g of cornmeal
145 ml of Karo Syrup
145 ml of Br'er Rabbit Molasses
2400 ml of distilled water
4.5 g of Dowicil-200 dissolved in 15 ml of distilled water[3]

[1] Instant medium is available from Carolina Biological Supply Co., 2700 York Road, Burlington, NC 27215.

[2] *Drosophila guide* may be purchased from Carnegie Institution, Department B, 1530 P Street NW, Washington, DC 20005.

[3] Dowicil-200 is available from Camile Products, 1776 Building, Midland, MI 48674.

First dissolve the agar by heating it with 1000 ml of the distilled water, and then add an additional 1000 ml of water. Carefully bring the mixture to a boil. Before adding the cornmeal to this mixture, mix the cornmeal with the remaining 400 ml of unheated distilled water. (Mixing the cornmeal with the unheated water prevents it from forming lumps when you add it to the hot agar suspension.) Add the cornmeal to the dissolved agar mixture, stirring continuously to prevent the medium from sticking. Now add the molasses and syrup and boil the whole mixture for about 15 minutes. Dissolve the Dowicil-200 (mold inhibitor) in 15 ml of distilled water; add this to the main mixture and mix thoroughly. The cornmeal should have no tendency to settle out in the final product.

Pour the medium into chemically clean bottles. These bottles may be of any size, but 4-oz. wide-mouthed bottles or half-pint milk bottles are satisfactory. Transfer the medium to a beaker for pouring, and fill the bottles to a depth of about 1 in. Take care to prevent the medium from coming into contact with the neck of the bottle. Place a piece of *nonabsorbent paper* such as brown wrapping paper in each bottle so that it extends down into the medium (a double piece of paper is more satisfactory). Use paper that is about 1 in. wide and be certain that it extends upward to a point about 0.5 in. below the neck of the bottle. This paper provides a dry place on which *Drosophila* larvae can pupate. Cotton plugs may be used to stopper the bottles, but foam diSPo plugs are more convenient.[4]

Once the bottles of medium are plugged, sterilize them in an autoclave at 20 lbs. pressure for 20 minutes. Other items to be used in handling the flies may be sterilized at the same time.

Water will condense on the insides of the bottles as they cool after sterilization. Allow 48 hours in a well-ventilated room for this water to evaporate. Immediately before you place flies into the bottles of medium, shake a small amount of dry yeast onto the medium. The yeast will grow and serve as food for the developing fly larvae.

II. DEVELOPMENT OF THE FLIES

The eggs of *Drosophila* are laid on or near the surface of the culture medium. They hatch about 1 day later, producing minute, white larvae, which burrow in the medium and feed on yeast cells. The larvae attain full size at the end of about 7 days. They then climb onto the side of the bottle or onto the brown paper and pupate. The pupal stage lasts about 2 days, after which the mature flies emerge. At 25°C, the entire life cycle from egg to adult is completed in about 10 days.

Materials needed for each student for this investigation (Figure 1.1):

stereo dissecting microscope
bottles of culture medium
gummed labels
etherizer
re-etherizer (petri dish)
ether in dropping bottle
dropper
teasing needle or fine camel's hair brush
morgue containing 70% ethyl alcohol

III. HANDLING THE FLIES

To etherize and examine adult flies, proceed as follows.

1. Place a few drops of ether on the absorbent material of the etherizer.

[4] Foam diSPo plugs are available from Baxter Scientific Products Division of VWR Scientific, 1430 Waukegan Road, McGaw Park, IL 60085-6787.

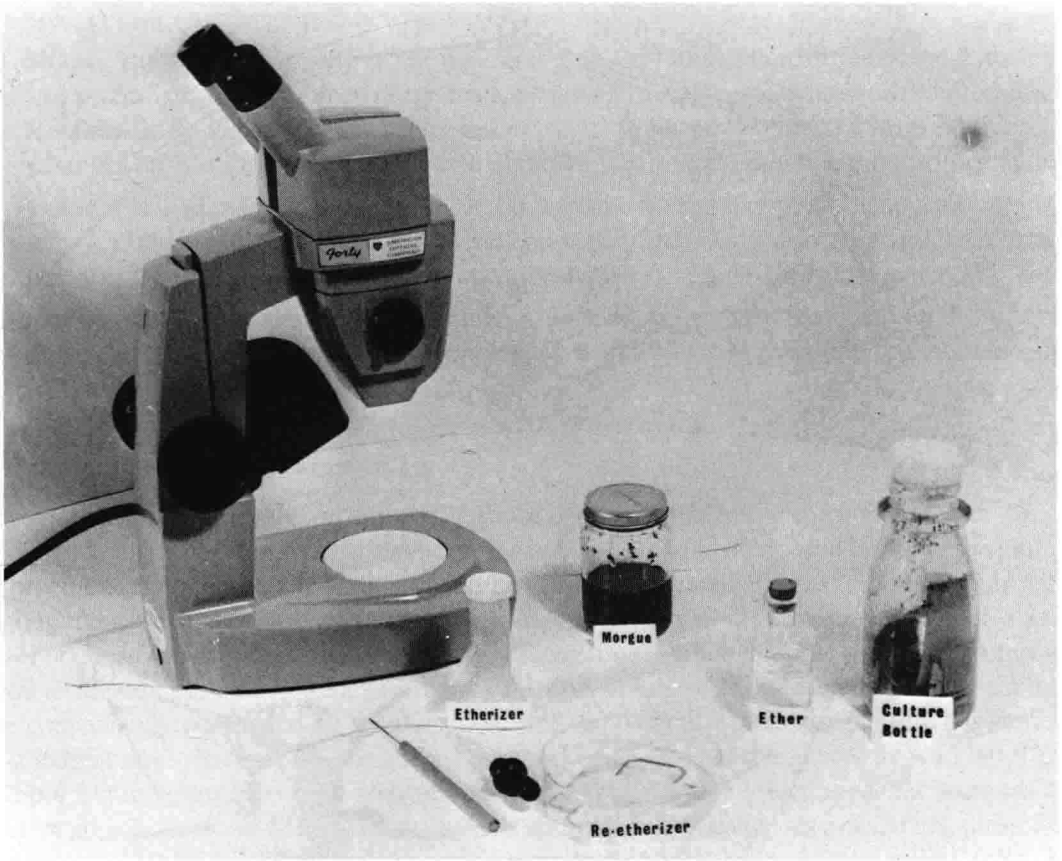

FIGURE 1.1. Equipment needed for handling and examining *Drosophila*.

2. Strike the base of the culture bottle lightly on the palm of the hand so the flies will drop to the bottom.
3. Remove the culture bottle plug, quickly replace it with the mouth of the etherizer, invert the bottle over the etherizer, and shake flies into the etherizer.
4. Subject the flies to ether for about 30 seconds after they cease moving. Avoid overetherization if they are to be used in further matings. The flies will die if left in the etherizer too long. Overetherized flies hold their wings vertically over their body (in contrast to the normal at-rest position shown in Figure 1.2).
5. Transfer the etherized flies to a clean white card. A 3-by-5 in. file card is ideal.
6. Examine the etherized flies with a dissecting microscope at 10× to 25× magnification. Use a soft brush or teasing needle for moving the flies about on the stage of the microscope.
7. If the flies revive before you finish examining them, add a few drops of ether to the absorbent pad on the re-etherizer and cover flies on the microscope stage for a few seconds.
8. If the flies are not needed after observation, they may be immediately discarded in the morgue. Etherized flies to be used for further matings should be permitted to recover in a dry vial or on a dry surface in the culture bottle before they come into contact with the moist medium.

IV. DISTINGUISHING SEX

Examination of the external genitalia under magnification is the best means of distinguishing the sex of flies. Only male flies exhibit darkly colored external genitalia, which are visible on the ventral side of the tip of the abdomen (Figure 1.3). The following characteristics, illustrated in Figures 1.2 and 1.3, can also help to distinguish males from females.

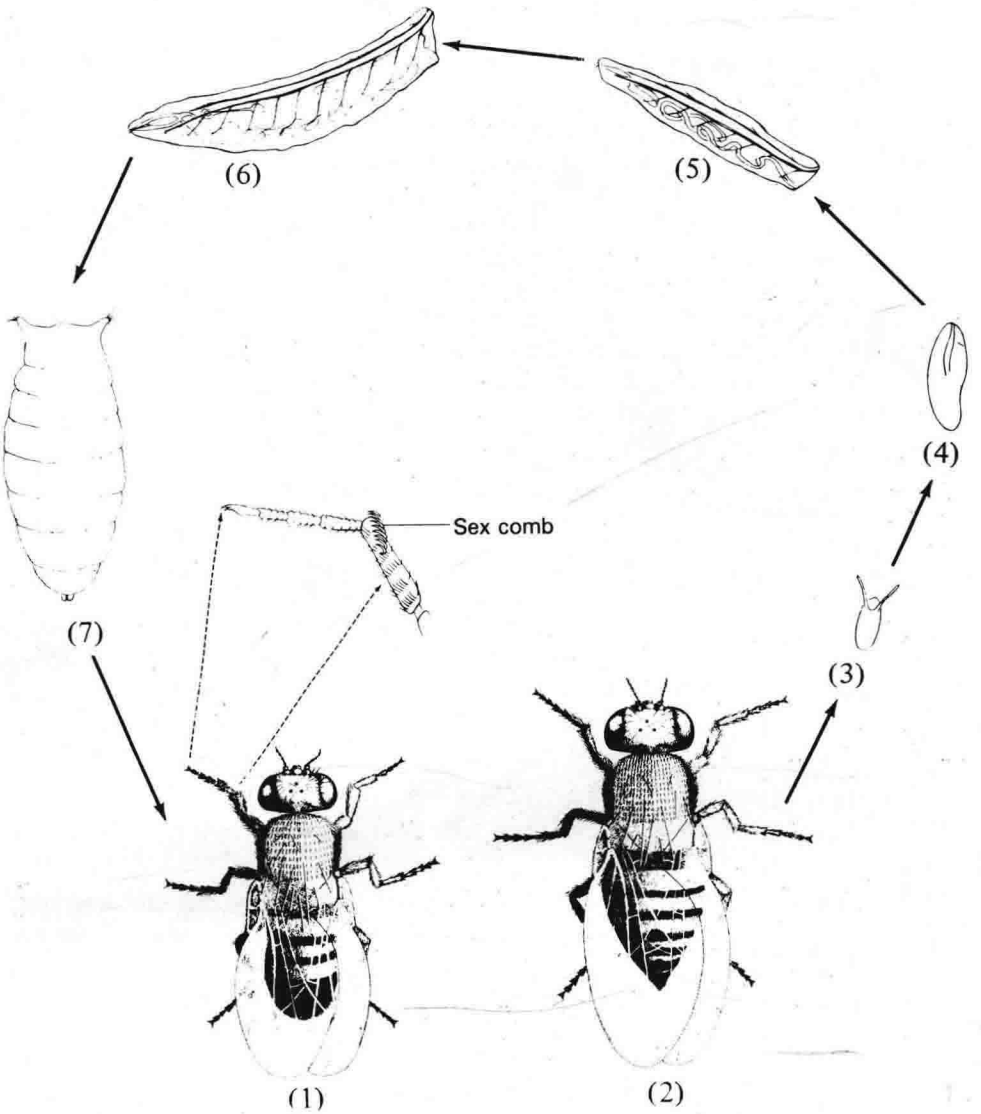

FIGURE 1.2. Life cycle and sexual dimorphism of the vinegar fly, *Drosophila melanogaster*. The male (1) is generally smaller than the female (2). The male also has a more rounded abdomen that is black-tipped rather than striped. The enlarged foreleg of the male reveals special bristles known as the sex comb, a characteristic lacking in the female. Metamorphosis is complete, from egg (3), larval stages (4, 5, and 6), and pupa (7) through adult.

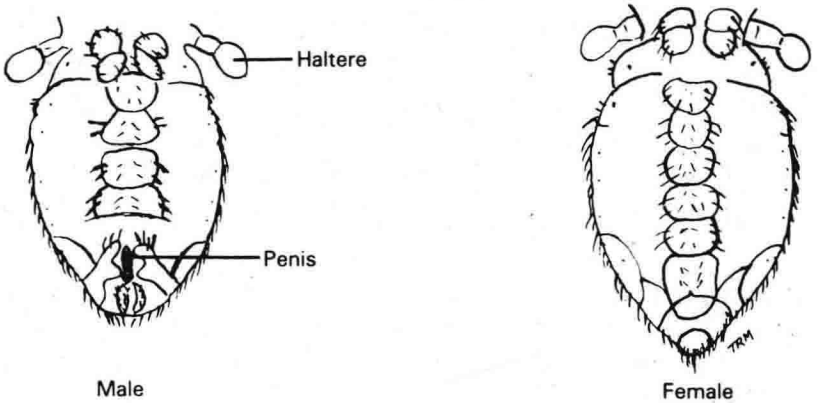

FIGURE 1.3. Ventral view of the abdomens of male and female *Drosophila melanogaster* showing external genitalia.

1. **Size.** Females are usually somewhat larger than males.
2. **Shape.** The caudal extremity of the male is round and blunt, whereas that of the female is sharp and protruding. The abdomen of the male is relatively narrow and cylindrical, whereas that of the female is distended and appears spherical or ovate. Adults newly emerged from the pupa case are relatively long and slender, and the sexual differences described here are not so readily noted.
3. **Color.** Black pigment is more extensive on the caudal extremity of the male than on that of the female. On the male, the markings extend completely around the abdomen and meet on the ventral side. On the female, the pigment occurs only in the dorsal region.
4. **Sex combs.** Only males have a small tuft of black bristles called a sex comb on the anterior margin at the basal tarsal joint of each front leg. Magnification is necessary to see the sex combs.

V. EXPERIMENTAL MATINGS

The methods outlined here are essentially those used in actual research problems with *Drosophila*. Pedigreed stocks carrying mutant genes are maintained in the laboratory. Flies in these cultures tend to breed true (i.e., produce offspring identical in appearance to the parents) as long as they are mated among themselves. Rarely, however, new hereditary variations—spontaneous mutations—do occur.

For classroom purposes, flies having easily recognizable differences are used. Names and symbols used to identify the mutant genes carried by the flies are those devised by research workers. Stocks are conveniently designated by symbols indicating the particular mutant gene or genes carried. Flies that exhibit traits that may be considered standard or normal are designated as wild type. The plus symbol (+) indicates wild type with reference to any gene. The lowercase letter indicates that the mutant gene is recessive to the wild-type allele. A capital letter designates a dominant allele. The symbol *e*, for example, represents the recessive mutant allele for ebony body color and e^+ (also, $+^e$ or E) the dominant allele for wild type, gray body; B symbolizes the dominant mutant allele for bar eye and B^+ ($+^B$ or b) the recessive allele for wild-type eye. Homozygous ebony flies are symbolized *ee* and homozygous wild-type flies, e^+e^+.

A. Becoming Familiar with Fly Characteristics

The first part of this investigation will provide an opportunity to become acquainted with the morphological characteristics of wild and mutant flies. Complete Table 1.1, recording with appropriate symbols, sketches, or descriptions the differences you see between mutant and wild-type flies. First examine wild-type flies that are considered normal for all traits listed. Place a plus sign in the appropriate space to signify the normal condition. Carefully examine flies from three or more mutant stocks prepared as unknowns in the laboratory and compare them with the wild type. If the unknown flies

TABLE 1.1. Comparison of Wild-Type and Mutant *Drosophila*

Trait	Wild Type	Unknown 1	Unknown 2	Unknown 3	Unknown 4
Body color					
Eye color					
Eye shape					
Wing shape and size					
Antenna shape and size					

are wild type for a given trait, place a plus sign in the appropriate square. If they are different from wild type, diagram the mutant trait or use one or two key words to describe it briefly.

B. Securing Virgin Females

To begin a cross between two varieties of flies, you must secure a virgin female. Once inseminated, females retain viable sperm for several days. Thus, the only way to ensure a controlled mating between different genetic stocks is to use virgin females.

The most common method of obtaining virgin females is to select those that have recently emerged from their pupa cases. These flies (*D. melanogaster*) do not mate for at least 8 hours (probably not for 10 hours) after emerging from the pupa case. Thus, 8-hour-old females will still be virgins even if male flies are present. To secure virgin females, follow this procedure.

1. Empty the appropriate stock bottle of all adult flies. Record the time on the bottle.
2. Within 8 hours of removing the adult flies, etherize any newly emerged adults. Such newly emerged flies are distinguished by their pale body color and a characteristic dark spot on the ventral side of the abdomen, slightly to the left of the midline.
3. Females among those newly emerged adults will be virgins that can be used in the appropriate cross.

Another method of obtaining virgin females is to isolate pupae from which the adult flies are about to emerge. Single pupa cases should be placed in small vials containing a narrow strip of moistened paper towel. The vial should be stoppered with cotton. Of necessity, any female hatching alone in a vial will be a virgin. This method of obtaining virgin females is laborious and can prove unwieldly because it requires many vials.

C. Making Crosses

When making a cross between two varieties, consider only those characters in which the parent flies differ. For example, in crossing flies having ebony body color with those having white eyes, only body color and eye color need to be observed carefully.

Secure etherized male flies of one variety and etherized virgin females of the other. While holding the culture bottle on its side, place these flies in the bottle. Be sure to add some dry yeast granules or yeast suspension to the medium before introducing the flies. Keep the bottle on its side until the flies have recovered from etherization. This position will prevent their becoming stuck in the medium. Label the bottle and record data in Table 1.2. After 7 or 8 days remove the parent flies (P_1) to prevent their being confused with or mated with their offspring. Flies of the first filial generation (F_1) will soon begin to emerge. After several F_1 flies have appeared, etherize and examine them, especially with regard to the characters by which the P_1 flies differed. Record in Table 1.2 the phenotypes of the F_1 flies of each sex; place these F_1 flies in a fresh bottle of medium. (Be sure to label the bottle!) This mating will allow for the production of a second filial generation (F_2). It is not necessary that the F_1 female flies be virgins for this mating.

1. Why? _____
   The instructor may wish to have you test cross an F_1 female fly. In this case you should use a virgin female.

2. Why is a virgin female needed in this case? _____

   _____

3. What is a test cross? _____

   _____

TABLE 1.2. Record of a *Drosophila* Experiment

Record both the phenotype and stock number of the parent flies.

Experiment number _____ **Name** _____

1. Cross _____ female × _____ male
2. Date P_1s mated: _____
3. Date P_1s removed: _____
4. Date F_1s first appeared: _____
5. Phenotype of F_1 males: _____
6. Phenotype of F_1 females: _____
7. Date F_1 male and female placed in fresh bottle (or date F_1 virgin female test crossed): _____
8. Date F_1 flies removed: _____
9. Date F_2 (or test cross) progeny appeared: _____
10. Record F_2 or test-cross data in the following table: _____

	Males		Females		
	Phenotype	Number	Phenotype	Number	Total
a.					
b.					
c.					
d.					
e.					
f.					
g.					
h.					
	Totals				

D. Laboratory Records

Much of Mendel's success in elucidating the basic principles of inheritance depended on his keeping accurate records about his experiments. Keeping accurate and detailed notes is also necessary to the successful completion of your laboratory work. To this end, you are provided with Table 1.2 in which to keep records as you do the various steps of this investigation.

E. Suggested Crosses

A number of crosses might be used to demonstrate Mendel's first law, the **law of segregation**. Examples follow.

P_1 Female	×	P_1 Male
1. sepia eye color	×	wild type (red)
2. dumpy wings	×	wild type (long)
3. vestigial wings	×	wild type (long)
4. ebony body color	×	wild type (gray-brown)
5. aristapedia antennae	×	wild type antennae
6. _____	×	_____

Your instructor may use one of these examples or perhaps the reciprocal crosses of those suggested. For example, the reciprocal of the first mating would consist of mating a wild-type female with a male having sepia eyes. An interesting and instructive experiment would be one comparing the results of such reciprocal matings in both F_1 and F_2 generations.

The first mating might have been prepared for you 5 or 6 days before today's laboratory period. If so, the bottles you receive will contain P_1 flies and F_1 larvae. In any case, on the basis of the cross that you make (or the one made for you), give the following information.

	Female	Male
Genotype of P_1	_____	_____
Genotype of F_1	_____	_____
Phenotype of F_1	_____	_____
Genotypes of F_2	_____	_____

1. What F_2 phenotypic ratio do you expect to obtain? _____
2. What F_2 genotypic ratio is expected? _____
3. Which trait is dominant in your experiment? _____
4. How do you know? _____

F. Maize Genetics

The extensive genetic variability in maize also obeys Mendelian principles; both seedling and aleurone (endosperm) characters lend themselves to classroom use. Figure 1.4 shows a flat of F_2 seedlings segregating for a recessive mutant allele for albinism (absence of chlorophyll). The classical Mendelian ratio of 3:1 is expected. Similarly, Figure 1.5 shows an ear of F_2 kernels of corn that are segregating in a ratio of 3 colored : 1 colorless aleurone.

Because maize has a relatively long life cycle (3 months or more to complete), you probably will not be able to conduct actual experimental matings with it. Your instructor might provide you with ears of corn or flats of F_2 seedlings and request that you determine the number of individuals having

FIGURE 1.4. Flat of F_2 corn seedlings segregating for a recessive albino mutation. The classical Mendelian ratio of 3:1 is expected.

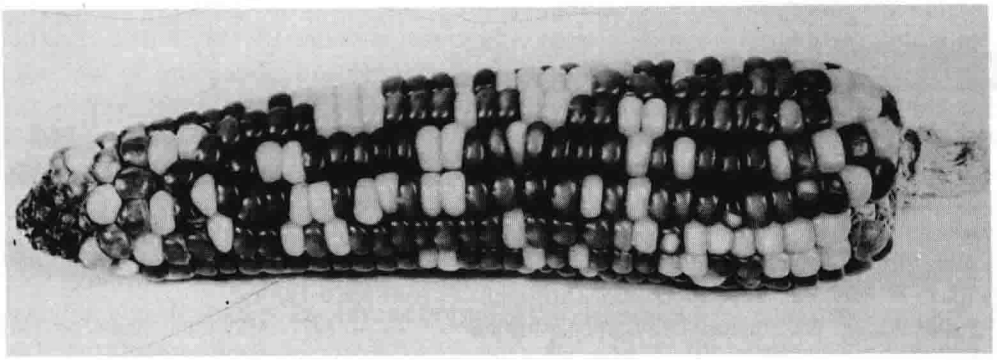

FIGURE 1.5. Ear of F_2 corn kernels segregating for colored versus colorless aleurone. A 3:1 ratio is expected.

the various phenotypes and then to interpret the data in terms of Mendelian principles. For example, suppose you counted 40 green and 12 albino seedlings in an F_2 population. How would you interpret these data?

Ears bearing F_2 kernels can be purchased from a number of biological supply companies. Without removing the kernels from the ears, you can count the number of kernels in the different phenotypes, record data in Table 1.3, and then formulate hypotheses to explain the data collected. Two possible illustrations of endosperm traits follow.