GENETICS

LABORATORY EXERCISES

ELDON J. GARDNER

GENETICS

LABORATORY EXERCISES

by

ELDON J. GARDNER

Department of Zoology

Utah State University

Logan, Utah

Burgess Publishing Company

426 South Sixth Street • Minneapolis 15, Minnesota

Fourth Edition 1964

Printed in the United States of America

Introduction

Terms and materials that are in common use by geneticists often are misunderstood by people who have not made a special study of the subject. Even students who have read textbooks and listened to lectures concerning objects familiar to geneticists frequently have uncertain or erroneous impressions of the appearance and significance of these objects. Expressions such as the following are commonly made by students taking lecture courses in genetics: "We have been talking about chromosomes all through the course. I'd like to see a chromosome." Even objects as familiar as "Mendel's peas" are often not clearly visualized by the student until he actually handles and classifies them and observes a Mendelian ratio. Students therefore, should not consider laboratory exercises an additional burden, but rather a valuable opportunity for amplifying and clarifying the principles of genetics.

Laboratory work in a beginning genetics course is planned to parallel or supplement a lecture presentation. Its major objective is to give the student first hand acquaintance with the relevant materials, methods, and terminology through experiments, problems, and demonstrations.

One reason for Mendel's great success in discovering basic principles of heredity was his inspired use of the scientific method. He was objective and precise in designing his experiments, observing and analyzing the data, and evaluating the significance of the results. On the other hand, the lack of success of some students in genetics, both before and since Mendel, has been attributable to a failure to apply this method properly to their observations and interpretations.

Modern genetics is increasingly dependent upon the use of mathematics for simplifying and interpreting data obtained from experiments. Therefore, provision is made for practice in the use of some of the more simple mathematical tools.

This laboratory manual has been designed on the assumption that the instructor will be in the laboratory during the entire period. Students should feel free to call on him for assistance at any time. They should be independent, however, in following through each exercise and ask for help only after they have conscientiously attempted to solve a problem. The learning experience rather than the final answer is the important objective. Students are advised to read carefully the exercise of the day, before coming to the laboratory. This procedure will enable them to understand the objective and method involved, and to proceed immediately with their work. Advance preparation will not only save the time of the student but will make the learning experience more meaningful.

This manual is an outgrowth of a series of laboratory exercises prepared by F. E. Stephens, W. W. Newby, Dorothea Mulaik, and others at the University of Utah. Over the years, most exercises have been modified considerably with the help of graduate assistants and students at Utah State University. John R. Simmons, Lois Cox, Gary M. Booth, and Larry L. Cox have assisted in checking the manuscript for the Fourth Edition. I am indebted to Professor Sir Ronald A. Fisher, Cambridge, and to Messrs. Oliver and Boyd Ltd. Edinburgh, for permission to abridge Tables No. 3 and 4 from their book, Statistical Methods for Research Workers.

Table of Contents

Exercise 1

MONOHYBRID CROSS AND BACKCROSS

This exercise demonstrates an experiment in cross breeding of living organisms under controlled laboratory conditions.

MATERIALS

The materials and equipment for this exercise are illustrated in Fig. 1 as follows:

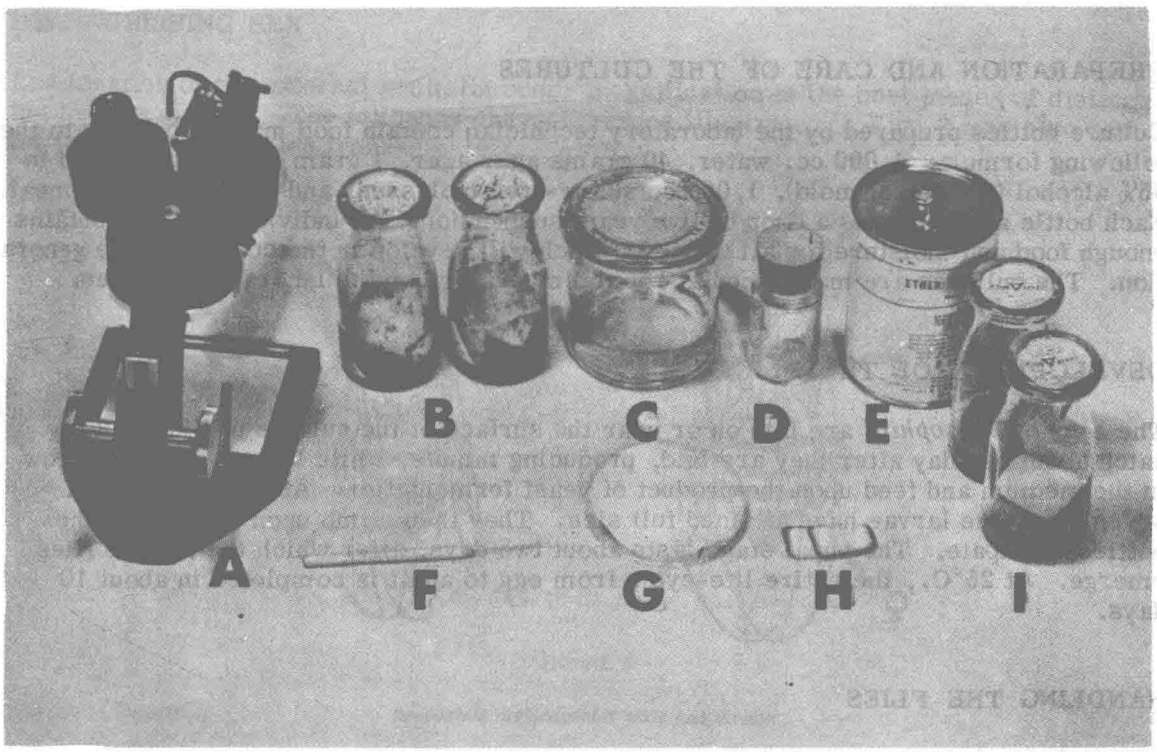


FIGURE 1

A. Dissecting microscope or magnifying glass. B. *Drosophila* wild and mutant stocks (ebony *(e)* is good). C. Morgue. D. Etherizing bottle with tight cork to which an absorbent pad has been attached. E. Ether. F. Teasing needle. G. Etherizing plate (a Petri dish cover with gauze or cotton pad inside). H. Gummed labels. I. Culture bottles prepared with food and moisture for one generation of flies.

Students should provide themselves with writing material for preparing the reports and a manila folder to be used for submitting reports.

METHODS OF CONDUCTING EXPERIMENTS

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 as long as they are mated among themselves. Very rarely, however, new heredity variations, i.e., spontaneous mutations, 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 which may be considered standard or normal are designated as wild. The plus symbol (+) indicates wild type with reference to any gene. A lower case 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^+ ($+^e$ *or E*) the dominant gene for wild type, gray body. *B* symbolizes the dominant allele for bar eye and B^+ ($+^B$ *or b*) the recessive gene for wild-type eye. Homozygous ebony flies are symbolized *e e* and homozygous wild type, e^+e^+.

PREPARATION AND CARE OF THE CULTURES

Culture bottles prepared by the laboratory technician contain food made according to the following formula: 4,000 cc. water, 40 grams agar agar, 1 gram moldex dissolved in 95% alcohol (to control mold), 1,000 cc sulfur-free molasses, and 1,000 cc. corn meal. Each bottle also contains a drop of live yeast suspension. An individual bottle contains enough food and moisture for all the flies which will develop in the culture in one generation. The cultures are maintained at room temperature on the laboratory shelves.

DEVELOPMENT OF THE FLIES

The eggs of *Drosophila* are laid on or near the surface of the culture medium. They hatch about one day after they are laid, producing minute, white larvae, which burrow in the medium and feed upon the product of yeast fermentation. At the end of about seven days, the larvae have attained full size. They then climb upon the side of the bottle and pupate. The pupal stage lasts about two days, after which the mature flies emerge. At 25°C., the entire life-cycle from egg to adult is completed in about 10 days.

HANDLING THE FLIES

Etherizing and examining adult flies:

1. Place a few drops of ether on the pad of the etherizer.
2. Jar the base of the culture bottle 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 and shake flies into etherizer.

4. Subject the flies to ether for about 30 seconds after they cease moving. If they are to be used in further matings, it is necessary to avoid overetherization. The flies will be killed if left in the etherizer too long.

5. Transfer etherized flies to a clean, white card or formica plate.

6. Examine with the dissecting microscope. A soft brush or teasing needle may be used for moving the flies about on the stage of the microscope.

7. If the flies wake up before the examination is completed, add a few drops of ether to the pad on the etherizing plate and cover the flies on the microscope stage for a few seconds.

8. Ordinarily flies are discarded in the morgue immediately following the observation. If etherized flies are to be used for further matings they should be permitted to recover in a dry vial or on a dry surface in the culture bottle before they come in contact with the moist food.

DISTINGUISHING SEX

Examination of the external genitalia under magnification is the best means of distinguishing the sex of flies. The following characteristics illustrated in Fig. 2 may also be helpful to distinguish males from females:

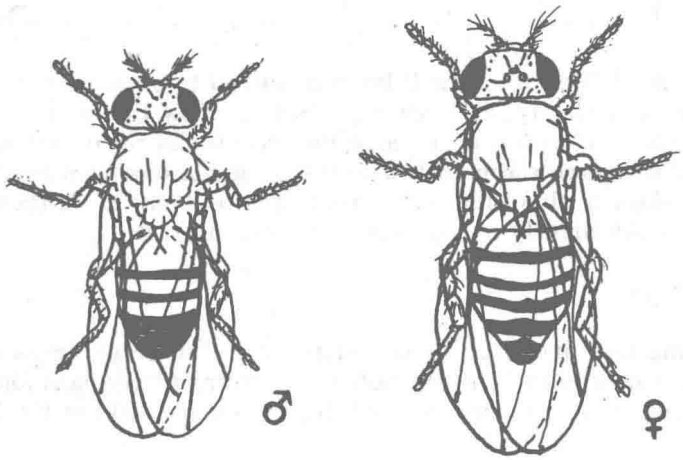


FIGURE 2

Drosophila melanogaster male and female

1. Size: Females are usually 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.

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: The male has a small tuft of black bristles called a sex comb on the anterior margin at the basal tarsal joint of each front leg, whereas the female has no such structure. Magnification is necessary to see the sex combs.

A. The first part of this exercise will provide an opportunity to become acquainted with the morphological characteristics of wild and mutant flies. Prepare a chart similar to the one below and record with appropriate symbols, sketches, or descriptions the differences you see between mutant and wild flies.

Trait	wild type	unknown 1	unknown 2	unknown 3	unknown 4
body color					
eye color					
eye shape					
wing shape					

First examine wild flies which will be considered to be normal for all traits listed. Place a plus sign in the appropriate square to signify the normal condition. Carefully examine flies from three or more mutant stocks prepared as unknowns in the laboratory and compare them with wild type. If the unknown flies are wild type for a given trait, place a plus sign in the appropriate square. If they are different from wild type, describe briefly or diagram the mutant trait.

B. Monohybrid cross

The first mating was prepared by the instructor five or six days before the class. Parent flies are now in the culture bottle, eggs have been laid and the larvae may be seen crawling in the surface of the food and on the side of the bottle.

1. Etherize the parent flies and examine them carefully. Observe the mutant trait expressed in one of the parents, distinguish between the sexes and verify the information on the label of the bottle. Discard the parent flies when the identifications have been made and place the culture bottle on the laboratory shelf.

2. Diagram the cross by representing with appropriate gene symbols the P (parents), gametes, F_1, F_1 gametes, and F_2.

3. Summarize the expected F_2 results in a summary chart under the headings: Phenotypes, Genotypes, Genotypic Frequency, and Phenotypic Ratio.

C. Diagram a backcross between an F_1 and the mutant parent and summarize the expected results.

ISOLATING VIRGIN FEMALES

Females once fertilized retain sperm for several days. Therefore, only virgin females should be used in breeding experiments involving different stocks. The most common method is to select young females which have recently emerged. They can be distinguished by their pale color and a characteristic dark spot in the ventral part of the abdomen slightly to the left of the midline. Another method of obtaining virgin females is to isolate pupae from which the adult flies are about to emerge, singly in small vials containing a narrow stip of moistened towel paper.

D. 1. During the next laboratory period, etherize all of the F_1 flies and examine them closely. Is the mutant trait which was expressed in the parent also expressed in the F_1? If a virgin female can be found, isolate her for the backcross. If you do not find a virgin female, check again at the end of the period for a newly emerged female. It may be necessary to spend extra time during the morning hours to secure a suitable female for the backcross.

2. Prepare a backcross between a virgin female from the F_1 culture and a male from the mutant stock used for the P cross.

3. Select about three females and three males from the F_1 progeny. Place them together in a new culture bottle to provide the F_2 generation.

4. Separate the F_1 males from the females and count the numbers of each sex. Tabulate the males and females from the F_1 progeny in a tabulation chart under the following headings: Classes (Male or Female), Observed, Calculated, and Deviation.

5. At the appropriate time classify, record and tabulate in chart form the F_2 population.

Hand in at the end of the first period:

A. Completed chart
B. 2 Diagram of cross
B. 3 Summary Chart
C. Diagram and summary
E. Concluding statement indicating how the Mendelian principle of segregation is illustrated by the monohybrid cross.

Hand in later:

D. 4 Tabulation chart.
D. 5 Tabulation chart.

Exercise 2

DIHYBRID CROSS AND BACKCROSS

This exercise will follow one of Mendel's experiments with garden peas and provide an opportunity to prepare a dihybrid cross with *Drosophila*.

MATERIALS

Two envelopes containing (A) pea seeds representing the F_2 (Fig. 3) and (B) backcross results of appropriate crosses; and two stocks of *Drosophila* with mutant genes located on different chromosomes which control easily recognized traits; culture bottles, and equipment for experimental work with *Drosophila*.

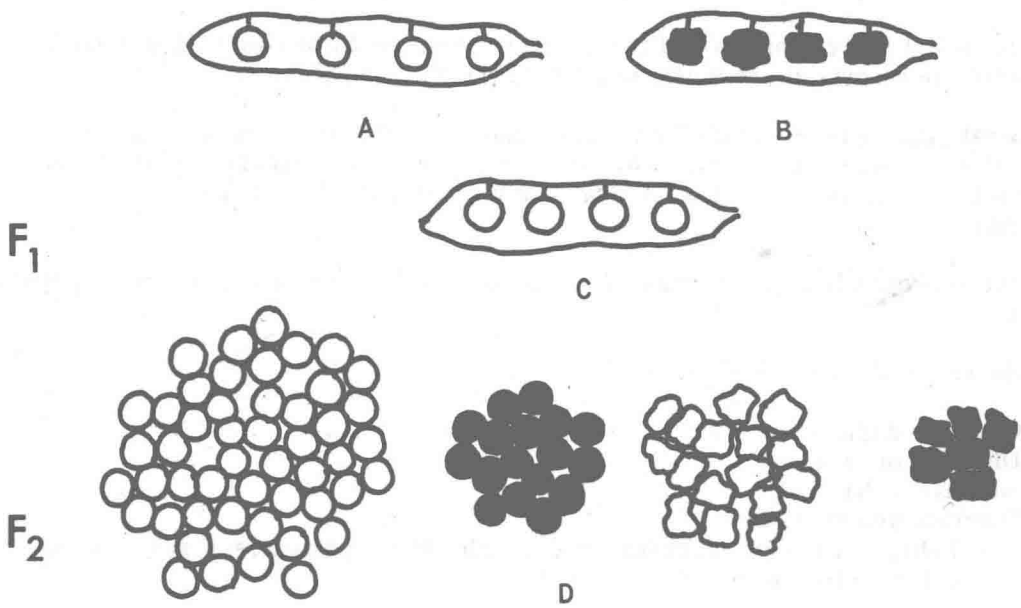


FIGURE 3

Illustration to represent a cross between a pea plant with yellow, round seeds and one with green, wrinkled seeds; A. four yellow, round seeds; B. four green, wrinkled seeds; C. four seeds from F_1 plants which are yellow and round; D. F_2 progeny showing proportion of approximately 9 yellow, round; 3 green, round; 3 yellow, wrinkled; 1 green, wrinkled.

A. Envelope A contains seeds representing an F_2 generation from a cross between a homozygous pea plant producing yellow, round seeds and a plant producing green, wrinkled seeds.

1. Segregate the four different phenotypes (Fig. 3. D). Count and record the number of each kind. Which characteristics are dependent upon dominant genes? Recessive genes?

2. Let *W* and *w* represent the genes for round and wrinkled, *G* and *g* the genes for yellow and green, respectively. Diagram the cross carrying it through the F_2 generation. Use a checkerboard square and summarize the expected F_2 offspring in a chart under the headings: Phenotypes, Genotypes, Genotypic Frequency, and Phenotypic Ratio.

3. Record and analyze the observed data (from counting the peas) in a tabulation chart under the headings: Phenotypes, Observed, Calculated, and Deviation.

B. Envelope B contains seeds representing the offspring from a backcross between an F_1 plant and the parent producing wrinkled seeds with green cotyledons.

1. Segregate the four different phenotypes. Count and record the number of each kind.

2. Diagram the backcross, and summarize the expected results.

3. Record and analyze the observed data in a tabulation chart.

C. Under the direction of the instructor prepare a mating between flies carrying homozygous mutant genes in the second and third chromosomes. F_2 results of such a cross should demonstrate independent assortment.

1. The gene *(vg)* for vestigial-wing is located on the second chromosome and the the gene *(e)* for ebony body is located on the third chromosome. Matings may be made between flies from these two stocks (be sure the females used have not already been fertilized). Reciprocal crosses, i.e., vestigial ♀ *(vg vg; e^+e^+)* X ebony ♂ *(vg^+vg^+; e e)* and ebony ♀ X vestigial ♂, may be made if time and facilities permit. After about eight days remove and discard the parent (P) flies.

2. Predict the results in the F_1 and F_2 generations from the matings prepared.

3. When the F_1 flies appear in the culture bottles, check to see if expectations have been obtained. Mate wild type appearing F_1 females with F_1 males in new culture bottles. After about eight days remove and discard the parent (F_1) flies.

4. When the F_2 adult flies are present in the culture bottles etherize and classify the flies for the four phenotypic combinations expected in F_2.

5. Record the results in a tabulation chart.

D. Define independent assortment and show how and under what conditions this principle can be demonstrated. Distinguish between segregation and independent assortment.

Exercise 3

GAMETOGENESIS

This exercise is designed to clarify the overall view of gametogenesis as a sequence of two cell divisions during which the chromosomes segregate in one cell division and duplicate themselves only once in the course of the two cell divisions.

MATERIALS

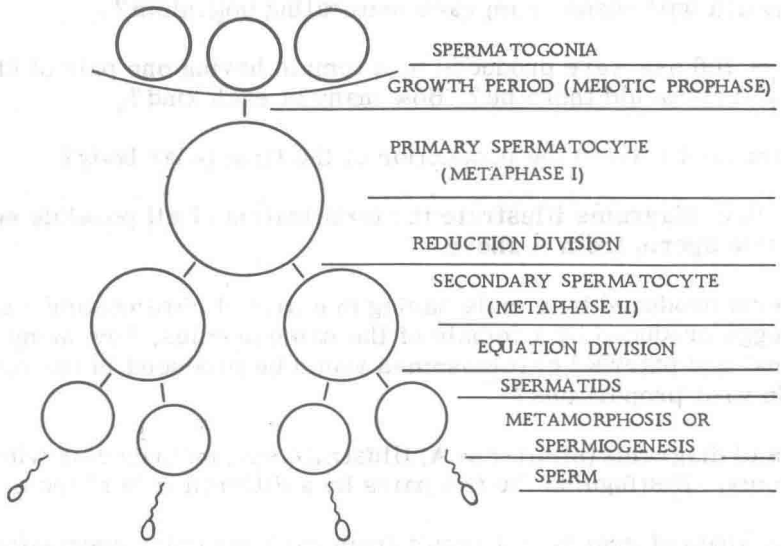
Blocks or pipe cleaners of different colors to represent chromosomes.

INTRODUCTORY STATEMENT

When higher animals reach sexual maturity, the germ cells within the reproductive glands (testes and ovaries) undergo changes which result in the formation of mature germ cells (gametes) which can function in fertilization. The general process of changing is known as gametogenesis. It continues throughout the period of sexual activity in both male and female animals giving rise to spermatozoa and eggs, respectively. The process in the male is called spermatogenesis and in the female oögenesis. In general the process is similar in all animals and plants. The details, however, vary according to sex and species.

Gametogenesis begins in certain germ cells called spermatogonia and oögonia in the male and female, respectively. A growth period of the cell is followed by two successive cell divisions (meiotic), one of which results in the reduction of the chromosome number. In the male the cells resulting from the two divisions must undergo additional changes to become mature, functional gametes. In the female only one of the four possible cells (resulting from the two divisions) becomes a functional gamete and the others (polar bodies) are resorbed by the surrounding cells. The reduction of the chromosomes provides for genetic variation and prevents a doubling in chromosome number which would otherwise occur with each fertilization. When reduced male and female germ cells combine at fertilization, the diploid number of chromosomes characteristic of the species is restored. Each maternal chromosome pairs with a particular (homologous) paternal chromosome. The position taken by each member of a chromosome pair on the equatorial plate of the spindle in the reduction division is a chance phenomenon. Each chromosome pair is ordinarily independent of other chromosome pairs in the cell. Therefore, the segregation which follows for members of pairs, as well as the assortment for different chromosomes, are random processes. At the time of fertilization, each zygote receives one member of each pair or one genome for each parent; those from the female parent are called maternal chromosomes; those from the male parent, paternal chromosomes.

A. Prepare a series of diagrams representing the cell phases of spermatogenesis (similar to the following) and show the sequence of changes for one pair of chromosomes. Make one member of the pair solid (black) and the other hollow (white). Diagram the spindles in the circles representing metaphase stages.



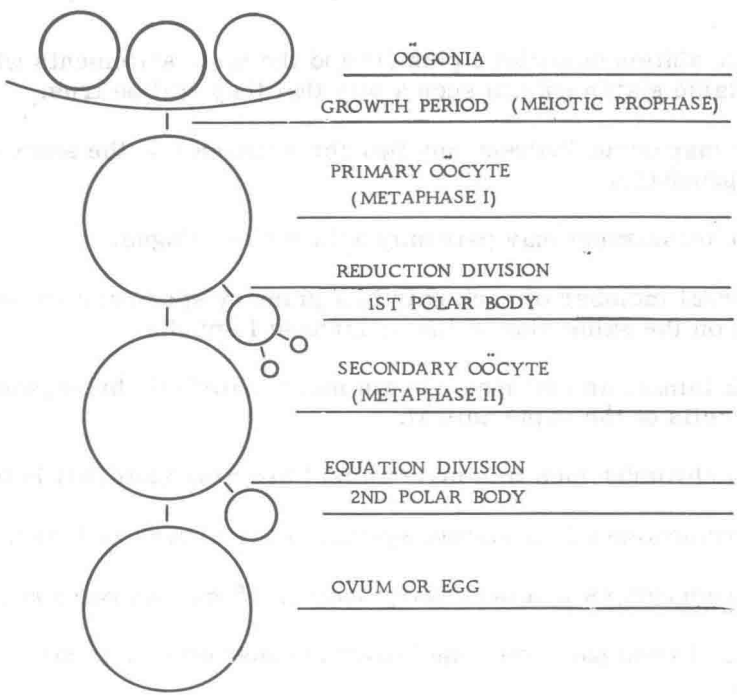
B. With blocks or pipe cleaners to represent chromosomes, demonstrate the chromosome arrangements in the two divisions.

1. How many sperm will result from each spermatogonium?

2. How many kinds of sperm will result from each maturating spermatogonium represented in the above diagram?

3. If a total of 200 sperm were produced by an animal having one pair of chromosomes, how many of each kind would there be?

C. Prepare a series of diagrams representing the cell phases of oögenesis (similar to the following) and fill in the division spindles and chromosome arrangements.



1. How many ova will result from each maturating oögonium?

2. If a total of 100 ova were produced by a female having one pair of chromosomes how many kinds would there be? How many of each kind?

3. What is accomplished in the production of the first polar body?

D. Using appropriate diagrams illustrate the fertilization of all possible eggs from C with all possible sperm from A above.

1. If 100 sperm produced by a male having one pair of chromosomes should fertilize 100 eggs produced by a female of the same species, how many combinations of maternal and paternal chromosomes would be produced in the resulting zygotes? In what proportions?

E. With a series of diagrams (similar to A) illustrate spermatogenesis with two pairs of chromosomes. Distinguish the two pairs by a difference in shape or size.

1. How many kinds of sperm will result from each maturing spermatogonium? How many of each kind?

2. If 400 sperm were produced how many kinds would there be? How many of each kind?

F. With appropriate diagrams (similar to C) fill in the details, including the spindle in each metaphase stage, to show the distribution of two pairs of chromosomes in oögenesis.

1. A female animal having two pairs of chromosomes produces how many kinds of eggs?

2. If 400 eggs were produced how many kinds would there be? How many of each kind?

G. Mark the true statements with a plus (+) and the false statements with a zero (0). Reword the false statements in such a way that they will be true.

1. Synapsis may occur between any two chromosomes in the same cell during the meiotic prophase.

2. A given chromosome may pair only with its homologue.

3. The paternal member of each pair in a primary spermatocyte will always take its place on the same side of the metaphase I spindle.

4. Eggs of a female animal may contain more maternal chromosomes than the somatic cells of the same animal.

5. Maternal chromosomes in a male animal are also maternal in his children.

6. Of 12 chromosomes in a mature sperm, 6 are always maternal.

7. Of 20 chromosomes in a secondary oöcyte, 15 may be paternal.

8. Members of gene pairs on homologous chromosomes lie opposite one another in synapsis.

9. Various combinations of maternal and paternal chromosomes are found in gametes.

10. Mendelian segregation occurs in the meiotic sequence.

H. Concluding statement: Identify in the gametogenesis sequence, a stage or stages in which the chance reflected in the genetic mechanism of segregation and independent combinations occurs.

Exercise 4

MITOSIS AND MEIOSIS*

MATERIALS

Compound microscope and microscope lamp; prepared slides of onion root tip showing mitosis; squash bug, grasshopper, or other material showing spermatogenesis: *Ascaris* showing oögenesis and cleavage; lily showing gamete formation and development; books and charts illustrating mitosis and meiosis.

A. Mitosis in Onion Root *(Allium cepa)*

Mitosis is the process of cell duplication through which growth is accomplished in animals and plants. From onion root tip preparations locate, study, sketch, and label the following stages:

1. Interphase. The metabolic stage sometimes called "resting stage" between divisions. Observe:

   a. Shape, size, and general appearance of the nucleus

   b. Nuclear membrane

   c. Number, size, and location of the nucleoli

2. Prophase. During this period scattered thread-like structures appear and develop into discrete chomosomes. Observe:

   a. Chromatin units which appear first as irregular threads in the early prophase. Some can be seen to be double. Each longitudinal duplicate is a chromatid.

   b. Results of further thickening and shortening of the chromatin elements. Look for the spindle, nuclear membrane, and nucleolar elements.

   c. Arrangements of the chromosomes on the equatorial plate in late prophase.

3. Metaphase. In this stage the spindle is completed with the equatorial plate in the center. Observe in several cells:

   a. Spindle fibers.

   b. Position of the chromosomes in the spindle.

   c. Size and shape of the chromosomes (each with two chromatids).

* This exercise is too long for one laboratory period. Selected parts of the exercise may be used or, if time permits, this exercise may cover two or more laboratory periods. If projection facilities are available, appropriate microscope slides or lantern slides may be projected on a screen to preview and review the microscope studies.