1963R/M656
2945

Name: John C. Mills Date of Degree: May 26, 1963

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: LABORATORY EXERCISES IN GENETICS FOR THE HIGH SCHOOL BIOLOGY CLASS

Pages in Study: 28 Candidate for Degree of Master of Science

Major Field: Natural Science

Scope of Study: The laboratory has been regarded as the heart of science by many teachers, yet the present day instruction in high school biology gives little time to the practical application and the learning experiences found in student laboratory periods. Opportunities to study organisms in the laboratory and in nature are most important, if students are to understand basic biological concepts. This paper presents a series of laboratory exercises and demonstrations selected from a group of genetics laboratory manuals, methods books, and source material pertinent to the study. Basic principles of Mendelian genetics are illustrated or offered as problems in the student exercises. Some of the specific areas covered are: the physical basis of heredity; the cell, the chromosome, the gene, the maintenance and variations of genetic make-up as related to mitosis and meiosis; the law of unit characters, the law of dominance, the law of segregation, and the law of independent assortment.

Findings and Conclusions: Many experiments, other than the ones presented in this study, could be used to enrich the study of high school genetics. The exercises used in this study were selected due to their conciseness and clarity. Some students will be limited by the scope of the proposed exercises, whereas other students will need to exert a maximum amount of effort to understand the material.

ADVISER'S APPROVAL

LABORATORY EXERCISES IN GENETICS FOR THE HIGH SCHOOL BIOLOGY CLASS

By

JOHN CORBIN MILLS

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1962

Submitted to the Faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1963

LABORATORY EXERCISES IN GENETICS FOR

THE HIGH SCHOOL BIOLOGY CLASS

REPORT APPROVED:

Report Adviser

Dean of Graduate School

ACKNOWLEDGEMENT

Indebtedness and gratitude are acknowledged to Dr. H. L. Bruneau for his advise, counseling, and assistance in securing reference materials.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

The laboratory has been regarded as the heart of science by many teachers, yet the present day instruction in high school biology gives little time to the practical application and the learning experiences found in student laboratory periods. Opportunities to study organisms in the laboratory and in nature are most important, if students are to understand basic biological concepts. Laboratory experiences can certainly stimulate greater interest in the life sciences and encourage students to learn biology.

Why is there a lack of laboratory studies in the high school biology courses taught today? Some may blame school systems, textbooks, individual teachers, or the shortage of teacher preparation time, but the author of this paper would place as much blame on the subject taught by the biology teacher. The making of good laboratory studies throughout the course requires an adequate background of training. Needless to say, biology is a very large field covering a scope far too wide for any one person to know many areas in detail. It is logical to assume most teachers have "special areas" of interest and training in which they use practical laboratory exercises for effective teaching. Other areas are usually covered by the factual text material with little or no laboratory enrichment. Thus, the goal of all high school biology teachers should be to improve those weak areas of background training or areas of noninterest, and not let them be expressed as a part of ineffective teaching.

Traditionally individual laboratory work has been used to illustrate and verify scientific principles and concepts. "To the extent that the laboratory work has been correlated with classroom activities, the laboratory has served these purposes."[1] Laboratory work is actually limited by serving the above purposes, and a much broader function could be utilized by accepting the point of view that one learns to solve problems by solving problems.

Students must be shown through the use of practical laboratory exercises that biology is a living science full of interesting and intriguing questions, not just a series of cut and dried school exercises. "Whenever possible, learning in biology should involve an active quest by students that makes them, for the moment at least, scientists in search of discovery."[2] It is true that some exercises must be concerned with observation, dissection, and the transmission of basic factual material, but many are constructed to present problems to the students, to give an opportunity to collect relevant data, and to lead students to relate the data to their own knowledge so they can formulate hypotheses for testing.

This paper will present a series of laboratory exercises and demonstrations for the study of genetics. Using both plant and animal material, genetic concepts are illustrated or offered as problems to the students. The interrelationship between heredity and environment is first presented, followed by studies concerned with the physical basis of heredity, the cell,

---

[1] John S. Richardson, Science Teaching in Secondary Schools (New Jersey, 1957), p. 70.

[2] Chester A. Lawson and Richard E. Paulson, Laboratory and Field Studies in Biology (New York, 1960), p. xii.

the chromosome, and the gene. The basis for maintenance and variations of genetic make-up are revealed in the studies of mitosis and meiosis. Mendel's law of unit characters, law of dominance, law of segregation, and law of independent assortment are used throughout six different exercises. Sex-linked characteristics and lethal genes make up important studies completing the series of exercises.

CHAPTER II

EXERCISES AND DEMONSTRATIONS

HEREDITY AND THE INFLUENCE OF ENVIRONMENT

One has probably heard the old argument about heredity and evnironment. The argument usually starts when someone asks, Which is more important, heredity or environment? This is a meaningless question because both are essential. Potentialities are inherited. What happens to these potentialities depends upon the environment to which the organism is exposed. There is a constant interplay between heredity and environment.

It is possible and often necessary to discover the extent to which the observed differences between the appearance of different individuals are due to heredity and the extent to which they are environmentally induced. The more reasonable question would be, How does environment modify the expression of our hereditary characteristics? One answer to this question may be found by modifying the environment of tobacco plants.

MATERIALS: Tobacco seed - heterozygous for the trait of albinism; (if tobacco seed is not available, corn or sorghum may be used); Petri dishes; light covers for germinating seed; paper towels.

PROCEDURE: The seeds are planted by sprinkling a liberal quantity over the moistened paper in four Petri dishes. Each seed may be as close as 1/8 inch to another. The covers are replaced and the dishes are put in a well-lighted place in the room, but not in the direct sunlight. Two of the Petri dishes are covered with a cigar box or other lightproof covering, and two are left exposed to the light. The uncovered seeds are the control group; the covered seeds are the experimental group. From the time of "planting" until almost all the seeds have germinated, daily observations are made describing the seedlings in both the uncovered and covered dishes. A daily record is kept for 4 or 5 days showing the number and kinds (green or albino) of seedlings in each group. When about half of the seeds have germinated, the cigar box cover is removed and all the seedlings are allowed to continue development in the light. Daily observations and a record of counts are continued.

APPLICATION OF RESULTS: After the students have collected all the necessary data, they are presented with a series of questions to see what conclusions can be drawn. Several conclusions that can be drawn from the data on the uncovered group are: (1) the hereditary control of plant color; (2) the approximate three-to-one ratio; (3) the closer approach to a three-to-one ratio as the number of individuals counted increases. Other conclusions can be drawn from the data on the covered group: (1) the environment can mimic the effect of a gene; (2) the expression of a gene-controlled trait depends on the proper environmental conditions; (3) considering the covered and uncovered dishes together, the effects of heredity and environment are shown to be interrelated.

HEREDITARY DIFFERENCES

This exercise helps students discover hereditary differences. Environmental factors can eliminate some of the hereditary differences between dwarf and tall plants, and students should also recognize this fact.

MATERIALS: Gibberellic acid - available from several drug companies and biological supply companies; pipettes - one for each concentration of gibberellic acid used; dwarf-strain and tall-strain peas; sterilized soil and containers for growing the plants.

PROCEDURE: Two containers are filled with sterilized soil. Five dwarf-strain peas are planted in one container and five tall-strain peas are planted in the other container. The containers are placed near a window and the soil is kept moist. After five to ten days it should be evident, by observing the plants, which are tall and which are dwarf. If possible, a relationship between the size of the pea seed and size of the plant should be noted.

Recent experimental work shows that plant growth is affected by a chemical called gibberellic acid. The effect of this chemical on the height of dwarf and tall pea plants can be noted by treating the plants just raised. This treatment may also help one to understand why some pea plants are dwarf while others are tall.

When most of the dwarf plants stand about two inches above the surface of the soil, a small drop of solution of gibberellic acid is placed on the growing stem tip of the tall plants in one container and on the stem tip of the short plants in another container. An equal number of dwarf and tall plants are left untreated. The treated and untreated plants are marked and a record of each plant is kept. The amount of plant growth-stimulation depends on the amount of gibberellic acid with which a plant is treated. Different groups of students will be given different concentrations of gibberellic acid in order to determine the relative effects. The growth of the plants is observed day-by-day following the treatment with gibberellic acid and a comparison made between the treated and the untreated plants. The average height of each group of plants, treated and untreated dwarfs, and treated and untreated talls, is computed. Further measurement of the lowest internode that is fully visible above the surface of the ground is made. The average length of this internode for each of the four groups is then computed and recorded. The same is done for the next higher internode. A graph of the average overall height of treated dwarf and tall plants for each of the concentrations of gibberellic acid is next constructed.

APPLICATION OF RESULTS: Students can discover that some of the hereditary differences between dwarf and tall plants can be eliminated by an environmental factor. Gibberellic acid can convert dwarf plants into tall ones, although their genetic constitution remains the same. The most obvious difference between dwarf and tall plants is the over-all height of the plant due to a difference in the length of the internodes. Gibberellic acid causes a lengthening of the internodes.

Using the collected data, the students may be asked to make the following observations about plant growth and hereditary versus environmental influences: (1) What is the ratio of the average height of treated to untreated dwarfs? Of treated to untreated talls? (2) Are any treated dwarf plants taller than untreated tall plants? (3) Compare the number of internodes in untreated dwarf and tall plants. (4) How does the number of internodes vary with gibberellic acid treatment? (5) Compare the relative effects of various concentrations of gibberellic acid treatment. (6) Of the two factors, environment and heredity, which is responsible for the normal differences between tall and dwarf pea plants? Which factor is responsible for the differences between treated and untreated plants?

THE CELL AND ITS CHROMOSOMES

The cell has been identified as the unit of structure of an organism, and also the unit of function since it is the seat of all activities of the organism. Characteristics of an organism are expressed through its cells, therefore the cell could be called, with certain qualifications, the unit of heredity. The following exercise is used to show the relationship between actively dividing plant cells and their important chromosomes. This study will also provide the student with some experience in microtechnique and staining procedures.

MATERIALS: Onions (substitute with *Tradescantia* roots, root tips of corn or rye; anthers from very young buds can be used); single-edge razor blades; watch glasses; three slides per student; cover glasses; dissecting needles; aceto-carmine stain; compound microscope.

PROCEDURE: Actively growing material is most essential for this exercise. Onion root tips make an easy source. To grow these tips, a fresh firm onion is selected and old roots are cut off close to the base of the bulb. The onion is suspended over a small vessel of water by sticking three toothpicks in the onion so they support it at the surface of the water. The onion is then set away from direct light for two or three days until new white root tips appear and grow to a length of one-half to one inch. The lower, denser half of the root tip is sliced off into a small Pyrex watch glass containing aceto-carmine stain, and these tips are heated in the stain for three to five minutes over a moderate flame. One root tip is placed on a slide with a drop of fresh stain. After cutting off the more deeply stained portion of the root (about one-sixteenth of an inch), the remaining portion is then minced with a razor blade on the slide until the largest pieces are smaller than half of a pin head. The minced root tissue is covered with a cover glass and pressure is applied to the cover glass so as to mash the material under it to form a thin layer. Care is taken to prevent slippage of the cover slip.

The student should first examine the preparation with the low power of the microscope in order to find the chromosomes or mitotic figures, and with high power to study individual cells more closely. The slides can also be used to study different stages of cell division.

GIANT CHROMOSOMES

This exercise has two main objectives: first to show the student the salivary chromosome preparation and second, to stimulate him to derive from his observations some thoughts about the role of chromosomes as vehicles of inheritance.

MATERIALS: 100 fly larvae - Drosophila, housefly, or blowfly; 2 sharp dissecting needles; 1 small scalpel; slides and cover glasses; aceto-orcein stain; insect Ringer's solution; compound microscope.

PROCEDURE: Large flies such as blowflies can easily be used. These are collected by leaving meat outdoors for a few days in fall or spring and collecting the larvae as they near the time of pupation. Using the blowfly or housefly larvae, the student can see and dissect salivary glands with the naked eye. The larva is placed on a glass slide and the blunt posterior end and pointed anterior end are specifically noticed. The black mouth parts are seen to move back and forth at the front end, and it is this part of the digestive system to which are attached the salivary glands. The larva is killed by dissecting off the mouth and to obtain the salivary glands. This is done by placing the larva on a slide and wetting it with a drop of Ringer's salt solution. Holding down the anterior end with the point of a dissecting needle, actually piercing the head, while also holding down the posterior end, the larva is thus slowly stretched until the mouth parts tear off. The salivary glands and digestive tube will come out at the same time. The salivary glands are observed under the microscope and can readily be distinguished from fat tissue and the digestive tube. After definite identification of the glands is made, they are dissected away from surrounding tissues. Excess salt solution around the glands is removed and one drop of aceto-orcein stain is then added to cover the glands. After five minutes of staining, a cover glass is dropped on top of the glands and pressed down hard on the stained material. Excess stain is removed. The salivary glands should be squashed thoroughly and some of its cells separated from others. In most of the cells students can see the nucleus and in the nuclei the coiled giant chromosomes.

The next step is to get the chromosomes out of the nucleus. This may be accomplished by gently tapping with a blunt instrument on the cover glass. Another observation under the microscope should reveal a nucleus which has broken and thereby released the giant chromosomes. When a chromosome or a piece of a chromosome is found, critical focusing will permit one to see their characteristic banded appearance.

MITOSIS AND MEIOSIS

Having associated heredity with cells and chromosomes, the necessity of mitosis in preserving the hereditary make-up of new cells is now demonstrated. Once an understanding of mitosis is realized, the next step is to provide a learning situation for meiosis. The term chromatid is omitted and chromosome is used throughout the exercise.

MATERIALS: "Poppit" beads - different colors in a strand two to three feet long; white paper for the diagramatic cells.

PROCEDURE: In the particular model used, each cell is assumed to have four chromosomes: two long chromosomes and two short chromosomes. A string of red beads represents one of the long chromosomes and a green string of beads represents the other long chromosome. A short pink string and a short yellow string represents the pair of short chromosomes.

The process of mitosis is first demonstrated as follows: In a cell that is going to divide by mitosis the first significant thing that happens is that each chromosome builds a duplicate of itself. This can be shown by doubling each of the four colored chromosome "beads". After this duplication is completed, the chromosomes line up in the middle of the cell. Each of the duplicated chromosomes separate from each other and members of the pair go to opposite sides of the cell. Finally a cell wall forms in the center, producing two cells that can be easily shown by arranging the eight chromosomes within two diagramatic cells drawn on a sheet of paper. The two cells with four chromosomes each represent daughter cells. It is pointed out that in the process of mitosis the number and kinds of chromosomes in a cell have not been changed.

Meiosis although being somewhat similar, does show important differences. Again starting with the four types of chromosomes, it is shown that the first significant thing that happens is again the duplication of the chromosomes. Instead of the individual chromosomes with their duplicate strands lining up on a plane through the middle of the cell, partnerships are formed. This is shown by placing the two long (red and green) chromosomes, each with its duplicate, together. The same situation is arranged for the two short yellow and pink chromosomes.

Meiosis involves two divisions. First, there is the separation of the partner chromosomes and the beads are arranged in such a way that the original red chromosome and its duplicate separate from the original green chromosome and its duplicate. The yellow and pink chromosomes separate in a similar manner with each of the resulting two cells containing two pairs of chromosomes, one short and one long. Second, there is separation of chromosome pairs with one of the red and one of the yellow chromosomes going to one cell. The other red and yellow chromosomes go into a second cell. The same thing occurs with the green and pink chromosomes. The arrangement can easily be shown with the beads on "paper cells". It is then emphasized that each of the four cells has two chromosomes, one half the number present in the cells from which they developed.

Crossing over can next be shown by illustrating how chromosomes intertwine and sometimes break. An interchange between the long red and green chromosomes may be used as an example with the four characteristic gametes resulting from the meiotic division. This condition is illustrated by breaking the strands and connecting them in any manner desired.

GENES AT WORK

This exercise is used to illustrate four points: (1) Genes are probably molecules located in specific positions in a chromosome. (2) These genes appear to control the chemical activities of cells. (3) These chemical activities sometimes result in clearly visible traits.

(4) If two different genes are present in a cell, the chemical process controlled by one gene may mask or dominate the activities of the other.

MATERIALS: test tubes; 10% HCl; pipe cleaners; brom thymol blue; 1% NaOH; test tube racks; toothpicks.

PROCEDURE: The students are provided with three cells (test tubes containing a liquid) and two different sets of chromosomes (white pipe cleaners). One type of chromosomes is known as A and the other as a. The A-gene chromosome pipe cleaners have been prepared by soaking them in 10% (HCl) acid for one half hour. The a-gene chromosome pipe cleaners were soaked in a weak solution of base (1% NaOH). The pipe cleaners are left in the respective solutions until the last munite, since some may turn brown if they dry out.

The 10% acid is diluted about one hundred times and a few drops of the base is diluted ten times. These solutions must then be used to adjust the color of an indicator, since it is important to supply the students with the indicator in its transition color. Brom thymol blue will be used in this particular exercise as follows: Take about as much brom thymol blue as will stick to a wet toothpick inserted one half its length in the powder. Dissolve this in a pint of hot tap water in a flask sufficiently large so that it is only one third full. Add a drop or two of the dilute alkali to the indicator solution, and it should turn deep blue. If the solution color is too concentrated, it should be diluted until light is transmitted through. Next, ten drops of the dilute acid is added to the indicator solution, and it should then turn bright yellow. While vigorously stirring the contents of the flask, add the dilute alkali one drop at a time and stop when the indicator turns blue-green. This is the end point or transition color. If the reactions go straight from blue to yellow and back, the acid and base solutions are too strong and must be diluted further.

The pipe cleaners are handled with forceps and not allowed to come in contact with anything except a paper towel, forceps, and test tubes. The students are reminded that the body contains two sets of chromosomes and in reproduction each parent contributes one of these sets. One member of each pair comes from the mother and the other member of the pair comes from the father.

Two A crhomosomes are placed in one of the test tube "cells". The student observes the change in the cytoplasm indicator. The indicator changes from blue-green to bright yellow. Next, two a chromosomes are placed in another cell and it is found that the indicator turns from blue-green to dark blue. In the third cell one A chromosome and one a chromosome are placed in the cytoplasm and the resulting color change observed. The A chromosome expresses itself over the a chromosome and the indicator turns yellow.

APPLICATION OF RESULTS: In an attempt to help the students understand this exercise, they are asked the following questions: (1) Even though A and a chromosomes looked alike and the cells were essentially the same, what can one conclude as to the cause of the different reactions? (2) When the cell contains both kinds of chromosomes, to which does it respond? (3) Does the cell respond to one A and one a chromosome in the same way it does to two A chromosomes?

The students are then asked to diagram and label the three cells with their chromosomes and indicate the color of the cytoplasm. Relating this exercise to a living cell, the gene (A) which can produce its effect even though another kind of gene (a) is present is known as a dominant gene. The other gene whose effect is not visible when it occurs in the same cell with the dominant gene is known as a recessive gene.

This information can be used to explain the inheritance of eye color in humans. A will stand for a gene which is dominant and causes the development of brown eyes. The recessive gene a causes the development of blue eyes when both recessive gene (aa) are present. Hypothetical cases can then be set up in order that the students may determine how eye color is inherited. Although the inheritance of eye color is not as simple as presented in this exercise, it is generally considered that genes for dark eyes are dominant over genes for light eyes.

FRUIT FLY - A GENETIC STUDY TOOL

The fruit fly Drosophila has been used for decades as a favorite organism in genetic studies. The animal can be bred easily; sexually mature offspring can be obtained in less than three weeks; chromosome number is low (2n = 8); giant chromosomes exist in the salivary glands; and precise microscopic investigation of experimental effects on Drosophila chromosomes are possible. In the course of years, mutations have appeared in the stocks of flies kept in various laboratories, and the inheritance of many such "mutant traits" will be analyzed here. The mutations to be studied are recessive, i.e., the wild-type normal condition is dominant.

MATERIALS: culture vials; plugging cotton; camel's hair brushes; ether; bananas; agar agar; malt extract; Brewer's yeast; propionic acid or moldex; ether-anesthesia bottles; Drosophila stock cultures - males and females of wild-type, vestigial-winged, white-eyed, and ebony body color; hand lens.

PROCEDURE: Flies are usually grown in half-pint milk bottles or shell vials containing food medium at the bottom. Larvae and pupae generally become well surrounded by food, and adult flies are found in the free space above the food. In investigating a given genetic problem a small number of the desired virgin (or nonmated) parental flies is introduced for about one week. During this time mating takes place, and fertilized eggs are deposited by the females. Parent flies are removed at the end of the week, the bottle is recapped, and nothing further is done for another week or two. By the end of this time offspring larvae have developed into adults, and the trait characteristics of these adults can then be studied.

Fly populations are examined under ether-anesthesia. The culture bottle containing the flies is inverted over another bottle which had been well saturated with ether vapor. Narcotized flies fall into the vapor bottle, and from there the organisms may be spread on a sheet of white paper for counting and examination. This is done with a hand lens or the unaided eye. The examination is carried out as rapidly as possible so that the flies remain well anesthetized throughout. When the work is done the organisms are returned to their culture bottle, where they are allowed to recover.

Wild-type flies represent the genetic norm for Drosophila. The wild-type appearance is as follows: wings reaching just beyond the tip of the abdomen, and the eyes being red in color. Males are slightly smaller than females. The tip of the abdomen in the male is more rounded than in the female, and is pigmented almost solid black. In the female the tip of the abdomen carries transverse stripes. Freshly emerged males have little body pigmentation and may easily be confused with the female if this criterion alone is used. The most reliable distinction is to be found in the genital organs as seen from a ventral view. The male organs are surrounded by heavy, dark bristles which are lacking in the females. Even in the flies which have just emerged from their pupa cases, this characteristic is clear-cut and it is recommended as the best method of sex distinction.

A gene for wing development is located on the second chromosome of Drosophila. The recessive mutant condition produces a highly reduced "vestigial wing", very easily distinguished from the wild-type wing. The recessive gene may be designated vg, and the dominant wild-type gene Vg. The students are then asked to identify male and female vestigial-winged flies, and compare with the wild-type of each sex.

A gene for body color is located on the third chromosome of Drosophila. This recessive mutant condition gives a body color of shining black known as ebony (e).

A gene controlling eye color is located on the first chromosome, i.e., the sex (X) chromosome of Drosophila. The recessive mutant condition produces white eye color which is due to the absence of any pigmentation, and is easily distinguished from the red of the wild-type. The recessive gene may be designated w, and the dominant wild-type gene W. The students then identify male and female white-eyed flies, and compare with the wild-type of each sex. It is also necessary to keep in mind that white eye, being produced by genes on the X chromosome, is a sex-linked recessive characteristic. In symbolic representation, a white-eyed female would be written X_wX_w, a white-eyed male X_wY, and normal-eyed females and males XX and XY, respectively.

MONOHYBRID CROSSES

The following exercises are used to especially illustrate Mendel's law of segregation and a situation of blended characters involving incomplete dominance.

MATERIALS: equipment necessary to maintain living cultures of fruit flies, as previously given; Drosophila stock cultures - males and females of wild-type and vestigial type; corn seed - with recessive albino characteristic; flats for growing plants; hand lens.

Exercise One

Vestigial wings is a favorite mutant for many monohybrid crosses because it is so easily recognized. This gene is expressed through the reduction of wings to mere stubs, but in many cases there is some reduction in the viability of the offspring. Some other mutant genes have a similar effect, and this should be kept in mind when one obtains his final ratios.

PROCEDURE: Virgin females of the wild-type and males of the vestigial type are put together in a bottle. If successful mating has taken place, small larvae should be crawling around by the end of four or five days. On the sixth day after mating the parents are removed from the bottles. When F_1 flies begin emerging, they are etherized and examined for mutant characteristics. If all the flies are alike, a notation to this effect should be made. Five to ten pairs of healthy looking F_1 flies are selected and put in a fresh bottle of food. This is known as an inter se cross (brother-sister mating). The females do not need to be virgin since, if they have mated, it will be with males which are the same geno-type as those placed in the vial with them. Five to ten virgin females from the F_1 cross are then placed in a bottle with mutant males which were saved P_1. This type cross is called the "back-cross" since, in effect daughters are mated to their fathers. When the flies from the two crosses begin to emerge, they are etherized and studied for identification of the characteristics in question. Careful tabulations are made on the work sheets of the results from each of the bottles.

Since the students are dealing with an autosomal gene in the first cross, there is no necessity for tabulation of the characteristics as they appear in the two sexes separately. Counting and tabulating will continue for about six days after the first flies emerge. After some time, flies which emerge from these bottles might possibly include some F_3 individuals and would tend to alter the ratio.

A test cross can be made to complete this series. Among the flies emerging from the bottle of the inter se cross one will find three genotypes: those homozygous for the mutant gene, those heterozygous for the mutant gene, and those homozygous for the wild-type allele. There will be only two phenotypes, since the heterozygous flies will express the dominant characteristic. Obtaining one virgin female that shows the dominant characteristic, a test cross is made to determine if she is heterozygous or homozygous. This is done by mating her to males which are homozygous for the recessive gene. Several males which express the recessive characteristic among the F_2 are selected and used for the parents of the test cross. The offspring of the cross will show whether the female was heterozygous or homozygous. A heterozygous female parent would give a progeny of two phenotypes, vestigial and wild-type. A homozygous female would give a progeny of one phenotype, wild-type.

<u>APPLICATION</u> <u>OF</u> <u>RESULTS</u>: Refering to the <u>inter</u> <u>se</u> cross, it can be shown that the progeny express themselves very close to the phenotypic ratio of three wild-type to one vestigial type and the genotypic ratio of one homozygous dominant to two heterozygous to one homozygous recessive. The expressed phenotypic ratio of 3 : 1 is the result of complete dominance.

Exercise Two

The following study uses plant material to show the effects of incomplete dominance. Although the task of distinguishing two different shades of green may create some difficulty, the author considers this study an effective teaching device.

PROCEDURE: Students plant about one hundred grains of corn from a cross between two green plants each carrying a recessive albino factor. Soon after emerging from the soil a count of the white and green seedlings is made to see what Mendelian ratio the results approximate. In about one week there appear two shades of green plants showing that the homozygous dominant and the heterozygous conditions are being differentially expressed. This would then illustrate a monohybrid cross with incomplete dominance and approximate a phenotypic ratio of 1 : 2 : 1.

INDEPENDENT INHERITANCE

What will happen when two mutant types not governed by genes at the same location, or even in the same chromosome, are crossed together? This is called a dihybrid cross. Suppose a fruit fly with vestigial wings but normal grayish body color is crossed with one that has normal wings but an intense shining black body color, known as ebony. The gene for vestigial wings is on chromosome two and the gene for ebony body color is on chromosome three. The following study provides a practical application and observation of independent assortment.

MATERIALS: equipment necessary to maintain living cultures of fruit flies, as previously given; *Drosophila* stock cultures - males and females of wild-type, vestigial wings, and ebony body color; hand lens.

PROCEDURE: The first cross is made between virgin females with ebony body color and long wings, males with gray body color and vestigial wings. As the F_1 progeny emerge the students examine at least fifty flies for wing condition and body color, and determine how many types exist. Three pair of F_1 flies are then mated by placing them in fresh culture bottles.

When the F_2 progeny emerge at least two hundred fifty flies are examined and classified into types by sorting them into different piles. After determining how many combinations of wing condition and body color exist, the student is then asked how many can be expected if all possible combinations occur. The ratio of all gray-bodied to all ebony-bodied and the ratio of all normal winged to all vestigial winged are figured and compared to see if they differ greatly from the 3 : 1 ratio expected if each abnormal trait depended on a single mutant gene.

<u>APPLICATION</u> <u>OF</u> <u>RESULTS</u>: If the two pairs of traits are inherited quite independently, then the combinations of the two wing types and the two body colors should occur with frequencies equal to the products of the frequencies of the simple type. If each pair of traits segregate into a 3 : 1 ratio, the product should be nine non-vestigial, non-ebony : three vestigial, non-ebony : three non-vestigial, ebony : one vestigial, ebony.

The students are then asked questions about their results as follows: (1) Were all four types found in the F_2? (2) Was the wild-type the most common? (3) Was the double mutant type the rarest? (4) Were the "single mutant" types about equally common? (5) Was the total distribution obtained quite different from a 9 : 3 : 3 : 1 ratio?

PROBABILITY

Students must realize that ratios obtained in breeding experiments represent averages and not definite numbers that will always appear. The fact that these ratios are accurate only when large numbers of individuals are concerned must be demonstrated. This exercise illustrates random or chance combination of two objects which can combine in only three ways. This is essentially what occurs during fertilization, when a pair of entities, the gene pair, recombine after being separated during meiosis.

MATERIALS: two small boxes for each group of students; 2000 dry red beans; 2000 dry white beans (it is important that red and white beans be of the same size).

PROCEDURE: Each group of students will be supplied with two small boxes containing dry beans. Each box contains equal numbers of red beans and white beans. Each student takes his turn in taking one bean from each box making sure he does not look at the beans while picking the bean from the box. The two beans are placed next to each other on the table. Several more pairs are picked and placed on the table. The students then decide into how many rows the pairs of beans can be grouped. Each member of the group continues picking beans until they are told to stop for the first round of bean picking. The group then counts how many pairs of beans there are in each row and records the results. After several rounds of bean picking the total of each of the three possible bean pairs is next figured. Varying the duration of the rounds will enable one to contrast the results when samples differ greatly in size. Each group will be allowed to pick one hundred or more pairs of beans, since larger samples are more likely to approach the expected 1 : 2 : 1 ratio. It can be explained that one box represents the male gonads, and the other box, the female gonads within which meiosis occurs. Next it can be pointed out that the sperm and the egg give only one gene from each pair of genes, and that the offspring gets one gene of each pair from the individual parent, except in sex-linked inheritance, etc. Using the principle of dominance, the expected 3 : 1 ratio of a monohybrid cross becomes evident.

A TEST CROSS

After a discussion of sex chromosomes, students should be prepared for the following problem dealing with sex-linked characteristics. Most of the important principles of heredity are also illustrated in this study.

MATERIALS: equipment necessary to maintain living cultures of fruit flies, as previously given; *Drosophila* stock cultures - F_2 of vestigial-winged female crossed with wild-type male and F_2 of vestigial-winged female crossed with white-eyed male; hand lens.

PROCEDURE: Six weeks in advance of the laboratory, two set of *Drosophila* mating cultures were started. Into each of about six culture bottles of one set were put five vestigial-winged, otherwise normal virgin females, and five wild-type males. This mating, cross I, can be represented symbolically by: (P) vgvg x VgVg. Into each of about six culture bottles of the second set were put five vestigial-winged, normal-eyed virgin females, and five normal-winged, white-eyed males. This mating, cross II, can be represented symbolically by: (P) vgvgXX x $VgVgX_wY$. The flies were allowed to mate and lay eggs into the culture bottles. One week after starting the cultures the parental flies were removed. F_1 generations hatched out subsequently.

Three weeks in advance of the laboratory the pair of culture bottles which will be studied was started. Five male and five female F_1 flies were put into each of the two bottles to produce F_1 (brother-sister) matings for cross II. A week after this the F_1 adults were removed. F_2 populations soon hatched out in each bottle, and these are the ones to be studied.

Four students work together as a team. Each team has available one F_2 culture bottle of cross I and one F_2 culture bottle of cross II. All four members of the team first deal with cross I as follows: Anesthetize the flies in the bottle, pour the narcotized flies on a sheet of white paper, and with a hairbrush divide the population into four roughly equal piles of flies. Each member of the team now types the flies in one pile. The flies are then examined one by one with the unaided eye, although the use of the hand lens may be necessary. For each fly a record of the sex and the appearance of the wings is made. Also the total number of flies in each trait category of each pile is recorded in a table. When all four piles have been counted, the cumulative totals are calculated and recorded.

Anesthetized F_2 flies of cross II are also divided into four piles in the same manner. Greater care will be required here than in cross I since a greater number of trait categories will have to be identified. Each fly is to be examined for sex, for eye color and for wing condition. Theoretically there will be eight trait categories. In the table, the total number of flies of each category is recorded. The cumulative totals are then figured.

APPLICATION OF RESULTS: In order that the students may discover what has happened in the two crosses, they are given the following directions:

Cross I

1. Determine the percentage of flies in each of the four categories.
2. Calculate the following percentage ratios:
   wild-type males : vestigial-winged males
   wild-type females : vestigial-winged females

all wild-type flies : all vestigial-winged flies.

3. Is there a significant difference between these ratios? Can it be concluded that inheritance of the vestigial wing is influenced by the sex of the fly?

4. What Mendelian ratio is exemplified by the F_2 of cross I? How does this experiment illustrate the law of segregation?

5. On the basis of all information, work out the genotypes of the P, F_1, and F_2 generations, and state what the phenotypes of these flies are.

Cross II

1. Calculate the percentage of flies in each of the trait categories.

2. Determine the following percentage ratios:
normal-winged males : vestigial-winged males
all male flies : all female flies
normal-winged females : vestigial-winged females
all normal-winged flies : all vestigial-winged flies
normal-eyed males : white-eyed males
all normal-eyed flies : all white-eyed flies
all wild-type : all white, all vestigial winged, all white-eyed and vestigial-winged

3. From these ratios and those determined for cross I, is the inheritance of the vestigial wing influenced by the simultaneous inheritance of (a) eye color traits and (b) sex?

4. Is the inheritance of white eye influenced by the simultaneous inheritance of (a) wing traits and (b) sex?

5. How does sex influence the inheritance of white eye?

6. What Mendelian ratio is exemplified by the F_2 of cross II? How does this experiment illustrate the law of independent assortment?

7. On the basis of all information, work out the genotypes of the P, F_1, and F_2 generations, and state what the phenotypes of these flies are.

LETHAL GENES

Occasionally, characteristics appear as a result of mutation which cause death of the organism. Using the data collected from the study of tobacco seedlings and the effects of environment and heredity on them (first exercise), the death dealing factor can be considered in detail.

PROCEDURE: The plants called white are called albinos by biologists. The 3 : 1 ratio can now be identified as one albino to three green plants. Suppose for the moment that there is a gene which causes the green condition and another gene that causes the albino condition. Assume further that the gene for green is dominant over the gene for albino. Parents GG x gg (G = green, g = albino) would produce G gamete and g gamete. The genotype of the F_1 offspring would be Gg and the color would be green. Next the students are asked to illustrate what will happen if two of the F_1 offspring are crossed. After it is shown that each offspring produces G and g gametes, a Punnett square is constructed to show how the genotypes of the F_2 offsprings can be determined.

APPLICATION OF RESULTS: The students are then in a position to answer the following questions: (1) What proportion of the F_2 offspring will be green? (2) What proportion will be albino? (3) What were the genotypes of the parents of the tobacco seed used in the study of environment and heredity? (4) Noting that the albino condition in plants is one in which no chlorophyll develops, would the albinos be expected to grow to maturity?

A gene which causes a condition that leads to the early death of the organism is called a lethal gene. Such a gene may result in the death of the embryo or may result in death during the early or later stages of development following the embryo. The students are finally presented with the following problem. If albino plants die off before reproduction, how can one account for the persistence of the gene for the albino condition?

CHAPTER III

SUMMARY

The solving of genetic problems on paper and the reading of textbook material are not always adequate means of teaching genetics with complete understanding. Students must be given the opportunities to personally observe and investigate genetic problems, while the teacher encourages them to ask questions about the problems that will reveal important genetic concepts and principles. Many other experiments were selected specifically to illustrate basic principles of Mendelian genetics.

The use of these exercises should afford students a greater appreciation and understanding of genetics and the related fields of study. Some students will be limited by the scope of these proposed studies whereas other students may need to exert a maximum amount of effort in order to comprehend the material.

BIBLIOGRAPHY

Hill, Ben J., and Helen D. Hill. Genetics and Human Heridity. New York: McGraw-Hill Book Company, 1955.

Ikenberry, Gilford J. Laboratory Manual in General Botany. Oklahoma: Oklahoma State University, 1957.

Lawson, Chester A., and Richard E. Paulson. Laboratory and Field Studies in Biology, A Source Book for Secondary Schools. New York: Holt, Rinehart and Winston, Inc., 1960.

Milne, Lorus J., and Margery Milne. Laboratory Manual in General Biology. New Jersey: Prentice-Hall, Inc., 1958.

Moon, Truman J., James H. Otto, and Albert Towle. Modern Biology. New York: Holt, Rinehart and Winston, Inc., 1960.

Richardson, John S. Science Teaching in Secondary Schools. New Jersey: Prentice-Hall, Inc., 1957.

Sinnott, Edmund W., L. C. Dunn, and Theodosius Dobzhansky. Principles of Genetics. New York: McGraw-Hill Book Company, 1958.

Weisz, Paul B. Laboratory Manual in the Science of Biology. New York: McGraw-Hill Book Company, 1959.

Winchester, A. M. Genetics. Iowa: Wm. C. Brown Company, 1958.

________. American Institute of Biological Sciences, Biological Sciences Curriculum Study, High School Biology. Colorado: Johnson Publishing Company, 1960.

VITA

John Corbin Mills

Candidate for the Degree of

Master of Science

Report: LABORATORY EXERCISES IN GENETICS FOR THE HIGH SCHOOL BIOLOGY CLASS

Major Field: Natural Science

Biographical:

Personal Data: Born in Enid, Oklahoma, August 14, 1937, the son of Harry Neal and Lois Edith Mills.

Education: Attended grade school in Stillwater, Oklahoma; graduated from Stillwater High School in 1955; received the Bachelor of Science degree from the Oklahoma State University, with a major in Natural Science, in May 1960; completed requirements for the Master of Science in May 1963.

Professional Experience: Entered the teaching profession in September 1961, and have since that time been employed by the Oklahoma City Public School System.