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ADVISER'S APPROVAL James H. Zant

EXPERIMENTS IN GENETICS DESIGNED TO PROMOTE SCIENTIFIC RESEARCH IN THE HIGH SCHOOL BIOLOGY COURSE

By

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EXPERIMENTS IN GENETICS DESIGNED TO
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CHAPTER I

Introduction

The purpose of this report is to provide a collection of experiments in genetics which will incorporate the scientific research method at the high school level. The study of genetics at the high school level often involves only the solving of a series of hypothetical and textbook problems. The students foundation in the basic principles of genetics is based entirely on the material they have read or what they have been told by the teacher.

The value of these experiments is based on the fact that they present an instrument for the high school biology teacher to use in introducing the principles of genetics in an interesting and challenging manner. The student is required to submit a report for each experiment which very closely resembles, in structure, the report that a research geneticist would make concerning his work. These experiments allow the student to work individually, taking careful notes of all his observations, and to use these notes to aid in drawing his conclusions.

The fruit fly or vinegar fly, Drosophila melanogaster, is the organism used in these experiments. Considerable

work has been done with this organism over the past forty years and abundant research literature is already in existance for reference. Many mutant types of Drosophila melanogaster have been established and the more common mutant types can be obtained from several biological supply houses. These flies are relatively hardy and easy to culture. They produce a large number of offspring each generation, and a new generation is produced every ten to twenty days.

CHAPTER II

THE CULTURING AND HANDING OF *Drosophila melanogaster*

List of Materials Needed

1. Basic containers (vials or bottles)
2. Plugging cotton (bale or batting cotton)
3. Small camel's - hair brushes
4. Ether
5. Karo syrup
6. Bananas
7. Agar agar
8. Malt extract (powder or liquid)
9. Brewer's yeast
10. Propionic acid or moldex
11. *Drosophila* stock cultures
    - (1) wild-type
    - (2) "brown" eye
    - (3) "sepia" eye
    - (4) "vestigial" wing
    - (5) "miniature" wing
    - (6) "curly" wing
    - (7) "black" body

Preparation of Culture Media

Many types of fermenting media have been devised for culturing Drosophila. Most of these media use agar to solidify the medium so that vials may be inverted. This is important in transferring flies from one vial to another. Demerec and Kaufmann[1] describe several agar-type media which have been found suitable for the proper development of these flies. The following medium has been found suitable for experiments conducted by the writer. This recipe furnishes enough medium for about one hundred and fifty of the 30 ml. shell vials.

Banana-Agar Medium

Water	1100 ml.
Malt extract	28 ml.
Karo syrup	28 ml.
Propionic acid	5½ ml.
Brewer's yeast	33 gms.
Banana pulp	300 gms.
Agar agar	22 gms.

Boil 22 gms. agar in 1000 ml. of water until dissolved. Add 28 ml. of malt extract and 28 ml. of karo syrup. Next add 33 gms. of brewer's yeast dissolved in 50 ml. of water. Then add 300 gms. of banana pulp and a solution of 5½ ml. of propionic acid in 50 ml. of water. Boil for a few

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[1] M. Demerec and B. P. Kaufmann, Drosophila Guide (Baltimore, 1950), pp. 6-8.

minutes and pour while hot into sterile vials. Each vial should contain 4 to 6 ml. of the medium.

Maintaining Stock Cultures

Many pure strains of specific mutations have been established for Drosophila melanogaster. A number of distinct characters are available which may be classified with the naked eye. Morholt, Brandwein, and Joseph[2] describe several pure stocks which have distinct and contrasting mutant characters for eye color, body color and wing type.

The stock cultures should be kept at a temperature range of 20° to 25° C. At 25° C. the life cycle may be completed in about ten days, while at 20° C. the life cycle will be lengthened to about fifteen days or longer. At lower temperature the life cycle is prolonged and viability is impaired. Continued exposure to high temperatures may cause sterility.

Stocks should be changed to new culture bottles once every ten days or two weeks, depending on the temperature at which they have been reared.

From four to six pairs of flies should be used as parents in making stock cultures and duplicate cultures of each stock should be kept, in case one culture is not suc-

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[2] Evelyn Morholt, Paul F. Brandwein, and Alexander Joseph, A Source Book for the Biological Sciences (New York, 1958), p. 196.

cessful. Each vial should be labeled with the name of the mutant character carried by the stock and the date of transfer should be recorded on the vial.

Examining and Crossing Fruit Flies

The students should first learn to identify male and female flies. The female has a slightly broader abdomen than the male and also has small lines across the tip of the abdomen. The male is smaller than the female, having a bluntly rounded, black-tipped abdomen.

Only virgin females should be used when making a cross between flies belonging to two different lines. If already mated, the females retain the sperm and use it in fertilizing a large number of eggs. One way to secure virgin females is to remove some of the pupae from the stock cultures at about the seventh day after mating and place them in separate vials.[3] As a rule, however, females do not mate within the first twelve hours after emergence. Since collecting females during subsequent twelve hour periods is much less laborious than isolating pupae, this method is suggested for high school biology students.

For convenience in examining, counting, and sorting, the flies must be etherized. A vial having the same

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[3] Turtox Service Department Publication, The Culture of Drosophila Flies and Their Use in Demonstrating Mendel's Law of Heredity (Chicago, 1944), p. 2.

circumference as the mouth of the stock vials can be used for the etherizing container. A cotton plug moistened with a few drops of ether should be placed in the etherizing vial. To transfer the flies, remove the plug from the etherizing vial and tap the culture vial on the table. When the flies fall to the bottom of the vial, quickly remove the plug and invert the stock vial with the mouth held closely against the mouth of the etherizer. Tap the etherizer on the table so that the flies fall down into the ether fumes. Then quickly separate the vials and plug both of them.

If the flies are to be separated for mating they should be etherized for about one minute. Excess or too rapid etherization should be avoided or the flies will die. Over-etherized flies have the wings obliquely extended. If the flies are to be counted and recorded only, they may be killed by being left for several minutes in the etherizing vial.

After the flies have been etherized they may be dumped onto a piece of white paper for examination. Since the flies are fragile, a fine camel's-hair brush should be used for sorting them into groups of male and female, as well as into groups according to the mutant characters they carry.

Five of the 30 ml. vials should be used for each experimental mating, four to six pairs of parent flies should be placed in each vial. It is important that each

vial be labeled. The date of the cross and the characteristics of the parent flies should be written on the label. Etherized flies should not be dropped directly onto the culture medium, because they will get stuck before they wake up. To prevent this, the culture vial may be kept on its side until the flies revive.

Parent flies must be removed from the culture vials by the seventh day, as suggested by Royle[4] in order that they will not be confused with the offspring when counts are made. When the offspring begin to emerge, they should be separated and counted each day for about ten days.

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[4]Howard A. Royle, Laboratory Exercises in Genetics (Minneapolis, 1953), p. 18.

CHAPTER III

METHOD TO BE USED BY THE STUDENT IN REPORTING EACH EXPERIMENT

Student reports of these experiments should include the items listed below:

1. Title
2. Author
3. Introduction
4. History or Review of Literature
5. Methods and Materials
6. Experimental Results
7. Discussion and Conclusions
8. Bibliography

The title of each report should be brief and indicate the content of the report. The student in placing his name on the report is to follow the example of scientific papers appearing in a journal in the field of genetics.

The introduction should include object or purpose of the experiment and the genetic description of the flies used. Bridges and Brehme[1] list the genes of

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[1] C. B. Bridges and K. S. Brehme, The Mutants of Drosophila melanogaster (Baltimore, 1944), pp. 238-252.

Drosophila melanogaster into four linkage groups corresponding to the four chromosomes of this organism. Students should refer to these chromosome maps for an indication of the position of the genes on the chromosomes. In this way students can see which genes are linked on the same chromosome and which genes are located on different chromosomes.

The student is expected to use high school library references to review the scientific literature concerning the experiment. This section of the report should help the student to relate his experiment to the larger field of genetics.

The materials and procedures section of the report should contain a brief description of what the student did in performing the experiment and the conditions under which the experiment was carried out. Such items as special techniques used in handling the flies, the type of medium used for their culture, and the temperature at which the cultures were reared should be discussed in this section.

The experimental results section of the report is the product of the careful notes that should be kept by the student during the course of the experiment. This section should indicate the various phenotypes observed and the number of flies counted for each phenotype. The percent of each phenotype present should also be included. A short, simple table can sometimes be used in presenting

the experimental results.

A diagramatic representation of the theoretical results expected may be incorporated into the discussion and conclusion section of the report. The student should make reference to the conditions of the experiment in the interpretation of the results. Variations from theoretical expectations should be explained.

The student should follow the example of scientific papers appearing in a journal in the field of genetics in choosing a method of citing and arranging references.

CHAPTER IV

EXPERIMENTS IN GENETICS DESIGNED FOR THE HIGH SCHOOL BIOLOGY COURSE

Foreword

These experiments are designed for individual work on the part of the high school biology student. It is not expected that each student will conduct all of these experiments. The writer's wish is to provide interesting and challenging experiences in genetics, for students of different levels of ability.

When several students are conducting the same experiment, the teacher may find it desirable to use a variety of mutant fly stocks. In this manner the students, while working with different characters, are solving the same basic problem.

Experiments five and six, which are more difficult, could also be used as individual projects for members of the high school science club.

Experiment Number One

OBJECT: To illustrate the law of segregation using a monohybrid cross.

PROCEDURE: Make a cross between flies bearing the mutant character "vestigial" wing and wild type flies. Set up the cross in the manner described on page seven. Remove the parent (P_1) flies on the seventh day. About twelve days after the cross is made the F_1 generation will begin to emerge. Note the appearance of the F_1 generation. Inbreed the F_1 generation and transfer these flies to vials containing fresh medium. Remove the parent flies on the seventh day after the F_1 cross is made. When the F_2 generation begins to emerge they should be counted each day for ten days or until approximately one thousand flies have been counted. Classify the flies into groups according to the characters they carry. Record the number of individuals found in each group.

Student Guide

1. Who discovered the law of segregation?
2. Is the mutant character "vestigial" wing dominant or recessive?
3. On which chromosome is the gene for the mutant character "vestigial" wing located?

4. How does this experiment illustrate the law of segregation?

5. How do the actual results of this experiment compare with the expected or theoretical results.

Experiment Number Two

OBJECT: To illustrate the law of independent assortment using a dihybrid cross.

PROCEDURE: Make a cross between flies bearing the mutant characters "vestigial" wing and "brown" eye. Remove the parent (P_1) flies on the seventh day. Note the appearance of the F_1 generation. Inbreed the F_1 generation and transfer these flies to vials containing fresh medium. Remove the parent flies on the seventh day after the F_1 cross is made. When the F_2 generation begins to emerge, they should be counted each day for ten days or until approximately one thousand flies have been counted. Classify the flies into groups according to the characters they carry. Record the number of individuals found in each group.

Student Guide

1. Who discovered the law of independent assortment?
2. Are the genes for the mutant characters "vestigial" wing and "brown" eye located on the same chromosome or on different chromosomes?
3. Let V represent a gene for normal or long wings; v represent a gene for vestigial wing; B represent a gene for normal or red eyes; b represent a gene for brown eyes. What gametes will be produced by the P_1? What gametes will be produced by the F_1?
4. Using the Punnett square, determine the theoretical

genotypes and phenotypes of the F_2 generation.

5. What is the theoretical phenotypic ratio of the offspring resulting from the F_1 dihybrid cross?

6. What is the actual phenotypic ratio of the offspring resulting from the F_1 dihybrid cross if one thousand flies are counted?

7. What is the actual phenotypic ratio of the offspring resulting from the F_1 dihybrid cross if only the first sixteen flies are counted?

Experiment Number Three

OBJECT: To learn the effect of a lethal mutation and to illustrate how the lethal factor modifies the expected Mendelian ratio.

PROCEDURE: This experiment requires that two crosses be made concurrently. Set up a cross between males bearing the balanced mutant character "curly" wings and females bearing the same mutant character. Set up a cross between flies bearing the mutant character "curly" wing and wild type flies. Remove the parent flies of both crosses on the seventh day. When the offspring begin to emerge, they should be counted every day until approximately five hundred flies for each cross have been counted. Record the results of each cross separately. Classify the flies into groups according to the characters they carry. Record the number of individuals found in each group.

Student Guide

1. What are some examples of lethal genes in other organisms?
2. Is the lethal mutant character "curly" wing dominant or recessive to normal?
3. Does this lethal mutant gene produce its non-lethal effect in the homozygous recessive, in the homozygous dominant, or in the heterozygous condition?

4. Letting C represent the dominant gene and c represent the recessive gene, use the Punnett square to diagram the theoretical genotypes of the offspring resulting from each cross.

Experiment Number Four

OBJECT: To determine whether an organism showing two dominant genes is pure or hybrid for one or both of these characters by using a test cross.

PROCEDURE: Make a cross between flies of unknown genotype that appear normal for eye and body characters and flies bearing the mutant characters "sepia" eye and "black" body. Seven days after the cross is made the parent flies should be removed. When the offspring begin to emerge they should be counted every day until approximately five hundred flies have been counted. Classify the flies into groups according to the characters they carry. Record the number of individuals found in each group.

Student Guide

1. What is the chief value of the test cross?
2. Could the genotype of the unknown flies be determined by some other type of cross?
3. Under what circumstances would the test cross be of little value in determining the genotype of unknown flies.
4. If the unknown flies were heterozygous for both the eye and body characters, how many kinds of offspring would be produced and in what proportions?
5. What would be the result of this cross if the unknown flies were homozygous dominant for both characters.
6. What results would be obtained if the unknown flies were heterozygous for one gene and homozygous dominant for the other gene?

7. Let S represent a gene for normal or red eyes: s represent a gene for sepia eyes; B represent a gene for normal or gray body; b represent a gene for black body. Using the Punnett square, diagram the theoretical results you would expect from the following crosses:

   (1) BbSs x bbss

   (2) BBSs x bbss

   (3) BbSS x bbss

   (4) BBSS x bbss

8. From a comparison of the theoretical results of the above crosses and the actual results of your test cross determine the genotype of the unknown flies.

Experiment Number Five

OBJECT: To learn how certain characters are inherited on the basis of XX- and XY- chromosome pairs.

PROCEDURE: This experiment requires that two crosses be made concurrently. Set up a cross between males bearing the mutant character "miniature" wing and females bearing the mutant characters "brown" eye. Set up the recriprocal cross (brown-eyed males x miniature-winged females). Remove the parent flies of both crosses on the seventh day. Note the appearance of the F_1 generation resulting from each of the crosses. Inbreed the F_1 generation from each cross and transfer the flies to vials containing fresh medium. When the F_2 generations begin to emerge they should be counted each day until approximately one thousand flies for each cross have been counted. Record the results of each cross separately. Classify the flies into groups according to the characters they carry. Record the number of male and female individuals found in each group.

Student Guide

1. Who discovered sex-linked genes in Drosophila? What mutant character did this involve?

2. On which chromosome does the gene for the mutant character "miniature" wing appear?

3. What is meant by the term X-linked gene?

4. Which of the mutant genes involved in this experiment could be referred to as an autosomal gene?

5. How do the individuals of the F_1 generation differ when the two crosses are compared?

6. Let M represent a gene for normal or long wings; m represent a gene for miniature wings; B represent a gene for normal or red eyes; b represent a gene for brown eyes. What gametes will be produced by the parents (P_1) in each cross? What gametes will be produced by the F_1 generation in each cross?

7. Using the Punnett square determine the theoretical genotype and phenotype of the F_2 generation for each cross.

8. In both crosses evaluate the actual results as to sex, eye color and wing length.

Experiment Number Six

OBJECT: To show the linkage and crossing over relationship of two mutant genes found together on the same chromosome.

PROCEDURE: Make a cross between flies bearing the mutant character "vestigial" wing and flies bearing the mutant character "black" body. Remove the parent flies on the seventh day. Note the appearance of the F_1 generation. Set up a cross between virgin females from the F_1 generation and black, vestigial males. Remove the parent flies seven days after making the cross. When the F_2 generation begin to emerge, they should be counted every day until approximately one thousand flies have been counted. Classify the flies into groups according to the characters they carry. Record the number of individuals found in each group.

Student Guide

1. How does the law of independent assortment apply to genes on different chromosomes? Does this law explain the mechanism by which genes, located on the same chromosome, are inherited?
2. Does crossing over occur in both sexes of the fruit fly?
3. On which chromosome are the genes for the mutant characters "vestigial" wing and "black" body located?

4. What percentage of crossing over would you expect between these genes according to their relative position on the chromosome?

5. Let V represent a gene for normal or long wing; v represent a gene for vestigial wings; B represent a gene for normal or gray body; b represent a gene for black body. What gametes will be produced by the P_1? What gametes will be produced by the F_1 females? What gametes will be produced by the F_1 males?

6. Using the Punnett square determine the theoretical genotype and phenotype for a cross between the F_1 females and black, vestigial males.

7. If the F_1 males and black, vestigial females are crossed, what types of individuals would be produced? In what proportion would they be produced?

8. Would you expect the results of a cross between males and females of the F_1 generation to indicate the percentage of crossing over between the genes for "vestigial" wing and "black" body?

CHAPTER V

EXAMPLE OF A RESEARCH REPORT INCORPORATING THE SCIENTIFIC METHOD AT THE HIGH SCHOOL LEVEL

Foreword

Experiment number five in chapter four has been conducted by the writer. The following report of this experiment is presented to illustrate the form to be used by the high school biology student. This form for reporting on experiments has been devised on the basis of a brief study of scientific research reports published in several journals in the field of genetics. While each report has its own individual characteristics, an attempt was made to select the factors common to all scientific reports.

Example Report of Experiment Number Five

The Use of *Drosophila melanogaster* to Illustrate Sex Linkage Relationships

William R. Duffer

The purpose of this experiment is to compare the theoretical and actual results of crosses involving sex linked characters and characters which assort independently. The mutant characters "miniature" wing, chromosome I, and "brown" eye, chromosome II, were used in this experiment. Miniature-winged males were crossed to brown-eyed females and the recriprocal cross was also made.

Review of Literature

Mendel's law of independent assortment did not fully explain the hereditary mechanism. This law is limited to genes in different chromosomes; genes on the same chromosome will not be assorted independently.

Linkage has been known since 1906 when Bateson and Punnett discovered the first case in sweet peas. Morgan (1911) discovered sex-linked genes in *Drosophila* and the results of his crosses were found to depend on the sex of the parent by which the trait was introduced into the cross. He supposed that the tendency of linked genes to

remain in their original combinations was due to their residence in the same chromosome.

Materials and Procedures

Banana-agar medium was used for the culturing of Drosophila in these experiments. Approximately 5 ml. of this medium was placed in each 30 ml. glass vial. Each was closed with a plug of absorbent cotton.

The flies to be separated for mating were etherized for about one minute, but if they were to be counted and recorded they were killed by being left for several minutes in the ether bottle. Demerec and Kaufmann (1950) found that all females collected during subsequent twelve hour periods are likely to be virgin. In this experiment the females to be used for mating were collected at eight hour intervals.

Five pairs of flies were placed in each vial and five vials were used for each cross. Parent flies were removed by the seventh day, as suggested by Royle (1953), in order that they would not be confused with the offspring when counts were made. All cultures were reared at room temperatures.

Results

When male "miniature" wing flies are crossed to female "brown" eye flies all of the offspring in the resulting generation (F_1) have long wings and red eyes. On the other

hand if the reciprocal cross is made the F_1 generation females have long wings and red eyes, but the males have miniature wings and red eyes. Tables I and II give a summary of the theoretical and actual results of the F_2 generation for the male "miniature" wing flies crossed to the female "brown" eye flies. Tables III and IV give a summary of the theoretical and actual results of the F_2 generation for the reciprocal cross. In both crosses the results are tabulated as to sex, eye color, and wing length.

TABLE I

THEORETICAL AND ACTUAL RESULTS

OF F_2 GENERATION MALES[1]

Phenotype	Number Counted	Percent Counted	Theoretical Percent
Long wing, red eye	213	40.2	37.5
Miniature wing, red eye	209	39.4	37.5
Long wing, brown eye	59	11.1	12.5
Miniature wing, brown eye	49	9.3	12.5
All male phenotypes	530	100.0	100.0

[1] P_1-male "miniature" wing x female "brown" eye with the F_1 being allowed to interbreed.

TABLE II

THEORETICAL AND ACTUAL RESULTS

OF F_2 GENERATION FEMALES[2]

Phenotype	Number Counted	Percent Counted	Theoretical Percent
Long wing, red eye	350	72.5	75.0
Miniature wing, red eye	0	0.0	0.0
Long wing, brown eye	134	27.5	25.0
Miniature wing, brown eye	0	0.0	0.0
All female phenotypes	487	100.0	100.0

TABLE III

THEORETICAL AND ACTUAL RESULTS

OF F_2 GENERATION MALES[3]

Phenotype	Number Counted	Percent Counted	Theoretical Percent
Long wing, red eye	219	41.8	37.5
Miniature wing, red eye	212	40.5	37.5
Long wing, brown eye	41	7.8	12.5
Miniature wing, brown eye	52	9.9	12.5
All male phenotypes	524	100.0	100.0

[2] P_1-male "miniature" wing x female "brown" eye with the F_1 being allowed to interbreed.

[3] P_1-male "brown" eye x female "miniature" wing with the F_1 being allowed to interbreed.

TABLE IV

THEORETICAL AND ACTUAL RESULTS

OF F_2 GENERATION FEMALES[4]

Phenotype	Number Counted	Percent Counted	Theoretical Percent
Long wing, red eye	217	44.7	37.5
Miniature wing, red eye	178	36.7	37.5
Long wing, brown eye	42	8.7	12.5
Miniature wing, brown eye	48	9.9	12.5
All female phenotypes	485	100.0	100.0

Discussion and Conclusions

The recessive mutant character "miniature" wing is located on the X- chromosome and is sex-linked. The recessive mutant character "brown" eye is located on chromosome II. When miniature-winged males are crossed to brown-eyed females the resulting generation (F_1) are all long-winged and red-eyed. In the F_2 generation the Drosophila males have the "long" wing and "miniature" wing characters in about the same proportion as indicated in Table I. All of the Drosophila females are long-winged (Table II) because they could recieve only the dominant allele from their fathers.

In contrast if the reciprocal cross is made, the males

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[4] P_1-male "brown" eye x female "miniature" wing with the F_1 being allowed to interbreed.

of the F_1 generation are miniature-winged and red-eyed while the females are long-winged and red-eyed. Since the F_1 females produce gametes for "long" wing character and the "miniature" wing character in about equal proportions, males and females of the F_2 generation show both of these characters (Table III and IV).

In Tables I, II, III, IV, if only the eye color is considered, 75 percent of the offspring are red-eyed and 25 percent of the offspring are brown-eyed. This is the expected 3:1 Mendelian Ratio.

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