Instructor's Manual

GENETICS

Laboratory Investigations

Fourteenth Edition

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To The Instructor

Many suggestions helpful to users of *Genetics Laboratory Investigations* have been incorporated directly into the fourteenth edition. Sources of genetic stocks and specific chemicals, supplies, and procedures are often included with the investigations. Additional information can be found in this *Instructor's Manual*.

We wish to call to the attention of those adopting *Genetics Laboratory Investigations* the need for long-range planning on the part of the course instructor or coordinator. For example, if you were to do one investigation per week and wait until the fourteenth week of the semester to begin Investigation 13 on "open-ended" *Drosophila* matings, it would be impossible to complete the investigation in the remaining weeks of the semester. Since the manual has been designed to meet instructional needs in a variety of academic settings, you can be selective in choosing which investigations to do and in determining the sequence in which to do them. We never use all of the 26 investigations in one semester, nor would we expect others to do so.

While the *Instructor's Manual* provides answers to many of the questions in the investigations, those questions dependant on data collected in the investigations are generally not answered here. In at least two instances (Investigations 3 and 24), we have provided sets of data collected by students in our classes that instructors may wish to share with their students. Finally, we invite your comments concerning the usefulness of our manual; please call to our attention errors or unclear writing that can be corrected in future printings. We hope that *Genetics Laboratory Investigations*, Fourteenth Edition will serve you and your students well.

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ANSWERS TO QUERIES AND PROBLEMS

Investigation 1

I. MEDIUM

Demerec and Kaufmann's *Drosophila* guide includes a number of recipes for different media.[1] The ingredients of another useful medium, which has been attributed to C.B. Bridges of the California Institute of Technology is as follows:

A. Ingredients of C. B. Bridge's *Drosophila* Medium

20 g of agar
200 g of cornmeal
145 ml of Karo Syrup
145 ml of Br'er Rabbit Molasses
2400 ml of distilled water
4.5 g of Dowicil-200 dissolved in 15 ml of distilled water[2]

B. Preparing The Medium

First, dissolve the agar by heating it with 1000 ml of the distilled water, and then add an additional 1000 ml of water. Carefully bring the mixture to a boil. Before adding the cornmeal to this mixture, mix the cornmeal with the remaining 400 ml of unheated distilled water. (Mixing the cornmeal with the unheated water prevents it from forming lumps when you add it to the hot agar suspension.) Add the cornmeal to the dissolved agar mixture, stirring continuously to prevent the medium from sticking. Now add the molasses and syrup, and boil the whole mixture for about 15 minutes. Dissolve the Dowicil-200 (mold inhibitor) in 15 ml of distilled water; add this to the main mixture and mix thoroughly. The cornmeal should not settle out in the final product.

Pour the medium into chemically clean bottles. These bottles may be of any size, but 4-oz. wide-mouthed bottles or half-pint milk bottles are satisfactory. Transfer the medium to a beaker for pouring and fill the bottles to a depth of about 1 in. Take care to prevent the medium from coming into contact with the neck of the bottle. Place a piece of nonabsorbent paper, such as brown wrapping paper, in each bottle so that it extends down into the medium (a double piece of paper is more satisfactory). Use paper that is about 1 in. wide and be certain that it extends upward to a point about 0.5 in. below the neck of the bottle. This paper provides a dry place on which Drosophila larvae can pupate. Cotton plugs may be used to stopper the bottles, but foam diSPo plugs are more convenient.[3]

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[1] *Drosophila Guide* may be downloaded from Carnegie Institution for Science, Books Online, http://carnegiescience. Edu/publications/books_online.

[2] Dowicil-200 is available from Sigma-Aldrich, P.O. Box 14508, St Louis, MO 63178

[3] Foam diSPo plugs are available from Baxter Scientific Products Division of VWR Scientific Products (Sargent-Welch), P.O. Box 5229, Buffalo Grove, IL 60089, and Carolina Biological Supply Company: www.carolina.com.

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

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

V. EXPERIMENTAL MATINGS

C. Making Crosses

1. F_1 females will be mated to F_1 males to produce the F_2. This mating can occur prior to or after placing flies in a fresh bottle.
2. A test cross is a controlled mating to a recessive homozygote; therefore, the F_1 female must not have mated before she is placed with the recessive male.
3. Any cross designed to determine the genotype of the individual tested; a cross to an individual homozygous recessive for genes in question.

VI. SUGGESTED MONOHYBRID CROSSES

A. *Drosophila* Crosses

3 and 4. Sample answer: red eyes. The F_1 flies all had red (wild type) eye color, and the F_2 consisted of about 3 red to 1 sepia (brown). Genes that behave as dominants may be symbolized with capital letters or with a + subscripted above the gene symbol.

VII. INDEPENDENT ASSORTMENT

A. Dihybrid Cross With *Drosophila*

2. F_1 phenotype: Wild type for both traits.

   F_2 phenotypic frequencies: 9 wild type (long wing, gray body) : 3 long, ebony : 3 vestigial, gray : 1 vestigial, ebony.

5. Table 1.5

Phenotype	Observed Number	Expected Number	Deviation O-E
long, gray			
vestigial, gray			
long, ebony			
vestigial, ebony			

6. P_1 *e/e* vg^+/vg^+ (ebony) × e^+/e^+ *vg/vg* (vestigial)

   F_1 e^+/e vg^+/vg (wild type)

   F_2 1 e^+/e^+ vg^+/vg^+, 2 e^+/e vg^+/vg^+, 2 e^+/e^+ vg^+/vg, 4 e^+/e vg^+/vg (all wild type); 1 e^+/e^+ *vg/vg*, 2 e^+/e *vg/vg* (both vestigial); 1 *e/e* vg^+/vg^+, 2 *e/e* vg^+/vg (both ebony); 1 *e/e vg/vg* (ebony, vestigial)

B. Dihybrid Crosses in Maize

A number of alternative corn experiments are available commercially and may be used to demonstrate the classical dihybrid F_2 ratio of 9:3:3:1. Either endosperm traits alone, seedling traits alone, or a combination of one endosperm and one seedling trait may be involved in such crosses. Consult the current catalogs of such firms as the Carolina Biological Supply Company to determine what materials are available. Among the possibilities are the following:

- *Y/Y wx/wx* × *y/y Wx/Wx* F_1 *Y/y Wx/wx* F_2 9:3:3:1
  yellow waxy white starchy yellow starchy
- *R/R Su/Su* × *r/r su/su* F_1 *R/r Su/su* F_2 9:3:3:1
  purple starchy yellow sweet purple starchy
- An F_2 segregating for two seedlings traits such as a gene for dwarf (e.g., d_5) and one for albinism (e.g., lw_3) would produce an F_2 ratio of 9 tall green : 3 tall albino : 3 dwarf green : 1 dwarf albino.
- An F_2 in which one trait is an endosperm trait (e.g., *Su*, starchy vs. *su*, sugary) and the other trait a seedling condition (e.g., *Gl*, normal vs. *gl*, glossy) will also assort into a 9:3:3:1 ratio.

2.

Gene Symbol	Phenotype
Su	starchy (smooth)
su	sugary (wrinkled)
R	colored (purple)
r	colorless (yellow -- no purple)

(a) Dominant: starchy (smooth) and colored (purple).

(b) Recessive: sugary (wrinkled) and colorless (yellow).

3. Genotypes of the two possibilities: (1) *R/R Su/Su* × *r/r su/su* or (2) *R/R su/su* × *r/r Su/Su.*

(a) Phenotypes: (1) purple starchy × yellow sugary or (2) purple sugary × yellow starchy.

(b) More than one original cross could have been used as shown above.

4. *R/r Su/su* purple, starchy.

Table 1.6

Phenotype	Observed Number	Expected Number	Deviation O-E
purple, starchy			
purple, sugary			
yellow, starchy			
yellow, sugary			

VIII. GENE INTERACTIONS

A. Gene Interactions in *Drosophila* (This particular mating can be part of an open-ended experiment in which different crosses involving eye color mutants can be made. See Investigation 13.)

Parental (scarlet) *sc/sc Bw/Bw* × (brown) *Sc/Sc bw/bw*

F1 *Sc/sc Bw/bw* Wild-type (brick-red)

F2 Symbol	Phenotype	Expected Ratio
Sc/- Bw/-	Wild-type	9/16
Sc/- bw/bw	brown	3/16
sc/sc Bw/-	scarlet	3/16
sc/sc bw/bw	white	1/16

B. Gene Interactions in Maize

Among the possible kinds of ears of corn that show epistasis or gene interaction are the following:

Phenotypes of the True-Breeding Parents	Phenotype of the F_1 Kernels	Phenotypes and Ratios of the F_2 Kernels
1. purple × white	purple	9 purple : 3 red : 4 white
2. yellow × yellow	yellow	13 yellow : 3 purple
3. white × white	purple	9 purple : 7 white
4. purple × yellow	purple	12 purple : 3 yellow : 1 white

Given below are the genotypes for the four crosses cited above. Note that official maize gene symbols are used.

1. *Pr/Pr R/R* x *pr/pr r/r* F_1 *Pr/pr R/r* F_2
   purple white purple

   9 *Pr/- R/-* (purple)
   3 *pr/pr R/-* (red)
   4 -- *r/r* (white)

2. C^I/C^I *R/R* × *C/C r/r* F_1 C^I*/ C R/r* F_2
   yellow yellow yellow

   9 C^I*/- R/-* (yellow)
   13 3 C^I*/- r/r* (yellow)
   1 *C/C r/r*
   3 *C/C R/-* (purple)

3. *C/C r/r* x *c/c R/R* F_1 *C/c R/r* F_2
   white white purple

   9 *C/- R/-* (purple)
   3 *C/- r/r*
   7 3 *c/c R/-* (white)
   1 *c/c r/r*

4.	*y/y R/R*	×	*Y/Y r/r*	F_1	*Y/y R/r*	F_2	9	*Y/- R/-*	
	purple		yellow		purple		12		(purple)
								3 *y/y* R/-	
							3	*Y/- r/r*	(yellow)
							1	*y/y r/r*	(white)

Other F_2 ears of "genetic corn" showing epistasis can be obtained. All of the ears cited in this investigation involve two segregating gene loci.

The epistasis ratios (9:3:4, 13:3, 9:7, and 12:3:1) are all modifications of the classical 9:3:3:1 F_2 ratio. Ears of genetic corn in which three gene loci are segregating may also be obtained. For example, the F_2 trihybrid epistasis ratio of 27:37 (a variation of the classical 27:9:9:9:3:3:3:1) could be used for this purpose.

The epistatic F_2 maize ears are becoming more difficult to obtain from vendors. For this reason, we have printed FIGURE 1.11 in a larger format so that students can count each of these different maize ears. Careful counting of kernels will provide reasonable data for analysis. For the ear in (B), hue of the kernel is more important than intensity of the color. For example, light red and dark red kernels are all counted as red, and the same is true for purple.