MENDELIAN GENETICS—INVESTIGATION II
Short Form

STUDENTS' WORKSHEET

Background

The principles and methods formulated by Gregor Mendel provide the basis for studies of inheritance in higher organisms. The events which Mendel studied—**segregation** and **independent assortment**—occur in nature, but are only visible to an investigator who makes controlled crosses between individuals and looks at the progeny. By observing regularities in the numerical relationships among the different forms of a trait in hybrid offspring, Mendel inferred both the behavior (segregation and independent assortment) and the presence of genetic factors that we now call genes. The principles established by Mendel help to explain heredity (the similarity of offspring to parents) and variation (the differences among parents and their offspring).

Segregating kernels on ears of corn and segregating seedling populations of corn, tobacco, and tomatoes have traditionally been used for genetic studies. For this lab, seeds of two types of *Brassica rapa* plants are provided as parents: wild type and mutant. *Brassica rapa*, with their rapid life cycle, will allow you to form a hypothesis about the inheritance of a trait, and test your hypothesis by obtaining data through two generations.

New Terms

cross-pollination The transfer of pollen from the anther of one plant to the stigma of another.

dominant A gene that exerts its full phenotypic effect regardless of its allelic partner, thus masking the partner's effect.

gene A sequence of nucleotides along a molecule of DNA (RNA in some viruses) that makes up a unit of inheritance.

independent assortment The normally random distribution of alleles during meiosis.

phenotype The observable properties (structural and functional) of an organism, produced by interaction of the organism's genetic potential (its *genotype*) and the environment. The term *phenotype* can be applied either to the totality of expressions of the genotype *or* to only a part (i.e., to particular characters or traits). The phenotypic range or expression is referred to as its *reaction norm*.

recessive A gene whose phenotypic expression is masked by its dominant allele.

segregation In genetics, the separation of allele pairs from one another and their distribution to different cells (usually at meiosis) observed only in heterozygous genotypes.

self-incompatibility The mechanism by which the stigma of a flower recognizes pollen from the same plant and prevents fertilization from taking place.

self-pollination The transfer of pollen from the anther of a flower to the stigma of the same flower or of another flower on the same plant; usually referred to as "selfed."

Objectives

In this lab you will investigate the inheritance of the gene(s) controlling a mutant trait. You will cross-pollinate F_1 plants to produce F_2 seeds. Then you will grow F_2 plants and observe segregation.

"Background" adapted by R.V. James, University of Wisconsin-Madison, Department of Plant Pathology, from Brassica campestris: *A Resource Book for Secondary Biology Teachers* by Robert S. Hafner, Department of Curriculum Instruction, University of Wisconsin–Madison.

Materials

For each group of students:

- 12 F_1 hybrid RCBr seeds
- quad and necessary planting materials

Pre-Lab Questions

1. How can you distinguish whether the mutant trait you are using is inheritable or is the result of environmental effects?

2. If the mutant trait is under genetic control, is it controlled by a single gene, a few genes, or many genes? What clues will help you determine how many genes are involved?

3. If a single gene controls the mutant trait, how will you determine the **dominant** and the **recessive** expression of the gene?

4. What is *your* hypothesis about the inheritance of the mutant trait?

Procedures and Observations

First Generation (F_1 Hybrid)

Day 1

Follow the "Growing Instructions" and plant F_1 seeds in all four cells of your quad. Be sure to label your quad.

Your instructor will plant a quad of each of the parental seed types (wild type and mutant type) for comparison.

Day 4 to 5

Compare the F_1 plants with both parental types. Record the number of wild-type and mutant plants that you observe among the F_1 plants in Table 1. Thin to the most vigorous plant in each cell.

Day 14 to 18

Pollinate plants. Gather pollen on a bee stick from one of your F_1 plants and use it to pollinate another F_1 plant (there should be two of three flowers open on most plants when you start pollinating). Pollen may be exchanged among as many of the F_1 plants as you wish (within your quad or among all plants in the class; the more, the better at this stage). Do not worry about removing the anthers from a plant being pollinated; because the RCBrs are **self-incompatible**, little **self-pollination** will occur. ***Be sure*** that pollen from the parental-type demonstration plants is not used for these pollinations.

After pollinating, insert the bee stick in the potting mix at the base of one of your plants.

Pollinate plants about 2 and 4 days later. It is best to pollinate six to eight flowers. After your *last* pollination, pinch or cut off *all* unopened buds and shoots so that uncontrolled pollinations cannot occur.

Day 20 to 45

Continue to remove new buds and shoots for the next 2 weeks. Keep plants watered and growing for 3 weeks after you finish pollinating to allow the seeds to ripen. Dry plants and harvest seeds as described in the "Growing Instructions." Seeds from your plants may be combined.

Second Generation (F_2)

Day 1

The mutant traits anl, ygr, and ros are all recognizable at the seedling stage. Place one filter paper disc in the lid of a petri dish. Write your name and the symbol of the mutant in pencil on the filter paper. Add water until the paper is wet; pour off excess water. Place 25 seeds in four neat rows on the upper two-thirds of the filter paper. Place the cover on the dish and stand the dish in about 2 cm of water in a reservoir. Tilt the dish slightly so that water collects at the bottom and place the dish under the light bank. Seeds should all be above the water line (Figure 1).

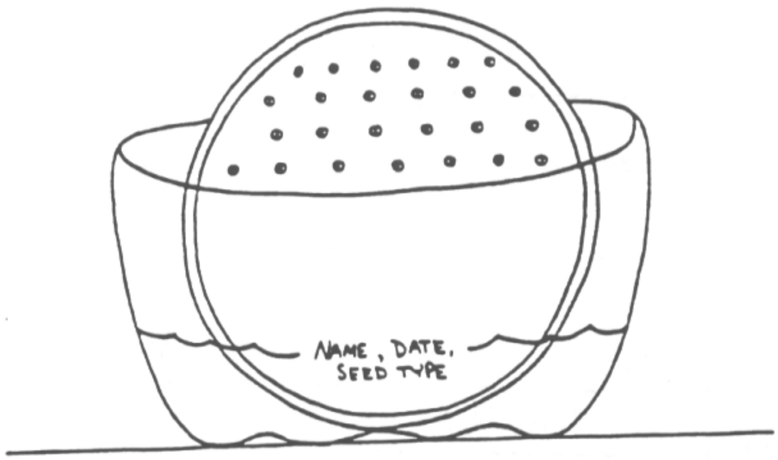

Figure 1. Reservoir with petri dish.

Day 3 to 5

Observe seedlings and record numbers of mutant and wild-type plants in Table 2.

Chi-Square Test

A test that is often applied to determine how well observed ratios fit expected statistical ratios is known as the chi-square (χ^2) test. This test calculates the deviations of observed numbers from expected numbers into a single numerical value called χ^2. The difference between the number observed and the number expected for a phenotype is squared and divided by the number expected. This is repeated for each phenotype class. The χ^2 value consists of the summation of these values for all classes. The formula for χ^2 (Suzuki, et al., 1986) is:

$$\chi^2 = \text{total of} \quad \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \quad \text{over all cases}$$

Associated with each χ^2 value is a probability that indicates the chance that, in repeated experiments, deviations from the expected would be as large or larger than the ones observed in this experiment. Table 3 lists probabilities and χ^2 values.

In Table 3, note the column "Degrees of Freedom." In an experiment, the degree of freedom is one less than the number of different phenotypes possible. In this experiment we have two possible phenotypes (the normal and the mutant) so there are 2 – 1 = 1 degree of freedom. If the probability is greater than 5% (0.05), we accept the observed data.

Calculate the χ^2 value for the class data observed in the F_2 generation. Assume simple dominance and an expected 3:1 ratio, wild type:mutant.

Example:

If, in an F_2 population of 100 plants, results are 60 wild type:40 mutant (expected ratio would be 75 wild type:25 mutant), then:

$$\chi^2 = \frac{(60-75)^2}{75} + \frac{(40-25)^2}{25} = 3 + 9 = 12.0$$

Looking in the χ^2 table for $\chi^2 = 12$ with 1 degree of freedom, probability = < 0.01, therefore, these results are not supportive of a 3:1 ratio since the probability is less than 5% (0.05).

Name: ______________________________

Table 1. *Observation of First Generation (F_1) Seedlings.*

Date planted __________

Date	Age of plants	Number of plants			
		Wild type		Mutant	
		Yours	Entire class	Yours	Entire class

Date pollination finished __________

Date seeds harvested __________

Table 2. *Observation of Second Generation (F_2) Seedlings.*

Date planted __________

Date	Age of plants	Number of plants			
		Wild type		Mutant	
		Yours	Entire class	Yours	Entire class

χ^2 value (entire class data) = __________ Probability = __________

Discussion

1. Describe the plants in the F_1. What does this suggest about the inheritance pattern of the mutant trait?

2. What do the combined class data for the F_2 suggest about the inheritance of the mutant trait? (Remember to consider the number of genes involved as well as dominance or recessiveness.)

3. What can you conclude from the χ^2 and probability values?

4. Are the deviations from a 3:1 ratio in the F_2 significant?

5. Do the results agree with your original hypothesis? If not, what is your modified hypothesis?

6. What additional cross(es) would you do to verify your hypothesis?

7. If the mutant trait you used was rosette, when counting the number of rosette plants germinating in the F_2 generation, the assumption is made that seeds not germinating are rosettes. Would this assumption be valid if you were using F_2 seeds which had been produced by a previous year's class instead of your own freshly harvested seeds? Explain.

Table 3. χ^2 *Values and Probabilities.*

Degrees of Freedom	Possibility of Chance Occurrence in Percentage (5% or Less Considered Significant)								
	90%	80%	70%	50%	30%	20%	10%	5% (sig.)	1%
1	0.016	0.064	0.148	0.455	1.074	1.642	2.706	3.841	6.635
2	0.211	0.446	0.713	1.386	2.408	3.219	4.605	5.991	9.210
3	0.584	1.005	1.424	2.366	3.665	4.642	6.251	7.815	11.341
4	1.064	1.649	2.195	3.357	4.878	5.989	7.779	9.488	13.277
5	1.610	2.343	3.000	4.351	6.064	7.289	9.236	11.070	15.086
6	2.204	3.070	3.828	5.348	7.231	8.558	10.645	12.592	16.812
7	2.833	3.822	4.671	6.346	8.383	9.083	12.017	14.067	18.475
8	3.490	4.594	5.527	7.344	9.524	11.030	13.362	15.507	20.090
9	4.168	5.380	6.393	8.343	10.656	12.242	14.684	16.919	21.666