

Chapter 7

The Power of Genetics: Using Classical and Molecular Genetics to Study “Real” Developmental Phenomena

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Introduction

Rationale

Traditionally, a course in genetics begins with Mendel and monohybrid/dihybrid crosses, progresses toward linkage, usually includes the basics of molecular genetics, and analyzes pedigrees. Typical lab exercises include *Drosophila* crosses, microscopic studies of meiosis and mitosis, and maybe a DNA isolation or polymerase chain reaction (PCR) analysis of polymorphisms among the student population.

Unfortunately, this traditional approach often fails to demonstrate the utility of a genetic approach in understanding the complex processes upon which all of life depends, and much of what is learned in the course is soon forgotten. As has been elegantly demonstrated repeatedly, genetic analyses can be used to dissect both biochemical and developmental pathways. In order for students to appreciate the power of the genetic approach, however, they need to see how it can be applied to “real life” investigations.

The goal of this laboratory exercise is to provide a laboratory experience for undergraduates in which they will use genetics to study a complex developmental process. In order to meet this goal, I have integrated some of my research into laboratory experiences for two of my courses: BIOL 365 (Plant Anatomy and Physiology) and BIOL 400 (Molecular Biology). The first run of this experience in the Plant A&P course occurred this past Spring 2001.

I am interested in understanding the process by which cell shape is determined. I use the simple plant *Arabidopsis thaliana* as my model system. In particular, I focus on root cell shape

development, because the root is a relatively simple structure and mutations that affect root cell shape can be easily distinguished from those that affect root cell number. In addition, cell shape in the root is relatively simple: root cells are generally cylindrical. Root cells can also be readily visualized after chemical treatment and their dimensions quantified.

The Experience

Fundamentally, students need to understand the connection between genotype and phenotype. Biochemical and developmental processes depend upon sequences of reactions that, in turn, are dependent upon the activities of the proteins produced from the genes within a cell. In order to understand a developmental process an investigator needs to know the proteins involved and the roles they play. In order to identify these proteins he/she can look for mutations that alter the process and then trace the phenotypic alterations to alterations in the genotype. Therefore, if he/she wants to understand how a specific organ of a plant develops, he/she can look for mutations that affect the development of this organ. Using genetic and molecular analyses, the gene at the locus that had been affected can be identified and further analyzed.

This exercise consists of three components, which together will require an entire semester. The class is divided into groups of three or four students.

I. Genetic Screen

In the first component, each group conducts a small-scale genetic screen (500 seeds) to identify mutants that are affected in the development of the root. Each group grows ethyl methanesulfonate (EMS)-treated *Arabidopsis* seeds on media containing Murashige & Skoog salts supplemented with sucrose. Each group then identifies any plants with aberrant roots and transfers these to soil. These mutants will be analyzed by later classes. This component takes approximately six weeks out of the semester and begins concurrently with the two other components.

II. Phenotypic Segregation

In the second component, the students analyze an F₂ population of seeds that are segregating for a mutation that results in aberrant roots. (This mutation was identified as a result of previous screens. I will have completed the crosses required for these analyses.) The students plate these F₂ seeds as above and score the population of seedlings for wild-type or mutant roots. From these numbers, they determine if the mutation is dominant or recessive. This component requires three weeks.

III. Genotypic Analysis

In the third component the students determine the map position of the mutation. The students collect the mutant F₂ plants and then isolate the DNA from each plant. The DNA from these plants is analyzed using the polymerase chain reaction (PCR) to determine the genotypes of the plants at specific locations on the chromosomes. If the genotypes observed at these locations do not show a 1:1 segregation for both of the parental ecotypes, we will know that the mutation and the marker are linked. If the marker and the mutation are linked, the approximate location of the locus in question can be determined by calculating the recombination frequency. This component requires most of the semester.

Modifications

Although the exercise outlined above focuses on root mutants, the lab can be easily modified to study other phenotypic mutants (*e.g.*, leaves, flowers) or nutrient/hormone mutants (*e.g.*, auxin, nitrate), both of which would be relevant in a plant anatomy and physiology course. Seed stocks for many different *Arabidopsis* mutants are also available from the *Arabidopsis* Biological Research Center (ABRC, Ohio State University) and could be used instead of conducting a genetic screen to identify additional mutants. Many of these mutants could be analyzed on soil, precluding the requirement for plates and sterile media. The third component of this lab exercise can be completed by anyone with a thermal cycler, PCR reagents, and the materials and equipment required to analyze the products.

Outline

(including notes for student and instructor)

a. Model Organism

Arabidopsis thaliana is a small weed of the family Brassicaceae (mustard) family, a family that includes such familiar members as cabbage (and its derivatives) and radish. *Arabidopsis* is of no significant economic importance, but it has become one of the most intensively studied plant model systems over the past ten years. *Arabidopsis* has a number of characteristics that make it a useful model system:

- it is a relatively small vegetative plant, consisting of a spiral of leaves in a rosette. The inflorescence appears in about four weeks and may become 18-24 inches or more in length;
- it reproduces fairly rapidly, setting first seeds in about six weeks;
- it has a relatively small genome (134 Mb) dispersed among five different chromosomes, and the genome has now been sequenced in its entirety;
- it can be transformed using *Agrobacterium tumefaciens*, and creating transgenic plants is relatively straight-forward.

As a result of the relative ease with which it can be grown and manipulated, many investigators around the world have identified a wide range of mutant *Arabidopsis*, affected in everything from organ morphology to hormone responses to nutrient uptake and assimilation. A centralized stock center collects and disseminates these seeds to the community for no or a low fee (The *Arabidopsis* Biological Resource Center, <http://aims.cps.msu.edu/aims/>). The *Arabidopsis* community convenes annually (The International *Arabidopsis* Meeting, now in its 12th year) and is also supported by its own website (The *Arabidopsis* Information Resource, www.arabidopsis.org).

b. First Component: Genetic Screen

Notes for Instructor:

Students can identify relatively easily mutant *Arabidopsis* plants. Instructors can obtain mutagenized seeds from Lehle Seed Co (<http://www.arabidopsis.com/>) (which gives a discount for bulk seeds used as teaching tools – call and ask for the current discount). The seeds can be spread on top of soil, where they will germinate within a few days, or they can be grown sterilely on solid media containing Murashige and Skoog salts in 10 cm x 10 cm square plates (Fisher catalog #08 757 11A). The media is relatively easy to prepare:

1x Murashige and Skoog salts (available from Invitrogen or Sigma)
 45 g/L sucrose*
 0.5 g/L MES (2-(4-morpholino)-ethane sulfonic acid)
 8.0 g agar (BBL product #211849 available from Fisher)
 pH adjusted to 5.7 with 1 M KOH

** I should comment that I use 4.5% sucrose in my plates, which is considered by many to be a fairly high level of sucrose. The standard in the community appears to be 1.0%. We use 4.5% in order to identify the desired root mutants. If you look for other mutants, then using 1.0% sucrose will work perfectly fine.*

One liter of this media makes approximately 25 plates. Between twenty and twenty-five seeds can be placed within a row on each plate and two rows of seeds can be placed on each plate. In the Spring 2001 Plant A&P course, I had my students do several plates of wild-type seeds for practice (one row/plate, so about 25 seeds/plate). For the genetic screen, I required them to plate 500 mutant seeds/group for their screen (which required about 10 plates with two rows of about 25 seeds apiece for each group).

Using plates is especially helpful when you want to conduct a genetic screen for plants that show altered sensitivities to specific compounds, such as hormones or nutrients, because these can be added sterilely to the media before the plates are poured.

At the same time the students are plating the mutagenized seeds, I also have them do several plates with the wild-type plants (as controls). The plates can be scored for mutants after the plants are 10-14 days old. First, I have them look carefully at the wild-type plants and become familiar with the general morphological characteristics of the plant, especially the roots. Then I have them look on the plates with the mutagenized seeds for anything that looks different and have them record in what way they look different. Among the population of seeds, they should be able to find several with white or yellow cotyledons (which are used to determine the efficiency of the mutagenesis). In my Spring 2001 BIOL 365 course, I had them focus on root mutants, but the students doing this exercise can be asked to look for any class (or classes) of mutants that the instructor is interested in.

Student Handout:

In the first part of this laboratory exercise, you will be identifying *Arabidopsis thaliana* plants that are mutant in a specific developmental process. In order to do this, you will need to obtain both wild-type and mutated seeds, sterilize them, plate them on sterile media, and then allow them to grow. After two weeks, you will compare the roots from the wild-type plants with the roots of the possibly mutant plants to see what kinds of mutants you can identify with respect to their root phenotypes. Many of the plants derived from your mutagenized seeds will not look any different from the wild-type. (*Why?*) I will not tell you what classes of mutants you will find – this part of the exercise is to sharpen your observation skills and use those skills to detect differences between the roots from some of the mutagenized seeds and those from the wild-type seeds.

You will be growing your plants on sterile media and will need to use sterile technique. In order to sterile your seeds:

1. Aliquot the amount of seeds that you want to sterilize into a 1.5-ml Eppendorff tube. DO NOT attempt to sterilize more than approximately 0.1 ml of seeds at a time – they will not become

sterile if you do so. Remember that the seeds will begin to germinate in the tubes if left in the water too long after sterilization.

2. Make the bleach solution by pouring 20 ml of bleach into a small beaker. Add two to three drops of Tween-20 to the bleach and swirl to mix.
3. Add about 1 ml of bleach solution to the seeds.
4. Close the lid – make sure it is closed securely – and rock the tube back and forth for two and a half minutes. Be sure that the seeds are not clumped together or else they will not become sterile.
5. After two and half minutes, place the tube in a rack and allow the seeds to settle to the bottom of the tube.
6. Remove the bleach solution using a sterile pipette and discard it into an appropriate waste container.
7. Add sterile water, close the lid, and rock 50 times.
8. Remove the water using a sterile pipet.
9. Repeat numbers 7 and 8 two more times.
10. After the final rinse, add about 0.5 ml water. Carefully draw some of the seeds into a new pipette and place the seeds one at a time on the surface of the plate by gently touching the tip of the pipette to the surface of the plate. Be sure to hold the plates away from you (media side toward the hood) so as to prevent contamination.
11. You should place the seeds in two rows, about twenty to twenty-five seeds per row. The top row should be approximately a centimeter from the top of the plate, the second approximately halfway down.
12. Once you have finished all of your plating, label them all with your name, the date, and the types of seeds (wild-type or mutant).
13. Wrap the plates with parafilm and place them in the refrigerator. These will be taken out about two days after you have put them into the refrigerator.

c. Second Component: Phenotypic Analysis

Student Handout:

Arabidopsis populations growing in specific geographic locations (referred to as “ecotypes”) have developed slight differences in their genetic material as a result of mutation and natural selection. These differences in the genetic material can be used to distinguish between the DNAs isolated from the different ecotypes: between the Columbia (Col) ecotype and the Wassilewskija (Ws) ecotype, for example. The genetic differences most commonly used to determine the map positions of mutations fall into two classes: simple sequence length polymorphisms (SSLPs) and cleaved amplified polymorphic sequences (CAPS). I will explain these in more detail below.

My research students and I have identified a number of root cell shape mutants that we are interested in studying further. Most of the root cell shape mutants that we have isolated are in the Columbia ecotype. For the F1 generation, we crossed these mutants to or with a wild-type plant from the Wassilewskija (Ws) ecotype. For BIOL 365 in Spring 2001 I crossed the mutant line designated Su99 #38 with a wild-type Ws line and obtained F1 seeds. (*I should note that the crosses in the majority of research labs are not Col x Ws, but instead Col x Landsberg, a much more common and more studied ecotype than Ws. We used Ws for historical reasons that are not relevant to this workshop.*)

The F1 generation of these plants is thus heterozygous for both of the parental ecotypes, having, for example, one “Col chromosome I,” and one “Ws chromosome I”. The same is true for all five chromosomes. Before the course began, I grew these F1 seeds on plates to score for the root phenotype: in this specific case, all of the roots of the F1 plants were wild-type, indicating that the mutation in the Su99 #38 line is recessive. (*Can you explain why this result indicates this conclusion?*)

Recombination then occurred during the production of gametes in the F1 plants. Thus the chromosomes within the gametes of the F1 plants are no longer either 100% Col or 100% Ws – but instead are patchworks of both Ws and Col. *Arabidopsis* is a self-fertilizing plant, therefore, the F2 seeds resulting from the combination of any two recombinant gametes may be homozygous Col, homozygous Ws, or heterozygous Col/Ws at any location on any chromosome.

Before the course began, I transferred the F1 plants to soil, allowed these plants to self, and harvested the F2 seeds. Each group now needs to sterile and plate 500 of these F2 seeds in order to determine if the mutation is segregating in the expected ratio. (*What is the expected ratio and why? What should you also plate for your controls?*)

Allow the plants to grow for 10-14 days and then score them for either wild-type or mutant roots. Once you have scored these plates, you should harvest the plants with the mutant roots, placing each one individually into 1.5 ml microcentrifuge tubes, which then should be frozen at –70°C.

Notes for Instructor:

The expected ratio of wild-type to mutant in this cross is 3:1, since it is a simple monohybrid cross. The actual numbers from the BIOL 365 course this spring were 580 wild-type: 131 mutant.

d. Third Component: Mapping the Mutation using Genotypic Analyses

Student Handout:

We can use the genetic differences between the ecotypes in order to determine the specific location of the mutation in these mutant plants. First, we isolate the DNA from the mutant plants in the F2 generation. Importantly, not only are these plants homozygous for the mutation, they are also homozygous Col at the location of the mutation (and probably for some distance around the mutation as well.) We can now systematically check the genotypes of these plants at specific locations on each of the five chromosomes using specific “markers” (pairs of primers used in polymerase chain reaction that distinguish between the ecotypes). If the mutation is located near one of the locations being analyzed, the frequency of the genotype of that location will be skewed toward the ecotype of the original mutant. Thus, in our case, we are looking for a location on a specific chromosome that shows a predominance of the Col ecotype over the Ws ecotype.

In order to determine the genotypes of the plants at these specific locations on the five chromosomes, we will use polymerase chain reaction (PCR). As I mentioned above, the genetic differences between the ecotypes used for mapping analyses fall, in general, into two classes: simple sequence length polymorphisms (SSLPs) and cleaved amplified polymorphic sequences (CAPS). SSLPs are microsatellites; these are simply repeats of simple sequences (usually di- or trimers) that differ in the number of repeats between the ecotypes.

For example, at a specific location on chromosome I in the DNA from a Col plant, the sequence might read

...ACCGGCCACACACACACACACGTTGGTGCAAAA...

while the DNA sequence from a *Ws* plant at the same location might read

...ACCGGCCACACACACACACACACACACACGTTGGTGCAAAA...

So, whereas the *Col* has the dimer AC repeated 7 times, the *Ws* has it repeated 11 times. If we use a set of primers (“a marker”) to amplify this region of chromosome I from a homozygous *Col* and a homozygous *Ws* plant and then analyze them using electrophoresis, we would see that both samples produce a single PCR product and that the PCR product from the *Col* plant is smaller than the *Ws* plant (by 8 nucleotides). A heterozygous plant would produce two bands – one the same size as the *Col* homozygote and one the same size as the *Ws* homozygote. We could then take DNA from any plant from a cross between *Col* and *Ws* and determine if the plant is homozygous *Ws*, homozygous *Col*, or heterozygous *at that specific location* on that chromosome using the same primers. In other words, we have determined the genotype of these plants using this molecular marker.

CAPS distinguish between the ecotypes as a result of different restriction endonuclease sites: one ecotype has more sites than the other does within a specific region of a chromosome. The region is amplified using PCR, and the PCR fragments are then digested and analyzed on an agarose or acrylamide gel. The genotype of the plant can be determined based on comparison with the homozygous plants from the two ecotypes used in the original cross.

Linkage. Two loci assort independently if they are unlinked. If the gene that is mutated in our root mutant is not physically linked to a specific location on one of the chromosomes, then when we analyze the results of the PCR using the marker at that location, we should see 50% *Columbia* alleles and 50% *Ws* alleles within that population. Therefore, if we analyzed the DNA from 40 of our F2 mutant plants using marker *nga139* and we saw that there were 40 *Col nga139* alleles and 40 *Ws nga139* alleles in that population, we would conclude that the mutation is not linked to marker *nga139*. Since we know that *nga139* is on chromosome V, we know that our mutation is NOT located on chromosome V close to *nga139*. (Note: It may still be on chromosome V, just not close to *nga139*.) If, however, we use *nga172* (located on chromosome II at position 6.9 cM), and we see that we have 70 *Col* alleles and only 10 *Ws* alleles, we have evidence of linkage. The two ecotypes are not segregating independently in our mutant population: the mutant population shows a predominance of the *Col* genotype at this location, indicating that the marker and the mutation are physically associated with each other. We can calculate the recombination frequency from this data and get a rough idea of where the mutation is located relative to the marker:

$$\begin{aligned} \text{recombination frequency} &= \# \text{ Ws alleles} / \# \text{ total chromosomes in population} \\ &= 10/80 \\ &= 0.125 \end{aligned}$$

Recombination frequency (Rf) is not identical to map distance as a consequence of double crossover events. However, the RF is sufficiently low and the population size large enough to strongly indicate that the mutation is roughly 12.5 cM away from the marker *nga111* – and is thus probably located around 20 cM on chromosome II.

In order to complete this component of the laboratory exercise, you will isolate the DNA from your mutant F2 plants, perform PCR using one of the above markers, and then genotype your plants by analyzing the results from your PCR reactions on acrylamide gels.

Notes for Instructor:

Sufficient numbers of SSLP and CAPS markers are available so that by using approximately twenty different ones, any mutation can be placed onto one of the five *Arabidopsis* chromosomes using the Col and Ws lines discussed above. Table 1 lists the markers and their genetic map locations that we used to analyze the Su99 #38 (Col) and Ws cross in Spring 2001. I should mention again that mapping is greatly facilitated if one uses the Columbia and Landsberg erecta ecotypes (rather than Col and Ws), as these are the two well-studied ecotypes in *Arabidopsis* and, as a consequence, many more markers are available to analyze these crosses. I should also point out that the table contains the primer pairs we have found useful, but other primer pairs may work as well or better than those listed. For an up-to-date list of current molecular markers and the specific sequences of the following markers, please see the TAIR website, <http://www.arabidopsis.org>.

Table 1. List of SSLP markers used to genotype the Su99 #38 x Ws F2 mutant population.

Chromosome I	Map Position	Chromosome IV	Map Position
nga59	1.0	JV30/31	c. 2
ciw12	39	nga8	27
nga280	81.7	ciw7	65
nga111	105.8	DHS1	106
Chromosome II		Chromosome V	
nga1145	9.6	ciw18	c. 15
F8P2-T7	c. 30	nga139	50.5
nga1126	50.7	nga76	68
nga168	73.8	ciw9	88
Chromosome III		nga129	105.4
nga172	6.9		
ciw11	43.0		
T16-K5-TGF	c. 60		
nga6	86.0		

*Student Handout:***Protocol: DNA Isolation**

In order to determine the genotypes of the plants using the PCR markers, we have to first isolate the DNA from the mutant plants in the F2 generation. This protocol is a modification of that published by Klimyuk *et al.* (1993).

1. Plate the F2 seeds of the cross between the mutant (Col) and the wild-type Ws (as described above).
2. Place the plates in the refrigerator for three days, then transfer to a 16 hr light:8 hr dark cycle. Be sure to position the plates vertically so that the roots grow along the surface of the medium.
3. After 7-10 days, the phenotypes of the roots should be obvious and you should see segregation in the expected 3:1 ratio.
4. Remove the parafilm and select the plants with the mutant roots, placing each one into a separate 1.5-ml microcentrifuge tube. These tubes can be stored at -70°C for preparation later or immediately processed.
5. When you choose to process your samples, keep them on ice as much as possible.

6. Grind the samples with the plastic pestles (Fisher catalog #K749520 0000). You do not need to grind them for very long – just mash them until they have turned to a green liquid. Be sure to leave the sample in the tube – it may sometimes stick to the pestle!
7. Add 10 microliters of 0.5 M NaOH and tap gently to get the drop to fall to the bottom of the tube.
8. Run in speed vac for 1 minute. Start timing from when the vacuum engages, not from the time you start the rotor.
9. Flick the tubes gently.
10. Run in speed vac for 2 minutes.
11. Heat the samples in 95°C heat block for 30 seconds. (Count the time from when you place the last sample in the heat block.)
12. Add 20 microliters 1 M TRIS, pH 8.0 and 80 microliters TE to your samples.
13. Flick gently to mix and store at –20°C.

Protocol: PCR

Once you have the DNA isolated, you are ready to perform PCR. The more samples you analyze the better your mapping data will be. For a rough map position, 40-50 samples are probably sufficient.

Notes for Instructor:

You can order primers from a number of companies, including Fisher, Sigma, Invitrogen, and Research Genetics. I purchase 50 nanomoles of each primer. Once I receive the primers, I resuspend them in 100 microliters of TE, pH 8.0, and aliquot 5 microliters into twenty labeled 1.5-ml microcentrifuge tubes. I spin these tubes in the speed vac and store these aliquots at –20°C. For each set of PCR reactions, I prepare the required number of Master Mixes, which contain all components required for PCR except the polymerase and the DNA.

I prepare all of the required Master Mixes beforehand and freeze them at –20°C. To prepare the Master Mix for a thermal cycler with twenty-four wells (23 samples + temperature sensor):

1. Add 500 microliters of water to each of the two primer aliquots (from above) and vortex well.
2. Mix:

60 microliters	10x PCR buffer (supplied with the Taq polymerase)
24 microliters	dNTP mix (0.25 mM @)
36 microliters*	25 mM Mg ⁺² solution (usually supplied with the Taq polymerase)
24 microliters	forward primer (prepared as in #1)
24 microliters	reverse primer (prepared as in #1)
406 microliters	sterile deionized water

Multiple tubes of Master Mix for each primer pair can be prepared at the same time. These can be stored at –20°C and used at a later time.

One of the major determinants of the success of PCR is the concentration of Mg⁺² ions. All of the primers listed in Table 1 work at a final concentration of 1.5 mM (the concentration indicated in step #2 above) unless otherwise noted at the TAIR site.

For most SSLPs, I use	1 cycle	92°C	3 minutes
	40 cycles	94°C	15 sec
		55°C	15 sec

	72°C	30 sec
1 cycle	72°C	1 minute
hold	room temperature	

Student Handout:

To perform PCR,

1. Label 23 0.2-ml tubes 1-23.
2. Thaw the appropriate Master Mix and add 1.2 microliters of Taq DNA Polymerase. Gently draw the solution up and down to mix well.
3. Aliquot 24 microliters of the Master Mix to each of the 23 tubes.
4. Add 1 microliter of the DNA samples to each successive tube. Include wild-type Col and Ws DNA samples as controls.
5. Place tubes in thermal cycler and run cycles.

Protocol: Electrophoresis*Notes for Instructor:*

The protocol outlined below is the one we use in our lab. There are multiple apparatuses and approaches to analyzing the results of the PCR so this may be modified to suit the equipment available.

Student Handout:

In order to determine the genotypes of your plants, you will need to run your PCR products on an acrylamide gel.

1. Clean a set of square and notched plates (gel size 20x20 cm) with water and alcohol.
2. Place the plates in the casting apparatus. Align the 1.5-mm spacers along the sides of the plates.
3. In a large flask mix
 - 18 ml 40% acrylamide solution (1bisacrylamide:29 acrylamide)
 - 12 ml 20x TAE
 - 96 ml deionized water
 - 120 microliters 30% ammonium persulfate (freshly made)
4. Place 5 ml of this solution in a 15-ml tube. Add 60 microliters of TEMED, mix quickly, and pour the solution into the plates. This solution will act as a plug to seal the bottom of the gel.
5. Once the plug has polymerized, add 120 microliters TEMED to remaining polyacrylamide solution and pour between plates.
6. Insert the combs into the solution and allow to polymerize.
7. Add 5 microliters of DNA sample loading dye to the PCR samples while the gel is polymerizing.
8. Once the gel has polymerized, remove the comb and rinse the wells with 1x TAE.
9. Set up the electrophoresis apparatus, add 10 microliters of your samples to the wells, and run the gels at 200 mV for two hours. A second set of reactions can be loaded onto the same gels at this point and the gel then run for two more hours.

10. Disassemble the electrophoresis apparatus and stain the gels in ethidium bromide.
11. Record the results of your gel using the Polaroid camera set-up in the darkroom.

Data Analysis:

By comparing the results from the two control DNAs (Col and Ws) with the results from your DNA samples, you should be able to determine if the plants you analyzed were homozygous Col, homozygous Ws, or heterozygous. Record your data in the following table. You can calculate recombination frequency using the following formula:

$$\text{recombination frequency} = \frac{\# \text{ WS chromosomes}}{\# \text{ total chromosomes analyzed}}$$

$$\text{where } \# \text{ WS chromosomes} = (2 \times \# \text{ Ws homozygotes}) + (1 \times \# \text{ heterozygotes})$$

		# Col homozygotes	# WS homozygotes	# heterozygous	RF
Chromosome I					
nga59	1.0				
ciw12	39				
nga280	81.7				
nga111	105.8				
Chromosome II					
nga1145	9.6				
F8P2-T7	c. 30				
nga1126	50.7				
nga168	73.8				
Chromosome III					
nga172	6.9				
ciw11	43.0				
T16-K5-TGF	c. 60				
nga6	86.0				
Chromosome IV					
JV30/31	c. 2				
nga8	27				
ciw7	65				
DHS1	106				
Chromosome V					
ciw18	c. 15				
nga139	50.5				
nga76	68				
ciw9	88				
nga129	105.4				

3. Results of the PCR Analyses:

The data we collected during the Spring 2001 semester is summarized in Table 2. Several of the markers that we used did not work well (designated “too faint” in the table), and several did not show any polymorphism between Col and Ws. As this marker set was based upon known polymorphisms between Col and Ler, these results were not unexpected in this first set of analyses. The mutation in the line we analyzed (Su99 #38) appears to be located on the bottom of chromosome V, since the marker at this location (nga129) showed the lowest recombination frequency (0.28).

Table 2. Results of the genotyping of the Su99 #38 x Ws lines.

		# Col homozygotes	# WS homozygotes	# heterozygous	RF
Chromosome I					
nga59	1.0	3	3	15	0.50
ciw12	39	5	3	13	0.45
nga280	81.7	4	6	11	0.55
nga111	105.8	5	5	11	0.50
Chromosome II					
nga1145	9.6	7	3	10	0.40
F8P2-T7	c. 30	NP			
nga1126	50.7	NP			
nga168	73.8	6	4	9	0.45
Chromosome III					
nga172	6.9	7	8	5	0.53
ciw11	43.0	4	5	11	0.53
T16-K5-TGF	c. 60	TOO FAINT			
nga6	86.0	3	7	10	0.60
Chromosome IV					
JV30/31	c. 2	7	4	9	0.43
nga8	27	TOO FAINT			
ciw7	65	0	8	9	0.74
DHS1	106	4	11	5	0.68
Chromosome V					
ciw18	c. 15	6	6	9	0.50
nga139	50.5	5	4	10	0.47
nga76	68	6	3	9	0.42
ciw9	88	9	3	9	0.35
nga129	105.4	12	3	5	0.28

4. Discussion of the Project:

Having the students apply the concepts that they have learned in previous courses enables them to develop their understanding of the scientific process. Although I found that several of my students had difficulties grasping the concept of using linkage analysis to determine the map position of a gene, I know that several of them were encouraged by their foray into “real world” science. As this was my first attempt at involving a larger group in an independent project I will be making several modifications to the experience in the spring of 2003:

- (1) I will have determined before the project begins which primers will work well and have found a set of twenty primer pairs (markers) that will yield useful information;
- (2) I will require them to operate in pairs rather than in groups of four so that more of them will be involved in all aspects of the project;
- (3) I will spend more time explaining the processes involved at the beginning of the lab and ensure that they have a better understanding before we do each of the steps involved;
- (4) We will do fewer DNA preparations as a class but analyze more of their samples. This year we prepared 142 samples but analyzed only 23;
- (5) I will ask them to do more short lab write-ups than a single laboratory report due at the end of the semester. This will enable me to assess better their comprehension of the techniques and of the project in general.

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