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Lesson

A Rapid Genetic Screen Using Wisconsin Fast Plants®: A Hands-On Approach to Inheritance of *de novo* Mutations

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Abstract

Some concepts in genetics, such as genetic screens, are complex for students to visualize in a classroom and can be cumbersome to undertake in the laboratory. Typically, very large populations are needed, which can be addressed by using micro-organisms. However, students can struggle with phenotyping microbes. For macroscopic organisms, the number of offspring produced, and the generation time can be challenging. I developed this lesson as a small-scale genetic screen of Fast Plants®. These plants are amenable to teaching labs as they have simple growth requirements, a short generation time, and produce numerous seeds that can be stored for years. Seeds used for this screen are purchased pre-treated with a DNA damaging agent, removing the need for in-house use of mutagens. Also, students can screen the phenotypes without specialized equipment. The initial lesson begins with an examination of the first generation of plants. Later their offspring are screened for altered phenotypes. Students responded well to having full-grown plants available on the first day of the lab project. This lesson fostered student collaboration, as they worked with class datasets. Differences in growth due to mutagenesis treatment in the first generation were clear to students who had not worked with plants before. Identifying plants with altered phenotypes in the next generation was more of a challenge. This lesson incorporates key concepts such as somatic and germline mutations, the impact of such mutations on phenotype, and the inheritance of mutation alleles, and provides a hands-on way to illustrate these concepts.

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Supporting Materials: S1. Fast Plant Screen – Introductory slides; S2. Fast Plant Screen – Supply list; S3. Fast Plant Screen – M1 planting scheme; S4. Fast Plant Screen – M1 data collection spreadsheet; S5. Fast Plant Screen – M1 generation lab packet; S6. Fast Plant Screen – M2 generation lab packet; S7. Fast Plant Screen – How to create a randomized planting plan; S8. Fast Plant Screen – Rubric for evaluating hypothesis and graphic, and example hypotheses.

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Learning Goals

Students will:

- Understand the difference between somatic and germline mutations and their effects on inheritance.
- Strengthen scientific processes and laboratory skills.
- Gain hands on experience with Fast Plants® as a model organism.

Learning Objectives

Students will be able to:

- Predict patterns of inheritance for recessive and dominant alleles in a screen of a self-fertile diploid organism.
- Calculate the probability of a particular gamete being produced from an individual.
- Write a hypothesis, draw a graphic to illustrate a hypothesis, and participate in peer-review of a hypothesis and graphic.
- Gain hands-on experience in data collection, analysis, error identification, graphical representation, and interpretation
- Map out a genetic screen showing three generations and the inheritance of germline DNA.

INTRODUCTION

I developed this lesson as part of a new Genetics lab course designed to pair with our current genetics lecture class. Students at our institution take genetics after they have completed a year-long introductory biology series for Biology majors with lab. These introductory classes have well-developed lab lessons on basic *Drosophila melanogaster* (fruit fly) genetics, and analysis of cell cycle regulation in *Schizosaccharomyces pombe* (fission yeast). I wanted to add plants to the new genetics lab curriculum to broaden student exposure to plants and capitalize on my expertise. I also sought to dispel the student misconception that plants are less complex or less important than animals, a topic previously referred to as "plant blindness" and now re-termed "plant disparity" (for review of this topic please see [1]). This problem can be partially traced to genetics textbooks, such as the one used in our genetics lecture (2), that introduces foundational genetics concepts with Mendel's work on peas then progresses to human and animal genetics, which could provide the mistaken impression that plant genetics are simple. Therefore, I designed a series of lessons using rapid cycling *Brassica rapa*, also known as Wisconsin Fast Plants®. The overall lab class includes a variety of methods and organisms to give students more experience and exposure. *B. rapa* plants are commonly used in K12 schools for lessons on plant reproduction, pollinators, the plant life cycle, and introductory Mendelian inheritance, as they have heritable variation in readily observable traits such as plant height (Lessons - Wisconsin Fast Plants®), as well as for college lessons on non-Mendelian inheritance due to the environmental influence on traits such as stem coloration (3). The irradiated seeds that I used for this genetic screen lesson are sold "for teacher demonstration, science fairs, or student research on the effects of irradiated seed on plant growth and development" (Carolina.com), but no supporting materials are provided. This lesson expands upon current *CourseSource* offerings using *B. rapa* that demonstrate the genotype to phenotype connection (4) and plant responses to carbon dioxide (5). This lesson will provide additional content to demonstrate inheritance of *de novo* mutations and germline versus somatic mutations.

Using Fast Plants® for a genetic screen has several advantages, including a very short generation time (as little as 40 days) making it possible for students to observe multiple generations in a relatively short amount of time. Unlike other eukaryotic model organisms, such as *Drosophila melanogaster* or *Caenorhabditis elegans*, no specialized organism handling skills or facilities are required. These plants are diploid and can be cross-pollinated as well as self-pollinated, allowing for flexibility in the types of offspring obtained. Although many previously published lesson plans for Fast Plants® state that these plants are self-incompatible and that seeds are only obtained via cross pollination, hand pollination of early flower buds (prior to anther maturation) does lead to self-seed production (6). In this manner, numerous self-seeds can be obtained from one plant, which simplifies analysis of patterns of inheritance.

Fast Plant® seeds can be purchased from stock centers, including Carolina Biological Supply (CBS) and the Fast Plant® stock center at the University of Wisconsin-Madison (UW-M). One seed stock available at CBS is the Wisconsin Fast Plant® Irradiated Seed set containing packets of seeds pre-treated with different levels of cobalt 60 radiation, along with non-irradiated control seeds. These seeds are intended for use with investigations of plant growth and development. I decided to use these seed stocks as the basis for a lab module on genetic screens, a classic genetic technique. A genetic screen relies on the use of random DNA damage to increase the chances of obtaining individuals with phenotypic alterations due to heritable mutations. As the M1 seeds come pre-treated with radiation, there is no need for in-house use of DNA damaging agents such as ethyl methanesulfonate (EMS) or ultraviolet light (UV light). Students have the flexibility to either look for changes in a trait they find interesting, such as plant size, or to observe the M2 population of plants (the offspring of the M1 plants that were grown from irradiated seeds) to seek out those that just "look weird." Along with the initial M1 population, I also planted out a few pots of the 'abnormal leaf' plant variety available from UW-M stock center as an example of a heritable phenotypic alteration in Fast Plants®.

This genetic screen lab provides an example of an active learning structure investigation using these seed resources, something previously lacking in CBS resources. The full lesson is intended to give students experience in the technique of genetic screens as a useful tool and to provide a review of how traits are passed on to generations. These plants also serve as an illustration of the importance of both germline and somatic mutations. While only mutations in the germline are heritable, the overall plant must be healthy enough to survive and reproduce. For example, many of the M1 plants had severe phenotype alterations due to damage to the overall plant but could not pass on any mutations as they did not produce seeds (died prior to flowering or were infertile). Students also gain hands-on experience in the process of science, such as writing a hypothesis, collecting data, analyzing data, and collaborating with peers.

Intended Audience

The intended audience is upper-level Biology major undergraduate students who have either completed a lecture course in genetics or are currently taking a lecture course in genetics. It could be modified to be suitable for new graduate students as well. The lesson is designed to be taught in a lab room with shared benchtops or tables for groups of students. A plant growth shelf with lights is required but this equipment can be in another room and the plants moved to and from the lab as needed. A lab cart is helpful for this purpose. The lesson also needs a workspace suitable for planting seeds, such as a work area or the lab cart itself. If the class also has a discussion day in the lab room, a computer and overhead projector would be useful for showing the introductory slides (found in S1. Fast Plant Screen – Introductory slides). Alternatively, printouts could be used. Due to the messy nature of trays and pots of plants, it would not be advisable to teach this lesson in a room with a carpeted floor.

Required Learning Time

This lesson was designed for a semester-long course with lab session of 160 minutes one time weekly. The full lesson takes place over 3 lab days in a 17-week semester. The timing of second lab day is dependent on how long the plants take to produce seeds, generally 3 weeks after the first lab. The third lab takes place one week after the second lab. As the plants

need time to mature and produce seeds, this full lesson is meant to be part of a genetics lab class with other lessons. The lesson can be expanded to include more weeks of analysis of the M2 generation, or to include a third generation.

Prerequisite Student Knowledge

This lesson was designed for students who have passed genetics lecture or who are currently enrolled in genetics lecture. Prior to this class, students should have the current skills of writing a hypothesis, collecting data, graphing data and analyzing data, and evaluating and interpreting data. Students should have background knowledge in predicting alleles and gametes, predicting the inheritance of alleles, the concepts of dominant and recessive alleles, and familiarity with how DNA is transmitted across generations. It is helpful if they are familiar with the concepts of germline and somatic cells. It is helpful but not necessary if students have experience in spreadsheet programs such as Microsoft Excel. Ideally students should be familiar with basic plant floral anatomy such as anthers, stigmas, and basic seed plant reproduction. A guide to Fast Plant® floral structure is included in the M1 lab packet.

Prerequisite Teacher Knowledge

The instructor should have background knowledge in basic principles of genetics such as inheritance of traits, and germline and somatic mutations in plants. If instructors do not have experience in growing and pollinating Fast Plants® (or similar species such as *Arabidopsis thaliana*), as well as using a spreadsheet program such as Excel, and in setting up a randomized plant growth plan, they may benefit from a trial run using the example planting schemes, data sheets, and instructions for creating a randomized planting scheme included in the supporting files of this lesson. Helpful resources include websites such as fastplants and Carolina - Wisconsin Fast Plants®.

SCIENTIFIC TEACHING THEMES

Active Learning

Activities outside of class: None.

Activities in class: Peer-review of student hypothesis and graphic, collection of plant growth and survival data, phenotyping and grouping plants, analysis of data, pollinations, collecting and planting seeds.

Assessment

Pre-assessments: None.

In-class assessments:

- Students give and receive peer-review on their initial hypothesis and graphic.
- Students revise their initial hypothesis and graphic.
- I assessed students on the basis of their completed lab packets including the revised hypothesis and graphic, data tables, graphs, and analysis questions. I made the lab packets worth 60 total points each (about 4% of their lab grade per packet). There were 2 lab packets for the M1 generation and M2 portions of the class, and 11 total lab packets for the entire semester.

Inclusive Teaching

As radiation was used as the DNA damaging agent, this lesson had many students discussing nuclear radiation incidents from around the globe and the long-term implications of radiation damage to both people and the environment.

While students collect measurements as individuals, the data are compiled into a class dataset. Students analyze this class data and thus need the contributions of their peers and class collaboration. Peer review also provided students with the chance to get feedback from someone with a different point of view, and who may have had different courses and scientific training. For example, some students were currently working in research labs, some had not performed any lab work since introductory biology (which may have been online), some had spent their entire undergraduate time at our university, and others were transfers from different institutions. Students also use multiple methods to collect data. They observe the plants visually, collect quantitative data with a ruler, and perform pollinations. They also gain experience in non-quantitative data, as they are asked to group M1 plants by their overall impression of how well the plants are growing.

LESSON PLAN

The entire lesson plan took place over three lab sessions as one part of a 17-week lab class and involves pre-class and post-class activities by the instructor class (see Table 1). The first lab is observing the M1 plants that are 3 weeks old. Ideally, the second lab (collecting and planting M2 seeds) can occur about 3 weeks later, but it can be delayed if seeds are not ready or if there is a break from classes. The third lab (screening M2 for phenotypes alterations) takes place one week after M2 seeds are planted. Again, this third lab can be delayed if more mature plant phenotypes are of interest.

The first lab day has several tasks for students. Some tasks (pollinating, measuring, entering data) can be done in any order. As students tend to complete activities at different rates it helps to meet with individuals or groups and go over the next task, rather than waiting for the entire class to be done at the same time. Our labs are 160 minutes in length so there is a generous amount of time to complete the in-class activities each day. If classes are of a shorter duration, the estimated timeline in Table 1 can be used to help break the lessons into smaller units.

Pre-class preparation lab 1 – M1 generation of plants

Order and gather supplies as listed in S2. Fast Plant Screen - Supply List. Plant the M1 generation as per the Table in S3. Fast Plant Screen – M1 planting scheme. On lab day 1, bring plants to the teaching lab (if they are grown in a separate location), and set out a few plants for each student to work with. Print out the data collection sheets and M1 generation lab packet (S4. Fast Plant Screen – M1 data collection spreadsheet, S5. Fast Plant Screen – M1 generation lab packet). The plants will be highly variable: controls should be large with open flowers, other plants will be small but have true leaves in addition to cotyledons (seed leaves), and many plants (those from the higher radiation levels) will have just cotyledons. Other seeds will have failed to germinate. Pots lacking any germinated

seeds are included in the initial data collection, but the soil should be discarded soon after to reduce the presence of fungi and other unwanted organisms in the plant growth area. I suggest giving each student a variety of plants so they can see the range of phenotypes. It is important to put a planting tray or paper towels under the plants as pots of well-watered plants will leak water and soil onto the work surface.

Class 1 – Analysis of M1 generation

Introduction to the genetic screen (mini lecture script). This mini lecture is broken up into sections based on activities students are undertaking at a given point in time. It is not meant to be delivered as one continuous introduction. A possible script for this lecture is included below.

> *"One method researchers use with model organisms is genetic screens. The purpose of this approach is to identify altered phenotypes. A phenotype is what the organism looks like. This change could be in any trait you are interested in, such as fly eye color, ability of bacteria to survive antibiotics, mouse susceptibility to colon cancer-- anything at all you can measure or characterize objectively.*
>
> *"While you can find phenotype differences due to existing natural variation, naturally occurring mutations are rare. So, to have more mutants to work with, the organisms in a genetic screen are treated with a DNA damaging reagent to increase the likelihood of mutations and of altered phenotypes. For this approach you damage the DNA of a large population of organisms in a random manner, and then look at their offspring for altered phenotypes. You do not know which gene was changed to give the trait of interest, but you can find that out. We do not do genetic screens on humans. Why?" (Pause here to ask for student input. If none is forthcoming here are some suggestions. Well, because you would need to mutate DNA on purpose, then look at one or two generations of offspring from purposeful mating. It is not ethical! Plus, two generations would take a long time). "For humans we look at family histories or pedigrees. We can sequence and compare genomes, also, and we have lots of model organisms with similar genes.*
>
> *"For this class we will be using Wisconsin Fast Plants®, these lovely plants you see here. As the name suggests, they grow very quickly, so we will have enough time to experiment with one or two generations of plants. The seeds that were planted for this first generation, the M1 generation, were treated with different levels of cobalt-60 radiation. The seeds and plants are not radioactive and were purchased from a biological supply company. The radiation damaged their DNA. The plant cells attempted to repair the damage, but some changes remained. These DNA changes are mutations. We don't know where the mutations occurred in each plant's genome or how many occurred. They are random. Some seeds, the controls, received no radiation. Others received 50, 150, 500 or 4,000 Krads. For context, in humans a full body dose of 1,000 Krads is considered lethal (7). We don't know what is lethal for these plants! The tricky part of a genetic screen is that there must be enough DNA damage to have a good chance of finding altered phenotypes but not so much damage that the organism fails to survive and reproduce.*
>
> *"The plants you will be working with today are about three weeks old. They grew from seeds that were treated with radiation. We refer to these seeds and the plants that grow from them as M1, meaning the initial generation of mutagenized plants. Their offspring (the seeds from M1 plants) are the M2 generation. We will be working with each of these generations. Irradiating the M1 seeds damaged DNA in the cells of these seeds. Inside each seed is a tiny plant embryo. These tiny plants grew up into the plants you see on your lab station. If these seeds received the radiation treatment, then they will have lots of mutations in their cells. As the embryo was already multicellular, different cells will have different mutations.* ***Only mutations that can be transmitted to the next generation are inheritable.****" (Ask these questions to class, if no ideas are forthcoming students may need a reminder about the plant lifecycle and about somatic vs. germline changes. For a mutation to be inherited, what cells need to have the mutation? What part of the plant makes those cells?) "So, to summarize, mutations are inheritable if they are in cells that give rise to the gametes (sperm and eggs); we call these* ***germline*** *mutations. Mutations in cells that grow up into other parts of the plant (like roots or leaves) are* ***somatic*** *mutations. These somatic mutations can impact the health of the M1 plants but would not be inherited. If a plant is too unhealthy to survive and reproduce then any mutations it has will not be passed on. While many animals have a set germline early in development, it is still unclear if plants have a set germline (8). In other words, it may be possible for a plant to pass on what appear to be "somatic" mutations if those cells later develop into the germline.*
>
> *"Your first task today is to observe the plants at your station and write a hypothesis. The hypothesis is a testable explanation regarding the impacts of radiation damage to DNA on plant health. We cannot directly see the DNA damage but instead are relying on the plant traits we will measure (germination, survival, and size) as indications of how much damage occurred. You can observe not only your plants, but any of the plants in this group. Write this hypothesis in your lab packet. The hypothesis must be related to the data we are collecting today (germination, size, and survival) and must be something we can test. Once you have your hypothesis written, draw a graphic (an image) that represents your hypothesis. Graphics are not limited to graphs; they are images that represent your idea. For example, if you write that increasing radiation levels will lead to decreasing plant size, sketch out plants of decreasing size, and label the radiation levels. If you have questions, I will be walking around the room to assist."*

[Pause here to allow students to work on their hypothesis and graphic, check in with individuals to see if they are stuck. Use the provided rubric and examples to quickly assess student work and help students with hypotheses in need of a major revision before they move on to the peer feedback portion (see S6. Fast Plant Screen – M2 generation lab packet).

Hopefully peers can identify needed minor revisions in the upcoming peer-review session.]

"Now that you have your hypothesis and graphic, it's time to get some peer feedback. Peer feedback is an important part of science. Can other scientists understand your ideas? So, for this you will turn to a partner and share what you wrote and drew. There are peer feedback questions in your lab packet. Once you have your feedback, you will make any needed revisions to your hypothesis and your graphic."

[Pause here to allow students to work on this task while you walk around to check in on all students and assist as needed. Once most students have finished, move the class forward to the next tasks.]

"Once you have finished your revisions, it is time to think about inheritance of mutations. We are going to assume that the M1 plants are heterozygous for any heritable mutations. Your task is to draw a picture or schematic of how one heterozygous mutation in the M1 plants could be inherited in the next two generations. Assume that all offspring are from self-seeds (meaning one plant is both the male and female parent). What proportion of each genotype would show up in each generation? Be sure to include all possible offspring genotypes. Don't worry about phenotypes, just focus on the DNA."

[Pause here to allow students to work on this task, walk around to check in on all students and assist as needed.]

*"Your first data collection task today is to score germination (*i.e., *did the seed sprout?) and survival (Is the plant alive?) of the plants. We score a seed as germinated if part of the plant is visible above the soil. We score the plant as alive if it has any green coloration. Your second data task is to collect size data on the plant: the height (soil level to top of the stem) and diameter (width across the two widest basal leaves). Only measure live plants. If the plant didn't germinate or is dead write the data as NA (not 0, as that will confound any numerical analysis). Please handle your plants gently, because they are very tender and easy to damage during measuring. As there are lots of plants everyone will have a few pots to work with. Use the provided printed datasheets to record your data. You will then enter your data into a class spreadsheet, and we will analyze it as a group. Notice that each pot has a number written on it. This is the* ***randomization number****, which indicates the position in the growth tray this pot occupied. There are three seeds per pot, numbered 1-3 (the* ***plant numbers****). It will be important to keep track carefully of which plant and which pot your data are from. Once you have all your data, enter it into the spreadsheet on the class computer. Each plant will have one set of data. While you are waiting your turn for data entry, you can work on your next task, pollinating your plants so they make more seeds. The flowers are small so I will demonstrate pollination for small groups so everyone can get a good view. Again, please handle your plants gently. We want them to survive and make seeds for use to study in the next generation."*

[Pause here to circulate around the room and check in on measuring. Double check to make sure everyone is using cm and not inches as the unit of measure, and ensure only live plants are measured. Check the data entry periodically as well, as there should be no 0 entries in the height or diameter categories.]

<u>Pollination demonstration (demonstration script)</u>

"Ok, the goal is to transfer pollen here from where it is produced in the anthers onto the stigma. These small yellow structures are the anthers, which make the pollen, and the little green structure here in the very center of the flower is the stigma, which is where the pollen needs to go. These plants do self-pollinate, but they are not very efficient at the process, and we don't have any pollinators (like bees) in our building to help in the transfer. Your job is to transfer pollen between the open flowers of one plant and not between different plants, which would be cross pollination. You will use a clean Q-tip for pollen transfer for each plant. Once you have used a Q-tip, throw it away in a trash can so no one else uses it by mistake. The youngest flowers are toward the top of the plant. You want to find flowers that are just starting to shed pollen as your pollen source, so that the pollen is fresh and alive. Dab up the pollen like this with your Q-tip. Now, look for flowers that are a bit younger (higher on the plant) than the one from which you just took the pollen. Gently dab the pollen onto the stigma. Ideally, you can see a little yellow color on the stigma. (It helps to have a magnifying glass for this part.) After you have finished pollinating a plant, move it off to the side so you know it is done. By next week we should have enlarged seed pods. Once the seeds are ready, we will collect them, plant them, then screen the M2 plants for any altered phenotypes."

[Pollination time is quite variable per student. Some will do a quick dab onto a flower and call it complete, while others will be more meticulous. Check for visible pollen on stigmas.]

<u>Grouping of plants by category (mini-introduction)</u>

"Now that everyone has finished collecting and entering our plant data, we have quantitative data on our plants. You will be drawing a graph of these data for part of your lab writeup. We can also get qualitative data on our plants regarding how they look. We are going to look over our entire set of plants as a class. What is your overall impression of the plants? Let's work as a class to come up with some groupings for our plants based on how we think a pot of plants looks overall. Then, we will place all the plants into groups by categories. Feel free to talk about where you are placing plants and why, and we can move pots around too. Once we have decided where the pots go, then we will see if our groupings follow a pattern regarding radiation exposure. So, what groups do we want to do?" (Pause here to get class feedback/voting/discussion. Then let students group their plants, they can move around plants placed by other students until most/all students agree on the groups. It is suggested to use an empty bench or lab cart as a work surface for this task.)

"Now that we have our pots all grouped, it is time to compare our groupings to the radiation levels and find out

if there were any patterns. Our datasheet has a hidden column with the radiation level on it. We are looking for general trends, not an exact match."

End of class instructions (to class)

"One you have completed all the tasks, wipe down your workstation and you are ready to go until next week. I will share a copy of the datasheet with the class. You will need all the survival, germination, and growth data for your analysis. You will be drawing one graph showing all these data. Then, you will evaluate your hypothesis. Do the data support it? Why or why not? There are also some questions to answer in your lab packet. If there is time, I encourage you to work on those now with your classmates. The analysis and writeup in your lab packet are to be turned in next week at the start of class."

Post-class lab 1 – cleanup and plant care

After the lab is finished, ensure that the plants are back in their proper randomized order. Then, return them to the growth shelf, and add water as needed. Unless students are very tidy, the floor will likely need sweeping to clean up spilled soil. At this point, pots of dead or non-germinated plants can be discarded. It is helpful to simply remove the soil and return the empty pot to the tray to help keep all pots in position.

Pre-class preparation lab 2 – collecting and planting M2 seeds

The M1 plants should set seeds and be ready for seed harvest in as little as 40 days after planting. However, if conditions are less than ideal, they will take a bit longer. The plants will need watering for about 2 weeks after pollination. Then, water is withheld. As a note, the seed pods do not open on their own so mature seeds can be retained on the plants until ready for harvest. If the plants remain too hydrated, seeds can start to germinate in the pods! Seeds germinated in pods have very poor rates of survival so try to avoid this outcome. Before the second class, observe the plants to see which ones are forming seeds have a general plan in mind for your goals of working with the M2 generation. Do you want students to observe early plant phenotypes? Do you want them to gain experience in seed collection and planting? If there are multiple sections, it may be desirable to have the instructor, or a teaching assistant perform the collection and planting steps, and then share one population of plants among the different classes. Before class begins, print the M2 generation lab packet (S6. Fast Plant Screen – M2 generation lab packet), gather needed supplies (listed in S6. Fast Plant Screen – M2 generation lab packet), and bring the plants to the lab room (if located elsewhere). You may want to divide the plants into groups to ensure that most students will have seeds to collect. This M2 generation of plants grown from the M1 seeds can have altered phenotypes. If the seed collection and planting are to be accomplished on the same lab day, the instructor will need to create the planting plan in real time based on seeds obtained. It is important to include M2 seeds from non-irradiated M1 parents to serve as a comparison for any putative altered M2 plants. Ideally, the pots for the M2 generation are randomized. However, they can be planted in class then randomized after the end of class. For direction on how to create a randomized planting plan using Excel, see S7. Fast Plant Screen – How to create a randomized planting plan.

M2 seed collection and planting (mini lecture script).

"Today we are going to continue with our genetic screen of Fast Plants®. Remember those plants you measured and pollinated a few weeks ago? Now they have seed pods (indicate pods on plants)! These are the self-seeds of our M1 generation, we call these seeds the M2 generation. The M2 plants have inherited the germline DNA of their parent plant. We will be observing the M2 plants for any changes to their phenotypes. This observing is the "screen" part of the genetic screen, and we are looking for changes of interest. In theory, our plants can have changes to any trait, like cold tolerance or root size. But, to check for these sorts of changes, we would need to grow our plants in a way that allows us to see that phenotype. For example, if we were interested in roots, we would either need to dig up the roots (without killing the plant) or grow them on Petri dishes. Instead, we will be looking for changes that we can easily see for the above-ground parts of the plants growing in standard conditions.

"Today we will collect the seeds, count our seeds, design a planting strategy, and plant the seeds. It is very important to collect the seeds of each plant individually and to keep track of which parent produced which seeds. We will carefully label, then later randomize the pots of seeds rather than growing them in groups by parent. That way the impact of the environment is minimized, and we can see more of the effect of the genotype on the phenotype.

"Your first task is to check your plants for seed pods. The pods do not open on their own so you will need to break them open. One way to do this is to carefully break or snip off the pods onto an envelope or a white paper towel. Then, press on the pods until they break open. Count how many seeds you obtained from each plant and put them into collection envelopes. Label them "M2 seeds, name of parent plant, # of seeds obtained, your initials." Then, write this information on the white board so we can plan our planting strategy. We will be planting all seeds of irradiated M1 plants to maximize our chances of finding altered phenotypes, but just some of the seeds of non-irradiated parents. Once everyone has finished collecting and seed counting, we will regroup to plan out which ones to plant. Once you have collected the seeds from your M1 plants, discard the plant and soil into a biological waste bin for proper disposal by autoclaving. Peel the labels off the pots, wash the pots in the sink, and leave the pots to air dry."

Post-class lab 2 – cleanup and plant care

Check to make sure plants are in the desired order. If they were not randomized when planted they can be randomized after class. Place trays of newly planted seeds in the growth area. Ensure all M1 plants were discarded. Store any unused M2 seeds for later (if wanted) by placing envelopes at room temperature in safe place; a closed cupboard works well. The floor will need sweeping after this class.

Class 2 – Collecting and Planting M2 seeds

Pre-class preparation lab 3 – Screening the M2 generation

The plants can be screened very early. This lesson was based on screening them while they are 1 week old. They

can be checked at an older age and/or for additional weeks (if desired). Create data collection sheets based on your planting scheme, gather supplies (listed in S6. Fast Plant Screen – M2 generation lab packet), and bring the plants to the classroom (if located elsewhere). Set out a group of plants for each student to observe. As a note, it is likely that not all seeds will germinate. This is normal and related to the health of the parent plants, the quality of the pollination, and the quality of the seeds.

Class 3 – Analysis of the M2 generation

M2 screening (lecture script)

> *"Welcome back everyone. Today you will be observing your one-week-old M2 plants to see if any appear to have altered phenotypes as compared to the control plants. Your first task today is to score germination and survival for the plants at your station, then we will combine our data for a class dataset. Then, observe your plants for potential phenotype alterations. Keep in mind that individual plants will have individual differences in overall size, leaf shape, and so forth. The easiest phenotypes to identify are those that are more extreme in appearance. Talk with your classmates, compare your plants to their plants. As we know which plants are siblings (*i.e., *plants that have the same M1 parent), we can look at sibling groups to see if any traits show up in more than one sibling. As always, please be gentle with the plants! We will regroup as a class to discuss what we are finding. We can also analyze the frequencies of any phenotypes to see which mode of inheritance is occurring."*

TEACHING DISCUSSION

I first used this lesson in Special Topics in Biology Fall 2020 and brought in the 3-week-old M1 plants on the first day of lab. Students liked being able to work on an actual experiment that first day and it was useful to show them one of the organisms they would be using throughout the semester. Such an in-person lab was exciting for students after spending the previous semester learning remotely. While most students were able to formulate a hypothesis in a timely manner, most students struggled with the peer review of the hypothesis and graphic. They tended to simply say "looks good" even where there were flaws in the hypothesis or misalignment between a hypothesis and graphic. For example, one student wrote that "the plants exposed to 150 Krads radiation would cause the next generation to change its DNA," implying that the DNA changes had yet to occur. Another student wrote a hypothesis about plant survival, but their graphic illustrated plant size. In reflecting on this part of the lesson it was clear that, despite prior experience in writing hypotheses, students required a review of those skills. Also because peer-review was a novel experience, I have now added a discussion of this component. Now the peer-review portion of the M1 lab packet asks students to list a strength and weakness of the hypothesis and of the figure. Students will also have to determine if the graphic matches the hypothesis, and to assess if the hypothesis is aligned with the data to be collected (plant germination, survival, and size). I also gave each student initial oral feedback as guidance for any hypotheses and graphics in need of major revision prior to obtaining peer feedback. I have included my scoring rubric for the hypothesis and graphic (see S8. Fast Plant Screen – Rubric for evaluating hypothesis and graphic, and example hypotheses). Students can use this rubric to guide their hypothesis and graphic and for scoring during peer-review.

Another goal for this lab was for students to collect and analyze data. Students were able to successfully collect data, but some were rough in overall plant handling. Despite instructions to handle the plants gently, some students pulled off leaves for easier measuring; or crushed stems trying to stake plants as straight as possible. One individual even dug down into the pot to see how deep the roots extended. This unanticipated damage may have been influenced by prior lab experiences, where teaching lab specimens were provided for observation and destructive sampling. In the future, I will emphasize that we want to keep the plants alive and healthy so that they can produce seeds for us to screen. I also added instructions to the lab packet to remind students to be gentle with the plants as we need them to survive to produce seeds. If plants are to be used by more than one group of students, I suggest planting a larger number of seeds so that loss of a few individual plants is of minimal impact. The data entry needed quality-checking to ensure that only live plants were measured for size, and that "NA" was recorded instead of 0 for sizes of dead or non-germinated plants to avoid confounding data analysis. Also, a quick accuracy check is helpful to ensure that all data are entered properly. (A suggestion is to simply look for largest and smallest values for height and diameter. A height of 20 cm is possible, but 200 cm is not plausible for this cultivar of *B. rapa*.) Data analysis was successful, and all students were able to calculate % survival and average height and diameter. Some required a refresher on how to perform these calculations. Students successfully graphed the data using a hand-drawn bar graph in their lab packet rather than using a computer program. Most students even went the extra step of color-coding the bars. The most common errors were omitting a title for the graph or leaving off one or more axis labels.

The grouping of M1 plants by broad phenotype categories worked very well and the class seemed to enjoy this part of the lab. The Fall 2020 class agreed on four general characterizations of plants, "look good, look ok, look bad, dead/no sprout." An example is shown in Figure 1. These plant size categories were in good alignment with the level of radiation received by plants. All the unirradiated controls pots grew well, except for one pot that had 2 seeds fail to sprout. The "look ok" group of plants were generally from seeds that had received a lower dose of radiation and the "look bad" and "dead/no growth" were from seeds that received the higher doses of radiation. Students were surprised to see that some of

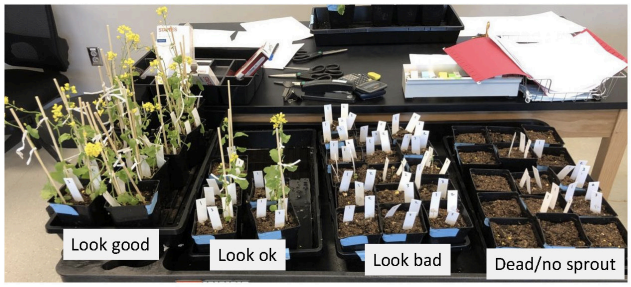

Figure 1. Example of M1 plant groupings by phenotypes. Three-week-old M1 plants were placed into broad phenotype classes by students based on their overall impressions of how the plants looked. Classification was based on the entire pot, which contained 0-3 live plants.

the live plants were from seeds that had received the highest level of radiation. We could have re-sorted the plants into groups by radiation level but in the interest of time did not do so. Students in both years seemed to like this activity of simply classifying plants based on an overall impression, rather than by strict numeric cutoffs. It was an effective way to talk about the challenge of quantifying an overall phenotype.

The lab also tasked students with predicting inheritance of a mutation across three generations of plants. This task allowed me to assess their familiarity with predicting alleles, allele proportions, and offspring genotypes. This task allowed me to quickly see how familiar students were with these concepts, addressing the learning goal of calculating the probability of particular gamete being produced by an individual. All should have been very familiar with this process due to having completed a lecture course in genetics, but some had taken genetics a few semesters ago, and during the unexpected transition to remote learning. Therefore, it was good for all to practice. Most students could draw Punnett squares. However, a common mistake was to omit predicting all possible genotypes of offspring. For simplicity, we assume the M1 was heterozygous for a heritable mutation. However, students forgot to account for the wild type gametes produced by the M1, and this omission was carried through to their predictions of the M2 and M3 generations. Many students focused in on the predicted ¼ of offspring that would be homozygous for the mutation in the M2, and then predicted all homozygous mutants in the M3. They ignored the possible wild-type or heterozygous M2 plants. I assessed the goal of comparing soma and germline by asking students to draw a plant and indicate where heritable and non-heritable M1 mutations would be located (M2 generation lab packet). This task was puzzling for some students until they realized that the flowers had the germline. The exercise helped them connect of physical structure of out organism with the concepts of soma and germline.

The screen portion of the lab was successful in identifying plants with potential phenotype alterations. In fall 2020, this lab had two sections. One lab section collected the M2 seeds, and the second lab section planted them. This approach worked well and gave me plenty of time to create the randomized planting plan between the two lab days. These tasks could be easily accomplished in one lab session or could be divided into different days or weeks. The seeds store for a long time, so the harvest and planting can occur at different times. Phenotyping of the M2 generation was harder than anticipated. In fall 2020, about ¼ of the offspring from one M1 parent plant had very elongated hypocotyls (the initial stem of the seedling, see the image at the start of this lesson). For me, this phenotype was very clear and striking. For some of my students, it was hard for them to identify this trait due looking at the overall plant size instead of the size of one region of the plant. If two plants were of a similar height, they called them both "tall," even if one plant had an elongated hypocotyl and no true leaves, and the other plant had a standard hypocotyl then an internode and a pair of true leaves, giving the overall plant a tall height due to reaching a more mature developmental stage. Next time, it may help for me to pre-screen the plants and incorporate a quantitative measure of some trait that appears altered in some plants, but such prescreening may detract from the explorative aspect of the screen. Some students in one class section also expressed the idea that as each plant was an individual, all plants were unique and therefore all plants must be mutants, even when no radiation was involved. The challenge of talking about individual variation versus inherited traits was a bit unexpected but was a good chance to discuss the importance of both DNA and the environment on overall phenotype. I did have students work analyze the M2 generation data to determine which mode of trait inheritance, dominance vs recessive, was most likely for the elongated hypocotyl plants. I had them try out Chi-squared analysis, a method they had used more than once in previous labs. Next time I will include a refresher on how to perform that calculation and how to interpret the data.

The goal of identifying possible sources of experimental error was successful. We teach that experimental error does not necessarily mean mistakes in the scientific process but can also be sources of unanticipated variation or challenge. Many students wrote about the difficulty in scoring plant survival, as many clearly unhealthy seedlings are still partially green at 3 weeks of age. Others identified plant size measuring and accurate data collection as possible sources of variation. Another source students identified was plant death due to damage in the experimental process. Others noted that as we did not perform the radiation exposure procedure ourselves, we had no way to verify the dosage received by the seeds prior to purchase.

Adaptations for increasing early student involvement

In fall 2020 the M1 seeds were planted 3 weeks prior to the first lab. In Fall 2021, I had the students plant the M1 seeds in the second week of class. The purpose of this change was to give students more ownership over the experiment and hopefully reduce damage to plants from rough handling. While this modification did remove the double-blind nature of the study (students could look up or recall which pots they planted) it was well-received. While plant care was positively impacted, the seed planting consistency was negatively impacted. Seed spacing was more irregular then when the instructor planted all seeds, and some pots from the no radiation control had fewer than 2 seeds germinate, indicative of possible accidental seed omission. The delayed planting time (week 2 instead of 3 weeks prior to the semester) also meant that only the M1 and M2 generations could be observed in a single semester. A year-long course would be able to follow M3 and subsequent generations.

Adaptations for graduate students

Neither year of this course included graduate students but, if appropriate, the lesson could be modified to include them. Typically, our institution asks graduate students to complete a more challenging addition to a course for it to count towards their graduate degree. For this lab, one graduate student addition could be to try to determine rough map locations of genetic changes for phenotypes of interest. This mapping may need to be accomplished with mutants identified in prior years and would be most suited to students with extensive prior molecular skills. It would also require additional reagents (DNA primers etc.) and equipment (such as micropipettes and a PCR machine). Alternatively, graduate students could perform a literature search to come up with a list of putative candidate genes for phenotypes of interest, provide justification for these genes, and suggest potential future experimental plans. As there are very few genetically identified Fast Plant® mutants this search would mostly focus on the genetics of related species such as *Arabidopsis thaliana*.

Adaptations for a shorter course

If desired, students could observe only the M1 generation of plants. Students could plant these seeds themselves and collect phenotype, survival, and growth data weekly. This approach may provide them with more ownership of the experiment and allow them to score survival and size over time. The plants germinate in about 2 days, meaning that germination and initial survival can be scored quickly. Alternatively, the instructor could provide plants in a ready-to-observe state and have students observe them on a single day. The identities of the plants could be kept hidden until the final set of measurements are taken. You can omit the pollination step if there are no plans for use of the M2 generation. These M1-generation-only lab alternatives would not be genetic screens as the next generation is not observed but would instead be an example of how mutations can impact health and survival of an organism. Another way to simplify this lesson is to plant just control (no radiation) and the lowest radiation level seeds. In this case, you could plant a larger number of seeds from each group, allowing students to see a wider array of phenotypes for each group. This approach would also provide a simple experimental setup; of one control group and one experimental group.

Adaptations for an expanded course

If this screen is performed more than once, you could keep seeds of interesting plants and plant them for additional analysis, such as complementation testing, or cross with wild-type plants to see if traits are inherited in a dominant or recessive manner. Mapping genetic changes is possible as there are Fast Plant® DNA markers and mapping lines available (9). However, this would be a time-consuming undertaking and may be beyond what is practical in a teaching lab. A dedicated and trained research assistant would be very helpful for such an undertaking. As mentioned above, graduate students could also take on this task. Another option would be to screen for alterations in specific traits of interest, such as the morphology of trichomes, or leaf architecture. In such cases, it would be helpful to have additional equipment, such as dissecting microscopes.

Adaptations for an online course

This lab was designed and taught in person. However, it could be adapted for an online lab class. For example, you could ship supplies to students to allow them to complete some experiments on their own. This would be easiest with the M1 generation only (omitting the pollination and M2 screening) as a study of the impacts of radiation on plant growth and health. You could hide the identities of the seeds (regarding radiation level) until after students collect data. Alternately, an instructor or teaching assistant could grow all plants and share data and images for analysis. As growth of plants is highly influenced by environment and Fast Plants® are meant to be cultivated with continuous illumination (which may be a challenge to provide to students due to cost), it is best for all plants to be grown at a single location. However, germination and survival data could be obtained for plants at different locations. That way, teams of students working remotely could collaborate on this lab project. Alternatively, you could send M2 seeds to students to screen at their location.

SUPPORTING MATERIALS

- S1. Fast Plant Screen – Introductory slides
- S2. Fast Plant Screen – Supply list
- S3. Fast Plant Screen – M1 planting scheme
- S4. Fast Plant Screen – M1 data collection spreadsheet
- S5. Fast Plant Screen – M1 generation lab packet
- S6. Fast Plant Screen – M2 generation lab packet
- S7. Fast Plant Screen – How to create a randomized planting plan
- S8. Fast Plant Screen – Rubric for evaluating hypothesis and graphic, and example hypotheses

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Table 1. Recommended timeline for the lesson.

Activity	Description	Estimated Time	Notes
Preparation for Class 1 – Analysis of M1 generation			
1. Plant seeds for M1 generation (instructor).	1. Plan or make use of the provided randomized planting scheme. 2. Label pots with randomization number. 3. Add soil and fertilizer pellets to pots. 4. Group pots by radiation level for easier planting. 5. Add seeds to top of soil. 6. Put plants into trays in order by random number for growing. 7. Add water to trays (not to pots). 8. Place humidity dome over trays. 9. Place trays in growth shelf with 24-hour lighting. 10. Check on plants every few days to ensure they have not dried out. 11. Remove humidity domes after about 1-1.5 weeks. providedrandomized planting scheme.	About 1 hour to label and fill pots and plant seeds	• Obtain irradiated seeds from Carolina Biological Supply, see S2. Fast Plant Screen – Supply list. • Label pots with only the randomization number to avoid unintentional bias in data collection. • Black ink permanent marker is suggested for labeling as plants will be kept for about 6 weeks and ink will fade under growth lights. • Mix the fertilizer with the soil, or add about 6 pellets to each pot; fertilizer should be located about 1/3 down in soil to avoid direct contact with seeds. • For a sample randomized planting scheme see S3. Fast Plant Screen – M1 planting scheme. • Plants grow well with 4 or fewer seeds per 3-inch pot; an example of a planting plan is found in Supplemental File 3. • Add one label stake with a number by each seed (1, 2, 3) in each pot. Or you can label seedlings (and blank spaces where seeds were planted but did not germinate) after seedlings emerge. • If the plan is for students to have mature plants to pollinate, score survival and take growth measurements, plant seeds about 3 weeks prior to the first lab. Plants can also be scored at younger growth stages. • Maintain moist but not soggy soil. • Water plants by pouring water into the trays, not into the pots as pouring water onto the soil will disrupt seed/plant positions and can damage plants.
Class Session 1 – Analysis of the M1 generation			
1. Introduce the concept of a genetic screen (mini lecture). 2. Students draft their first hypothesis and graphic. 3. Students do peer review and revision of the hypothesis and graphic. 4. Students draw a schematic depicting how a mutation originating in the M1 plants would be inherited in the next two generations of plants. 5. Students collect data on germination, survival, and plant size and enter data into a class datasheet 6. Students self-pollinate plants to improve seed yield.	1. Mini lecture 2. S5. Fast Plant Screen – M1 generation lab packet 3. Students work in pairs for peer review. 4. Students work as individuals for lab tasks 4-7.	~10 minutes ~15 minutes ~15 minutes Activities 4-8 are worked on concurrently as students take different amounts of time to finish. Drawing the schematic takes 5-20 minutes, data collection takes about 15 minutes, data entry takes about 5-10 minutes per student, pollination 5-20 minutes. Class discussion took about 10 minutes.	• This lesson was run with two lab sessions of 15 total students. • Students for this course had 1 year of introductory biology with experience in hypothesis writing. • Many students struggled with providing any criticism. • Students often could draw a Punnett square but had a hard time turning this information into a schematic or diagram • Only live plants are measured for size. One set of plants can be used for multiple lab sessions. • Each student collects data from a set of plants. The goal is to give each student examples from the range of plant survival and health.

Activity	Description	Estimated Time	Notes
7. Students enter all measurements into a class spreadsheet. 8. Students arrange pots of plants into class-decided categories and discuss as a class any needed changes. 9. Students compare plant categories to radiation levels. 10. Students analyze the class data, draw graphs of the data, and evaluate their hypothesis.		~10 minutes Remainder of lab time, often competed after class All hands-on activities listed take place in one lab session of 160 minutes. Students may need time after class to complete data analysis.	• I highly suggest that you quality check the data entry! Check that all size data are present for live plants (and only for live ones), and check for errors in data entry such as a misplaced decimal point. • Instructor demos pollination. • It is helpful for the instructor to demonstrate how to sort data in Excel. • These categories can be very broad, like "plants look good," "plants look sick" and "plants are dead." • After plants are arranged, give students a key to radiation levels and plant numbering – found in S3. Fast Plant Screen – M1 planting scheme. • The class data analysis and evaluation can be moved to a post lab activity if needed if not all students finish prior to the end of a lab session.
Plant care of the M1 generation – after class session 1			
1. Care for M1 plants until seeds form.	1. Plants need watering for about 2 weeks after pollinations are complete, then water is withheld to allow for seed maturation. 2. Successful pollinations will show enlarged seed pods within a day. 3. Be sure to stop watering after seed pods are well-formed (about 2 weeks post pollination).	About 3 weeks for plant growth; pollinations take ~15 minutes per day	• Pots with no live plants should be discarded to reduce the chance of mold infestation. • The instructor may choose to perform extra pollinations in the days after student pollination to increase seed yield. • If plants are watered too long the mature seeds can germinate while in the seed pods.
Class session 2: Harvest of M2 seeds from the M1 plants and analysis of seed yield			
1. Collect seeds of M1 plants (instructor or students). If the students are to complete this part, use the mini lecture in the lesson plan.	1. Use scissors to snip plants out of pots for harvest. 2. Smaller plants can be collected whole and placed into envelopes. 3. Count the number of seeds produced from each plant to make planning for planting easier.	About 1 hour total for collection and counting	• Seeds are ready for harvest when pods are brown. • Pods do not shatter on their own and must be mechanically opened by gentle pressure. • The seeds of the M1 plants are the M2 generation. • Collect seeds from individual plants. • Most of the no radiation (control) plants will give good seed set but few of the irradiated seeds will give rise to plants with seeds. • Label envelopes with the radiation level of the M1 parent, pot number and plant number of the M1 parent, and number of seeds collected. • Seeds can be planted immediately or stored in a cool dry environment for years.

Activity	Description	Estimated Time	Notes
Class session 2: Planting of M2 generation of seeds			
1. Students compile seed yield data. 2. Decide on how many seeds to plant; only a subset of control (non-irradiated parent) seeds is needed. 3. Instructor designs randomized planting scheme. 4. Students plant M2 seeds in soil.	1. Instructions for using Excel to randomize the order are in S8. Fast Plant Screen – Rubric for evaluating hypothesis and graphic, and example hypotheses 2. Seed planting instructions are found in this table (above)	About 2 hours	• Plant all seeds from irradiated M1 parents, and about 15-20 seeds from a subset non-irradiated M1 parents • About three seeds can be planted in each three-inch pot. Only plant seeds from the same M1 parent in each pot. • When possible, plant the same number of seeds per pot (3 or fewer).
Plant care of the M2 generation – after class session 2			
1. Care for M2 plants until next class.		About 1 week, unless class will take place later	• If plants are grown for just 1 week, humidity domes can be left on the entire time. • Make sure soil stays moist by adding water to trays, not to pots.
Class Session 3 – Analysis of the M2 generation			
1. Mini lecture to introduce the topic 2. Students score germination and survival. 3. Students observe plants to look for altered phenotypes. 4. Students answer questions in lab packet discussing findings.	1. Germination is defined as any part of the plant showing above the soil, and survival is scored by plant appearance.	About 10 minutes About 30 minutes for scoring and data entry A long time can be devoted to phenotyping and talking about inheritance. Remainder of lab session	• It is expected that not all seeds will germinate, even from control plants • Students can have a hard time scoring survival. • Phenotypes will vary between plants; it is easiest to look for striking changes rather than subtle differences. • Siblings (M2s from the same M1 parent) will have DNA in common, so you might expect to see the same altered trait in a sibling group. • Students do well working in groups for answering these questions.
Plant care of M2 generation - optional			
1. If you are keeping the M2 generation for seeds the plants will need self-pollination and watering. Otherwise, dispose of plants and clean pots and trays with soap and water.			• Seeds will keep for years in cool dry conditions.