

Who's The Father?

Investigating Mendelian Genetics with Wisconsin Fast Plants[™] *Two Genes (Dihybrid)*



The Offspring Purple Stem Green Leaf

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Written by Sarah Lauffer and illustrated by Amy Kelley. For more information about Wisconsin Fast Plants[™], go to www.fastplants.org or www.carolina.com/fastplants. See back page for ordering information.

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WHO'S THE FATHER?

HOW ARE TRAITS PASSED FROM ONE GENERATION TO THE NEXT?

Since the dawn of agriculture, humans have been trying to improve and increase their food supply. Historically, this was done by selecting the best plants and animals, then breeding them to get the tastiest, hardiest, most nutritious, or most appealing offspring. But no one really understood how these traits were inherited. Many people believed that traits "blended" in the offspring, or that only one parent passed a trait on to its offspring.

In the mid-1800's, a monk named Gregor Mendel gathered evidence that began explaining how inheritance works. Mendel studied discrete and contrasting traits. For example, the traits that he explored in pea plants included tall vs. short plants and purple vs. white flowers. His careful, repetitious work yielded two principles of genetics: the Law of Segregation and the Law of Independent Assortment.

In this investigation, students will gather their own evidence to explain how inheritance works. As they observe three generations of Wisconsin Fast Plants[™], students will unravel a mystery of paternity: What is the father's phenotype? Is it the same as the mother's phenotype or the offspring's phenotype? Or is it something entirely different? As the generations of plants grow, students will make observations that will serve as evidence to support or refute their explanations about the inheritance of two genes.

THE INVESTIGATION

This investigation spans the entire life cycle of the first-generation offpsring (F_1) of plants; an additional nine days allow students to observe the second-generation offspring (F_2) and the father generation (P_2). *Who's the Father?* is designed to provide students with the opportunity to investigate the inheritance of two genes in Wisconsin Fast PlantsTM. One gene, anthocyaninless (*anl*), determines whether the stem color of each plant will be purple or non-purple. A second gene, yellow-green (*ygr*), determines whether the leaf color of each plant will be green or yellow-green.

To ensure success of the plants — and to optimize conditions for expression of the genetic traits used in this investigation — it is important that you carefully follow the enclosed *Wisconsin Fast PlantsTM Growing Instructions* flyer.



OBJECTIVES

At the end of this activity, students will be able to:

- Explain the inheritance of two genes
- Observe Mendel's Law of Segregation and Law of Independent Assortment
- Identify and quantify the phenotypes that are conditioned by two genes
- Use scientific skills effectively

LIFE SCIENCE STANDARDS

Met by the Objectives of Who's the Father?

• Reproduction and heredity

MATERIALS

The following materials are included with the *Who's the Father?* Kit.

Each kit contains enough materials for 8 groups of 2-4 students.

WRITTEN MATERIALS

- Who's the Father? Book, which includes: a detailed life cycle description (pages 8–9), a Teacher's Schedule of observation days and plant care (page 10), and Student Notebook Pages (pages 11-13).
- Wisconsin Fast Plants[™] Growing Instructions

WISCONSIN FAST PLANTS™ MATERIALS

- Wisconsin Fast Plants[™] seeds Seeds of three generations are included: Non-Purple Stem (P₁) Yellow-Green Leaf (P₂)
 - F_1 Non-Purple Stem, Yellow-Green Leaf
- 8 growing systems
 - Each growing system includes:
 - 1 Circular watermat
 - 1 Long watermat wick
 - 1 Small container
 - 1 Large container (reservoir)
 - 4 Pots
 - 4 Small blue watermat wicks
- Potting mix
- Fertilizer
- Stakes and ties
- Labels

MATERIALS THAT YOU MAY NEED TO SUPPLY OR ORDER SEPARATELY

• Plant Light House or Plant Light Bank (Purchase through Carolina Biological Supply Company, Catalog Numbers 15-8997 and 15-8998) or make your own (see www.fastplants.org for instructions)

TIME REQUIREMENTS

- It is recommended (but not necessary) that you begin this activity on a Monday.
- Total days to complete the activity: About 53

The F_1 generation will complete the full Wisconsin Fast PlantsTM life cycle, which is approximately 40–45 days. An additional nine days allows students to observe the second-generation offspring (F_2) and the father generation (P_2). The actual time of this exploration may vary, depending on the environmental conditions in each classroom.

• See page 10 for a detailed Teacher's Schedule.

Observation days—Students tend the plants, make observations, and record their observations on the Student Notebook Pages. On these days, plan 10-30 minutes.



Twice a week—Verify that there is enough nutrient solution in the plant reservoirs (especially before a weekend), and that the plant tops are 10 cm below the lights.



BACKGROUND: INHERITANCE AND MENDEL

Every cell in plants and animals contains DNA that is made up of thousands of genes. And every cell (except the gametes) has two copies of each gene—one from each parent—and the two sets of genes are different from each other. Cells with both sets of genes are referred to as **diploid**.

During meiosis, each gene pair is split apart randomly — and independenly of the other genes — so that the resulting gametes (eggs or sperm/ pollen) have a single set of reassorted genes. Cells that have only one set of genes are referred to as **haploid**.

During mating, two parents each contribute a single gamete. When the two gametes fuse, the two sets of genes are then united in the diploid offspring. The new assortment of genes contains all the information for a whole new organism.

GENE ALIASES: ALLELES

Genes can have different forms, called **alleles**. Each allele codes for a slightly different form of a gene. Different alleles of the same gene are represented with the same letters, which are differentiated by capitalization. Example: Mendel described a gene for flower color with the following alleles: "P" for purple and "p" for white. Another way to describe the alleles is with three-letter names. Example: The two alleles for anthocyaninless gene in Wisconsin Fast PlantsTM can be represented as "ANL" (purple) or "anl" (non-purple). Together, the two alleles describe the **genotype** of a specific **trait**.

The interaction of the paired alleles, along with the environment, determines the **phenotype** of the offspring. The interaction can be simple (one allele is dominant over a recessive allele), or the interaction can be more complex (the two alleles both contribute to the apparent phenotype). In the case of Mendel's flower color, the dominant P allele masks expression of the recessive p allele; if the dominant P allele is present, the flowers appear purple. So if the genotype is either PP or Pp, then the phenotype is a purple flower; if the genotype is pp, then the phenotype is a white flower.



MENDELIAN GENETICS

- Law of Segregation: Paired alleles separate during the formation of gametes.
- Law of Independent Assortment: Each allele segregates independently of the other allele in gamete formation.
- Alleles retain their identity during gamete production and pollination/ mating.
- Two alleles (one from each parent) are paired in the offspring; this constitues the **genotype** of a trait.
- The offspring's **phenotype** is based on the expression of both alleles; one allele may "mask" the expression of another in a dominant/recessive relationship, or both alleles may contribute to the apparent phenotype.

THE TRAITS: ANTHOCYANINLESS (NON-PURPLE) AND YELLOW-GREEN

Anthocyanin is a purple pigment found in many plants. Anthocyanin is best observed when the plants are 4–7 days old. Look for anthocyanin expression on the stems and hypocotyls, under the cotyledons, and at the leaf tips.

The anthocyaninless gene (*anl*) in Wisconsin Fast PlantsTM regulates whether or not anthocyanin will be expressed. In the homozygous recessive form (*anl/anl*), anthocyanin expression is completely suppressed, and the plants appear a bright green color (which is the "non-purple stem" phenotype). If the genotype is heterozygous (*anl/ANL*) or homozygous dominant (*ANL/ANL*), then anthocyanin is expressed at varying levels (which is the "purple stem" phenotype).

The **yellow-green** gene (*ygr*) in Wisconsin Fast PlantsTM determines whether the leaves will be yellowgreen or green in color. In the homozygous, recessive form (*ygr/ygr*), the leaves appear a pale, yellowgreen color (which is the "yellow-green leaf" phenotype). If the genotype is heterozygous (*ygr/YGR*) or homozygous dominant (*YGR/YGR*), then the leaves appear green (which is the "green leaf" phenotype).

The P_1 and P_2 plants used for this investigation are true-breeding for their phenotypes. The F_1 plants are the hybrid offspring that result from crossing the two parents.

MOTHER GENERATION (P₁)

Seed Packet Name: Non-Purple Stem Phenotype: non-purple stem, green leaf Genotype: *anl/anl*, YGR/YGR

The P_1 plants are homozygous with the recessive alleles for anthocyanin expression (*anl*) and the dominant allele for yellow green leaf color (*YGR*). Anthocyanin expression is suppressed, so the stems appear bright green. The leaves and cotyledons also appear green.

FIRST-GENERATION OFFSPRING (F₁)

Seed Packet Name:

F₁ Non-Purple Stem, Yellow-Green Leaf Phenotype: purple stem, green leaf Genotype: *anl/ANL*, YGR/ygr

The F_1 plants are the result of crossing the P_1 and P_2 plants. They are heterozygous with both a dominant and a recessive allele for anthocyanin expression. Anthocyanin is expressed, so the stems show some purple color. They are also heterozygous with both a dominant and a recessive allele for yellow-green color. The leaves and cotyledons appear green. These plants are phenotypically different from both parents.

Father Generation (P_2)

Seed Packet Name: Yellow-Green Leaf Phenotype: purple stem, yellow-green leaf Genotype: ANL/ANL, ygr/ygr

The P_2 plants are homozygous with the dominant allele for anthocyanin expression (*ANL*) and the recessive allele for yellow-green leaf color (*ygr*). Anthocyanin is expressed, so the stems show some purple color. The leaves and cotyledons are a pale, yellow-green color.

SECOND-GENERATION OFFSPRING (F₂)

There is no seed packet for this generation. See page 16 for a list of genotypes.

The F_2 plants are a result of intermating the F_1 plants. They represent all the genotypes listed in the P_1 , P_2 , and F_1 generations. The phenotypic ratio is **approximately*** 9:3:3:1 as follows:

9 purple stem, green leaf3 purple stem, yellow-green leaf3 non-purple stem, green leaf1 non-purple stem, yellow-green leaf

* Ratio is approximate, due to the random nature of independent assortment and segregation of gametes.



GETTING STARTED

In this activity, students will have the opportunity to investigate how two genes are inherited in Wisconsin Fast PlantsTM. The students are responsible for:

- 1. Tending the plants.
- 2. Articulating hypotheses about the inheritance of genes.
- 3. Gathering evidence (by recording their observations on Student Notebook Pages).
- 4. Determining whether or not their evidence supports their hypotheses.
- 5. Explaining inheritance of two traits in Wisconsin Fast Plants[™], based on their evidence.
- 6. Determining the father's (P₂) stem and leaf colors, based on their explanation of inheritance.

Students will observe and record the stem and leaf colors of both generations, but they will tend only the first-generation plants (F_1) through the entire life cycle. During the investigation, students will tend the plants, pollinate the flowers, harvest the seeds of the second-generation offspring (F_2), and plant the seeds. A few days after planting the second generation seeds, students will observe the stem and leaf colors of the F_2 seedlings, then plant seeds of the father generation (P_2). Finally, they will observe the stem and leaf color of the P_2 plants. (See page 10 for the Teacher's Schedule.)

The week prior to starting, assemble a 24-hour fluorescent light source (the Plant Light House or Plant Light Bank). Read the Wisconsin Fast Plants[™] Growing Instructions (included with this kit.)

On day 0, assemble the growing systems and plant seeds of the mother generation (P_1) and the first-generation offspring (F_1).

DATA ANALYSIS AND DISCUSSION

Throughout the activity, students can record their observations, predictions, hypotheses, and results by using the Student Notebook Pages (pages 11–13). After they have recorded the stem color of the F_2 plants, students can do a χ^2 test with data from the entire class (pages 14–15). The χ^2 results will provide additional strength to help them determine whether or not their evidence supports their hypotheses.

By explaining how their observations agree or disagree with their hypotheses and sharing their analyses with fellow students, they will unravel the mystery of paternity and come to understand the inheritance of two genes in Wisconsin Fast Plants[™], and the Laws of Segregation and Independent Assortment.



THE LIFE CYCLE OF WISCONSIN FAST PLANTS[™]

WEEK 1 (O-6 DAYS OLD)

Each seed contains a tiny, new plant, called an embryo. The outside of the seed is called the seed coat. A seed can remain quiescent (sleeping) for years, as long as it stays dry and cool.

A day or two after planting and watering, the tiny seeds germinate. During germination, the seed takes up water and swells until its seed coat cracks. The radicle (embryonic root) comes out first, followed by the hypocotyl (stem) and two cotyledons (seed leaves). A few days later, the hypocotyl pushes through the soil, pulling the seed leaves along with it.

WEEK 2 (7-14 DAYS OLD)

Above ground, the true leaves, stems, and flowers originate at a point at the very top of the plant, called the shoot meristem (growth tip). Each part emerges gradually, then grows larger — and the growth is measurable from day to day.

Stems elongate at the internodes, which is the space between the nodes (where the leaves attach). The elongation allows the plant to grow taller and spread out the leaves and flowers so they are in the best position to do their jobs. Stems allow food, water, and minerals to move throughout the plant.

Leaves contain many pores (called stomata) on their surfaces that allow the plant to "breathe" by uptaking carbon dioxide (CO₂) from the air, and then expelling oxygen (O_2) . A green pigment, called chlorophyll, causes the leaves to appear green and captures energy from light. When CO, and water are combined in the presence of light, the plant makes its own food, called carbohydrates (or sugar). This amazing process is called photosynthesis.

Under the soil, the roots grow downward. Roots anchor the plants into the soil so they don't blow or wash away. Root hairs absorb water and nutrients from the surrounding soil and bring them to the rest of the plant. Most of the absorbed water used to cool the plant as water evaporates from the leaves; some is used for other cellular processes, including photosynthesis.



WEEK 3 (14-20 DAYS OLD)

The **flowers** bloom. At the growth tip, new flower buds begin to appear. Each bud is protected by four green sepals. Once a flower opens, the sepals are hidden beneath four bright yellow petals. The flower's center boasts a single pistil, which is the female part of the flower. The pistil is surrounded by six yellow stamens, which are the male parts of the flower. Each stamen is covered with lots of powdery, yellow pollen.

The bright yellow petals may catch your eye — and the eye of insects. The petals form a beacon that lets insects know that there is food available. Hidden deep inside the flower are nectaries, which produce nectar. Nectar is a sweet, sugar-rich substance that insects love to eat. That's why bees and butterflies are attracted to flowers — they're hungry!

In exchange for food, insects pollinate flowers. When an insect moves from flower to flower looking for nectar, pollen from each flower gets caught in the insect's body hairs and is transferred to other flowers. After pollen has landed on the tip of another flower's pistil, it grows a tube down into the pistil, where the eggs are housed. Sperm (from inside the pollen) are then able move down the tube until they reach the eggs and fertilize them. The fertilized eggs then become the embryos of new seeds.



WEEKS 4, 5, AND 6 (21-40 DAYS OLD)

As the seeds mature and ripen, the outside of the pistil swells to become the **seed pod** (or **fruit**) that encases several seeds. The leaves and flowers slowly wilt and fall off, one by one.

After the seeds have dried out completely, they are ready to be planted or stored. Inside each seed is a tiny **embryo**, waiting for water and warmth so it can germinate into a new plant, and another life cycle can begin.

ABOUT WISCONSIN FAST PLANTS[™]

Wisconsin Fast Plants TM (*Brassica rapa*) are rapid-cycling brassicas. They are members of the crucifer family of plants, closely related to cabbage and turnips that have been bred over 30 years at the University of Wisconsin - Madison.

These petite plants (-20 cm tall at maturity) whiz through an ultra-short life cycle in about 40 days. In just over a month, students can plant seeds, tend plants, pollinate flowers, and harvest new seeds. The seeds can be immediately planted or stored for up to 10 years. Easily grown in the classroom, Wisconsin Fast Plants[™] require little more attention than continuous fluorescent light, water, and fertilizer.

For details about growing Wisconsin Fast Plants[™], refer to the Wisconsin Fast Plants[™] Growing Instructions. For classroom activities and investigation ideas, go to www.fastplants.org.

TEACHER'S SCHEDULE

The objective of this investigation is to understand how two traits are inherited. Students will make observations about stem and leaf color that will serve as evidence to explain how those traits are inherited. From their evidence and explanations, they will predict the phenotype of the father (P₂) generation.

DAY ACTIVITY

Students work in groups of 2–4. Each student should record observations, notes, hypotheses, and explanations on the Student Notebook Pages (pages 11–13). All student groups (except one) **plant** the seeds of the first-generation offspring (F_1). One student group plants seeds from the same population as the mother plants (P_1). Refer to the Wisconsin Fast PlantsTM Growing Instructions for details.

4–7 Observe the stem and leaf colors of the young P₁ and F₁ plants. Students decide how they will quantify the differences in stem and leaf colors. (Example: Students may count the number of plants that have certain phenotypes, or they may try to quantify the variation of the traits.) Record observations in a table. Discard the P₁ plants, but continue to maintain the F₁ plants according to the *Growing Instructions*. Thin the F₁ plants to 2 per pot. Explain how you think stem and leaf colors are inherited in Wisconsin Fast Plants[™]. State a testable hypothesis, then predict the father's (P₂) stem and leaf colors based on your hypothesis.

- 15–17 Intermate the entire population of F₁ plants over a 3–day period. (Intermate means to pollinate plants from the same generation.) Be sure that all flowers receive pollen from several different plants. See Growing Instructions for information about making beesticks and pollinating flowers.
- **Terminate** (cut off) any new flower buds that were not pollinated on days 15–17.

As the investigation progresses, students may reevaluate how they measure phenotypes in order to get data that is more meaningful for testing their hypotheses. If you prefer a more structured investigation, tell the students that the four phenotypes are: (1) purple stem, yellowgreen leaf (2) purple stem, green leaf (3) non-purple stem, yellowgreen leaf (4) non-purple stem, green leaf

- 37 **Stop watering** the plants. Let them dry out for a full week, until they are brown and crispy.
- Harvest the seeds from the pods of the F_1 plants, according to the *Growing Instructions*. These are the seeds of the second (F_2) generation. **Predict** the stem and leaf colors of the F_2 plants, based on your hypothesis.
- 45 **Plant** the F_2 seeds.
- 49 **Observe** the stem and leaf colors of the young F_2 plants. **Record** observations. **Perform** a χ^2 test to analyze your evidence. Do you accept or reject your hypothesis? **Predict** the stem and leaf colors of the P_2 plants, based on your (revised) hypothesis.
 - Refer to Growing Instructions

- 50 **Plant** the P_2 seeds.
- 53 **Observe** the stem and leaf colors of the young P₂ plants. **Record** observations. Does the evidence support your (revised) hypothesis?



Who's the Father?

Student Notebook Pages

YOUR CHALLENGE

The goal of this investigation is to determine... Who's the Father? You and your classmates will gather evidence to explain how stem and leaf color in Wisconsin Fast PlantsTM are inherited. From your evidence and explanations, you will predict the phenotype of the father (P_2) generation.

DAY ACTIVITY

0 Work in groups of 2–4. All student groups (except one) plant the seeds of the first-generation offspring (F_1) . One student group plants seeds from the same population as the mother plants (P_1) .

Refer to the Wisconsin Fast Plants™ Growing Instructions for details.



4–7 **Observe** the stem and leaf colors of the young P_1 and F_1 plants. **Record** your observations in the table below.

NUMBER OF PLANTS WITH EACH TRAIT IN THE \mathbf{P}_1 and \mathbf{F}_1 GENERATIONS

List Each Phenotype:			
P ₁ Generation			
F_1 Generation			

Discard the P_1 plants, but continue to **maintain** the F_1 plants according to the *Growing Instructions* by thinning the F_1 plants to 2 plants per pot.

Explain how you think stem and leaf colors are inherited in Wisconsin Fast Plants[™].



State a testable hypothesis, then **predict** the father's (P_2) stem and leaf colors based on your hypothesis.

DAY ACTIVITY

15–17 Intermate the entire population of F_1 plants over a 3–day period. (*Intermate* means to pollinate plants from the same generation.) Be sure that all flowers receive pollen from several different plants. See *Growing Instructions* for information about making beesticks and pollinating flowers.

Based on your hypothesis (from day 4-7), **predict** the stem and leaf colors of the second-generation offspring (F_2) that will result from the pollination you did today.

- **Terminate** (cut off) any new flower buds that were not pollinated on days 15–17.
- 37 **Stop watering** the plants. Let them dry out for a full week, until they are brown and crispy.
- 44 **Harvest** the seeds from the pods of the F_1 plants, according to the *Growing Instructions*. These are the seeds of the second (F_2) generation. **Notes or observations:**
- 45 **Plant** the F_2 seeds.
- 49 **Observe** the stem and leaf colors of the young F_2 plants. **Record** your observations in the table below.

NUMBER OF PLANTS WITH EACH PHENOTYPE IN THE F₂ GENERATION

List Each Phenotype:			
F ₂ Generation			









DAY ACTIVITY

- 49 Share your results with your classmates.
- (cont) **Perform** a χ^2 test to analyze your evidence, using results from the entire class.

Describe your $\chi^{\scriptscriptstyle 2}$ results:



Do you accept or reject your hypothesis, based on your evidence? If your hypothesis was accepted, are you convinced that it is correct, or do you need more evidence? If your hypothesis was rejected, what is your new hypothesis? Explain.

Predict the stem and leaf colors of the P_2 plants, based on your (revised) hypothesis.

- 50 So...Who's the Father? Plant the P₂ seeds.
- 53 **Observe** the stem and leaf colors of the young P_2 plants. **Record** your observations in the table below.



NUMBER OF PLANTS WITH EACH PHENOTYPE IN THE $\rm P_2$ GENERATION

List Each Phenotype:			
P ₂ Generation			

Does the evidence support your (revised) hypothesis? Justify your answer with evidence.

Put Your Results to the Test! Probability and the χ^2 Test for *Who's The Father*?

Put your claims to the test! Was the ratio of the phenotypes in the F_2 generation what you predicted it would be? Was it even close? A χ^2 test will compare your observations with your hypothesis.

The χ^2 test calculates (1) the deviation between your observed numbers and your expected numbers and (2) the probability that the deviation is due to **chance** or that the deviation is **significant**. If the deviation is merely due to chance, then your results support your hypothesis, and you can **accept** your hypothesis. If the deviation is significant, then your results do not support your hypothesis, and you **reject** your hypothesis.

STEP 1 Determine the ratio of phenotypes you <u>expected</u> in the F_2 generation, based on your hypothesis.

Phenotype	Expected Ratio	Expected Number of Plants (e)
1		
2		
3		
4		

STEP 2 Record the ratio of phenotypes you <u>observed</u> in the F_2 generation.

Phenotype	Observed Number of Plants (o)
1	
2	
3	
4	

STEP 3 Calculate the χ^2 value. Fill out the following table, using numbers from the entire class. For *o* and *e* values, use the actual numbers of plants, not percentages or ratios.

List Each Phenotype:	1	2	3	4
Observed Value (<i>o</i>)				
Expected Value (e)				
Deviation (d) = $o - e$				
Deviation Squared (d^2)				
d²/e				

STEP 3 Calculate the χ^2 value, continued.

Add all of the d²/e values together to get the χ^2 value:

STEP 4 Calculate the degrees of freedom.

To calculate the degrees of freedom, subtract one from the number of phenotypes possible.

Number of phenotypes possible			Degre	es of fre	edom
	_ 1	=			

STEP 5 Determine whether to accept or reject your hypothesis.

Find the probability that the deviation of the observed values from the expected values was a chance occurrence. Look up your degrees of freedom in the table below. Find where your χ^2 value falls in that row.

Degrees of		Probability of Chance Occurrence							
Freedom	90%	80%	70%	50%	30%	20%	10%	5%	1%
1									6.635
2	0.211	0.446	0.713	1.386	2.408	3.219	4.605	5.991	9.210
3	0.584	1.005	1.424	2.366	3.665	4.642	6.251	7.815	11.341
4	1.064	1.649	2.195	3.357	4.878	5.989	7.779	9.488	13.277

Probability value:

If the probability is 5% or greater, then you can **accept** your hypothesis. If the probability is less than 5%, then **reject** your hypothesis.

Do you accept or reject your hypothesis?

