

GENETICS

Move over fruit flies—fungus is taking over the genetics lab

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Demonstrations

GENETICS LABS ARE INTERESTING WHEN done with living organisms, but not all teachers want to use fruit flies as subjects. Therefore, I developed a series of classroom experiments using the nonpathogenic fungus *Aspergillus nidulans*. Like fruit flies, *A. nidulans* is inexpensive to grow, has a short life cycle, and has many genetic mutants available for study. But it has the added advantages of being easier to grow than fruit flies, easy to cross and analyze, and a good subject for molecular analysis. The *A. nidulans* genetic system can be used to demonstrate mitosis and meiosis, typical Mendelian principles, statistical analysis, genetic linkage, and gene interactions.

MITOSIS AND MEIOSIS

The processes of mitosis and meiosis are similar, and as a result students sometimes confuse them. Because *A. nidulans* has both asexual (mitotic) and sexual (meiotic) life cycles (Figure 1), it is an excellent tool for students to use when studying differences between mitosis and meiosis. In the laboratory the asexual (mitotic) cycle is used to propagate strains, resulting between the production of millions of identical spores called conidia. The conidia are identical because they are the products of mitotic division, in which daughter cells are identical to the cells from which they derived. The sexual (meiotic) cycle, in which progeny produced are not genetically identical to their parent cells due to crossing-over and independent assortment of chromosomes, is used to perform genetic crosses.

A. nidulans is haploid, having only one set of chromosomes for most of its life. On a simple microbiological medium (Figure 2), a conidium will grow by forming a finger-like cytoplasmic projection called a hypha; the single nucleus divides by mitosis to produce many nuclei in a common cytoplasm. When the hyphae are in contact with air, they develop spore-forming structures called conidiophores that project upward from the surface of the agar and contain many haploid conidia. This is the asexual life cycle, which takes about 3 days at room temperature (25° to 27°C) or 2 days at 37°C.

The sexual life cycle occurs under conditions of stress, which can be induced in the laboratory by placing a ring of tape all the way around the petri plate to limit oxygen. Under this condition, two hyphae will fuse such that their nuclei mix together into a common cytoplasm. Two of the nuclei will then be partitioned off into an individual cell where the nuclei fuse to form a diploid cell.

This cell then divides many times by mitosis, producing numerous identical diploid cells that undergo meiosis. Each diploid cell produces meiotic progeny called ascospores. Meiosis occurs inside a structure called a cleistothecium, which develops around the initial diploid cell. The sexual cycle takes about 3 weeks at room temperature or 2 weeks at 37°C.

MENDEL'S LAWS

A. nidulans is an excellent subject to use for studying independent assortment and gene interactions. Any two strains of *A. nidulans* may fuse together to form mixed hyphae and enter into the sexual cycle; it is therefore possible to perform genetic tests by simply growing two genetically different strains close together on an appropriate medium (Figure 2) and taping the plates to induce the sexual cycle. Each cleistothecium may contain millions of ascospores, and these can be easily scored for genetic traits of interest. (See Figure 3 for a detailed protocol.) The easiest of the genetic traits to score is spore color (conidia color)—wild-type *A. nidulans* is dark green, and mutant strains with white, yellow, or chartreuse spores are readily available.

Independent assortment may be demonstrated by crossing a yellow strain with a chartreuse strain, which will produce a 1:1:1:1 ratio of colors: parental colors (yellow or chartreuse) and colors due to recombination (wild-type green known as brownish). Wild-type green occurs when neither the yellow nor chartreuse mutation is present in the cell, and the brownish color occurs when both yellow and chartreuse mutations are present in the cell (Figure 4). This cross not only demonstrates that different genes assort independently of each other (hence the 1:1:1:1 ratio) but also demonstrates a type of gene interaction known as epistasis. By definition, epistasis occurs when two genes (here, the yellow gene and the chartreuse gene) affect the same trait (spore color). When both mutations are present in the cell, the result is a combination of color that looks amazingly like the color one gets when blending yellow and chartreuse crayons.

A similar cross between white and chartreuse strains demonstrates a second type of epistasis: one in which the effects of one gene completely mask the effects of another gene. In this cross, only three classes of progeny are obtained—2 white: 1 chartreuse: 1 green. This is because progeny containing both white and chartreuse mutations are white in color. The white mutation covers up the effect of the chartreuse mutation. A cross between white and yellow strains gives similar results.

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These types of experiments are examples of discovery-based learning. Students do not expect to see wild-type green (or brown) colonies from the crosses, and when they do their first reaction is to think they did something wrong. However, when students write out the genetic crosses they performed (the genotypes and the phenotypes, as in Figure 4), they discover not only that they performed the experiment correctly but that they observed independent assortment in action. Many students will then devise new experiments to test their hypotheses.

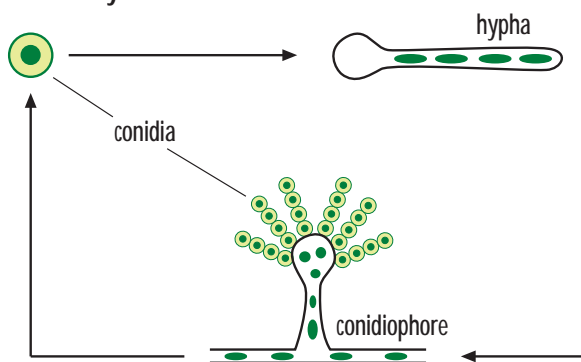
CROSSING-OVER, GENETIC LINKAGE, AND MAPPING

To demonstrate crossing-over and genetic linkage, two different traits must be examined. In addition to differing spore colors, nutritional requirements are easily scored traits that may be used to demonstrate linkage. A truly wild-type *A. nidulans* strain does not require the addition of vitamins to its media because it makes its own vitamins. However, many strains have mutations that cause them to require specific vitamins, such as para-aminobenzoic acid (paba), biotin (bi), riboflavin (ribo), and so forth. If a strain has a specific nutritional requirement, that nutrient must be added to the culture medium or the strain will not grow. The progeny of sexual crosses between strains with differ-

FIGURE 1.

A. nidulans life cycles.

Asexual cycle:



Sexual cycle:

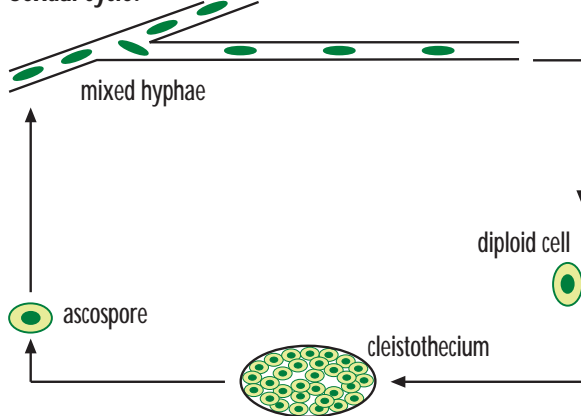


FIGURE 2.

Directions for making media.

Rich media:

Malt extract	20 g/L	Vitamin mix	2 mL/L
Peptone	2 g/L	Agar	20 g/L
Trace elements	1 mL/L		

Mix all components in 1 L water, autoclave, cool to 55°C, and pour approximately 20 mL into a 100 mm² petri dish. The vitamin mix must contain all vitamins required by the strains being used. If it does not, either add these to the vitamin mix or add separately to the media.

Minimal media:

Sodium nitrate	6 g/L
Potassium chloride	0.52 g/L
1 M Potassium phosphate buffer	12.0 mL/L
Dextrose	20 g/L
Trace elements	1 mL/L
1 M Magnesium sulfate	2 mL/L (Add after autoclaving and cooling to 55°C.)

Vitamins as required by strains

Mix all components in 1 L water, autoclave, cool to 55°C, and pour 20 mL into a 100 mm² petri dish.

Crossing media:

This media contains everything listed above (for minimal media); no vitamin mix is added. Only vitamins required by both strains are added to the crossing media. Prepare as above, but pour 15 mL into a 60 mm² petri dish.

Vitamin mix:

Vitamin	stock concentration	add per liter
Nicotinic acid	2.0 mg/mL	1.0 mL
Para-aminobenzoic acid	1.0 mg/mL	1.0 mL
Riboflavin HCl	2.5 mg/mL	1.0 mL
Pyroxidine HCl	0.5 mg/mL	1.0 mL
D-biotin	20 µg/mL	1.0 mL
Choline HCl	20 mg/mL	1.0 mL

Each supplement may be made separately, or a single "vitamin mix" containing each of the above vitamins in the concentrations listed may be prepared. Autoclave after preparing and store at 4°C.

Trace elements:

FeSO ₄ •7H ₂ O	1 g/L
ZnSO ₄ •7H ₂ O	8.8 g/L
CuSO ₄ •4H ₂ O	0.4 g/L
MnSO ₄ •7H ₂ O	0.15 g/L
Na ₂ B ₄ O ₇ •10H ₂ O	0.1 g/L
(NH ₄) ₆ Mo ₇ O ₄ •4H ₂ O	0.05 g/L

Dissolve each fully in the order given. This stock is a 1000-fold concentrate; add 1 mL/L of media.

Phosphate buffer:

KH ₂ PO ₄	68 g	Water to 1 L
K ₂ HPO ₄	114 g	pH to 6.5–7.0 with HCl

Autoclave after preparing and store at 4°C.

FIGURE 3.**Crossing protocol.**

1. Sterilize toothpicks by autoclaving them in a 100 mL beaker covered with aluminum foil. Label rich media plates with the strains being crossed.
2. Inoculate rich media plates with the strains of interest by stabbing a sterile toothpick into a stock colony of one strain and then gently running the toothpick in a straight line down the middle of the agar. Repeat with a clean, sterile toothpick for the second strain, inoculating this strain approximately 5 mm away from the first strain.
3. Incubate at 37°C, upside down, for 2 to 3 days. Hyphae from both strains should fuse at the interface between the two strains. Cut out a small (5 mm²) piece of agar from the area where the mycelia are mixed and place it in the center of a labeled crossing media plate. Incubate the plate for 2 days at 37°C.
4. Tape the lid of the plate to the bottom all the way around to restrict oxygen; incubate upside-down at 37°C for 7 to 10 days. This induces the sexual (meiotic) cycle.
5. The sexual cycle results in the formation of small, round ball-shaped structures called cleistothecia that contain numerous ascospores, or products of meiosis. These are black when mature and may be slightly obscured by vegetative growth. They are easily observed using a dissecting microscope. Using a sterile toothpick, remove a cleistothecium from the crossing plate and place it onto a 3 percent water agar plate (made by adding 30 g agar to 1 L water, autoclaving, and pouring into a 100 mm² petri dish). Roll the cleistothecium gently on the surface of the water agar until the vegetative cells are removed and the ball is shiny black.
6. Using another sterile toothpick, pick up the cleaned cleistothecium and break it into a tube containing 400 µL sterile water (cleistothecia will break by pressing against the wall of the tube beneath the water surface).
7. Dilute the spore suspension by placing 0.1 mL into 0.9 mL sterile water, then spread 0.1 mL of the diluted suspension evenly onto a rich media plate, invert, and incubate 2 to 3 days at 37°C. Strains that have crossed will have colonies of both parental types as well as wild-type color.
8. Score the colonies for color. If analyzing colonies for nutritional requirements, use a sterile toothpick to transfer spores from the offspring plate in a grid pattern onto a rich media plate and onto minimal media plates that lack the specific nutrient for which you are testing. For example, if testing for paba deficiency, stab the toothpick into a minimal media plate that contains any vitamins other than para-aminobenzoic acid, which are required by both strains—do not add paba. Anything that fails to grow on this medium would be considered to have a paba-requiring mutation. When scoring mutations, be sure to score all traits for each colony.

ent nutritional mutations are scored by determining whether they grow in the absence of that nutrient. For example, a cross between a paba-requiring strain and a ribo-requiring strain will produce some progeny that require both paba and ribo (and thus die on media that lack either), some progeny that require paba, some that require ribo, and some that require neither paba nor ribo.

Genetic linkage and crossing-over can be demonstrated easily because many nutritional genes are on the same chromosomes as the spore color genes—that is, they are genetically linked. For example, one paba mutant gene (*paba1*) is on the same chromosome as a mutant gene (*yA2*) that causes yellow spore color. By crossing a yellow, paba-requiring strain with a non-yellow, non-paba-requiring strain and scoring the progeny, it is easily seen that the yellow and paba mutations usually move together during meiosis (because they are close together on the same chromosome). How close together are they? By simultaneously scoring offspring for both color and nutritional requirements, the percentage of progeny in which a crossover event occurred can be determined—that is, the genes can be mapped. Offspring that are not both yellow and paba-requiring are the result of cross-over events, and these are few because the genes are closely linked. Results of a typical student cross are shown in Figure 5.

Statistical analysis, specifically chi-squared analysis, may also be used in analyzing the crosses. As with any other cross, students determine what they expect and then use statistics to demonstrate whether their observed results agree with what they expected. We have found that counting 100 progeny for a cross gives a good statistical sampling.

MORE ABOUT MEIOSIS

Much more can be done using *A. nidulans* as a model system for discovery-based learning. By using mutant strains that are affected at various stages of the cell cycle, students can learn more about the specifics of mitosis. By exposing conidia to chemical mutagens (this should be

FIGURE 4.

Cross demonstrating independent assortment and epistasis.

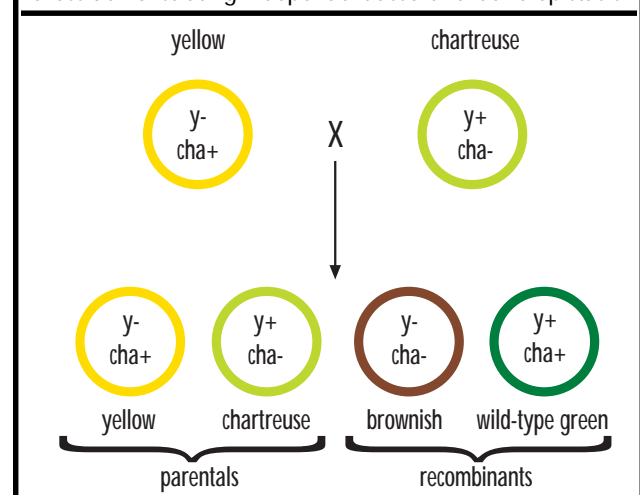


FIGURE 5.

Typical cross results.

Cross 1: Chartreuse x yellow strains

	Progeny phenotypes			
	Wild-type	yellow	chartreuse	brownish
Observed (o)	24	20	28	24
Expected (e)	24	24	24	24
(o-e) ² /e	0	0.66	0.66	0

$X^2 = 1.33$

P (0.05), df(3) = 7.82

Data are within expected range; genes *y* and *cha* assort independently.

Cross 2: Yellow, Paba x wild-type

	Progeny phenotypes			
	(Parentals)		(recombinants)	
	<i>y</i> +/ <i>paba</i> +	<i>y</i> -/ <i>paba</i> -	<i>y</i> -/ <i>paba</i> +	<i>y</i> +/ <i>paba</i> -
Observed (o)	41	45	6	8
Expected (e)	25	25	25	25
(o-e) ² /e	10.24	16.0	14.44	11.56

$X^2 = 52.25$

P(0.05), df(3) = 7.82

Data deviate from expected values; genes *y* and *paba* do not assort independently; 14 percent of progeny are recombinants; therefore, the genes are 14 map units apart on the same chromosome.

done by the instructor to prevent student exposure), new or different mutants can be produced. Also, students learn about the structure of DNA and can even perform experiments similar to those of George Beadle and Edward Tatum when they devised their “one gene-one enzyme” hypothesis (Raven and Johnson, 1996). A combination of protein structure, genetics, and cell biology can be studied by advanced students using a technique known as extragenic suppression analysis (Engle, 1998).

One advantage of the *A. nidulans* system is that the same strains and often the same crosses can be used to demonstrate numerous genetic principles. The cross used to demonstrate independent assortment and gene interactions (yellow x chartreuse) can also be used to demonstrate crossing-over, provided the yellow strain also has a *paba* mutation. Advanced students can analyze the chartreuse color as they analyze the yellow and *paba* mutations. An additional advantage is that the strains only have to be purchased once—spores are simply stored in a freezer long-term suspended in 50 percent glycerol at -20°C. The strains are then grown out by streaking a loopful onto rich medium.

With few exceptions, the media components are frequently available in school stocks, but those that are not

may be purchased relatively inexpensively from most chemical suppliers. The only specialized equipment required are an autoclave or pressure cooker for media sterilization and a dissecting microscope; an incubator will speed up the life cycles but is not required. And best of all, students (or teachers) do not have to wake up at strange hours to harvest flies—so move over fruit flies, fungi are fun! ✧

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REFERENCES

Engle, D. B. 1998. Extragenic suppression: A research method in genetics for undergraduates. *The American Biology Teacher* 60:297-306.

Raven, P. H., and G. B. Johnson. 1996. *Biology*. Dubuque, Iowa: William C. Brown Publishers.

Timberlake, W. E., and M. A. Marshall. 1988. Genetic regulation of development in *Aspergillus nidulans*. *Trends in Genetics* 4:162-169.