Name: **Date:**

HANDS-ON LAB

Investigating Meiosis in *Sordaria fimicola*

The fungus *Sordaria fimicola* is a sac fungus that spends most of its life cycle in a haploid state (containing only one set of chromosomes). Its main body is composed of haploid cells attached end-to-end in a mass of intertwined filaments called a *mycelium.*

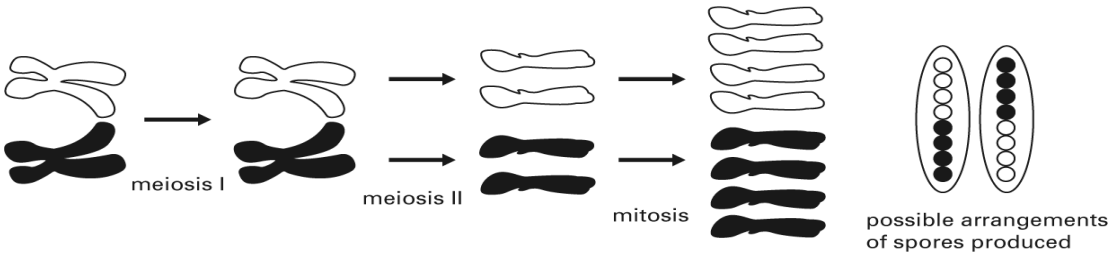
During sexual reproduction, haploid cells from two different filaments, either from one individual mycelium or two different strains, can fuse to form a diploid cell. The diploid cell then immediately undergoes meiosis to form four haploid cells, which in turn go through mitosis, resulting in eight haploid cells. These cells are arranged in sacs called *asci* (singular, *ascus)* inside a fruiting body, where they will mature into spores.

Sordaria spores are normally black (the wild type), but a mutation in one of the genes that determines color can make the spores look tan. Because the spores are haploid, only one allele of this gene is present. This allele can be determined by looking at the spores. If they are black, it is the wild type allele (+). If they are tan, it is a mutant allele (*t*). If wild type and tan mutant haploid cells fuse, four black and four tan spores will ultimately be produced. How the spores are arranged in an ascus, however, depends on whether chromosomes cross over during meiosis. If they do not cross over, the tan spores and black spores will be grouped together in foursomes. If crossing over occurs, the spores will not be grouped together in fours.

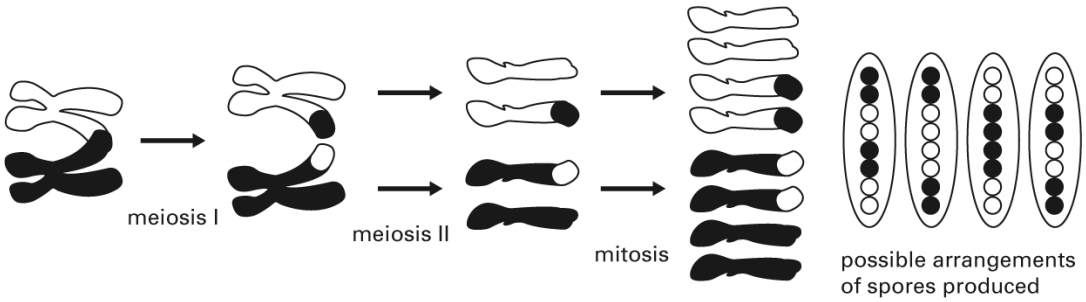
MATERIALS

- agar plate, with *Sordaria* culture
- cover slip (10)
- cup, large paper (for disposal of microscope slides)
- cup, small paper (for disposal of toothpicks)
- microscope
- microscope slide (10)
- pencil with eraser
- pipette, plastic disposable
- toothpick (10)

Formation of non-crossover asci:



Formation of crossover (recombinant) asci:



In this exercise, you will examine *Sordaria* asci to determine if crossing over occurred during meiosis.

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PREDICT

How often do you think crossover occurs in *Sordaria* during meiosis?

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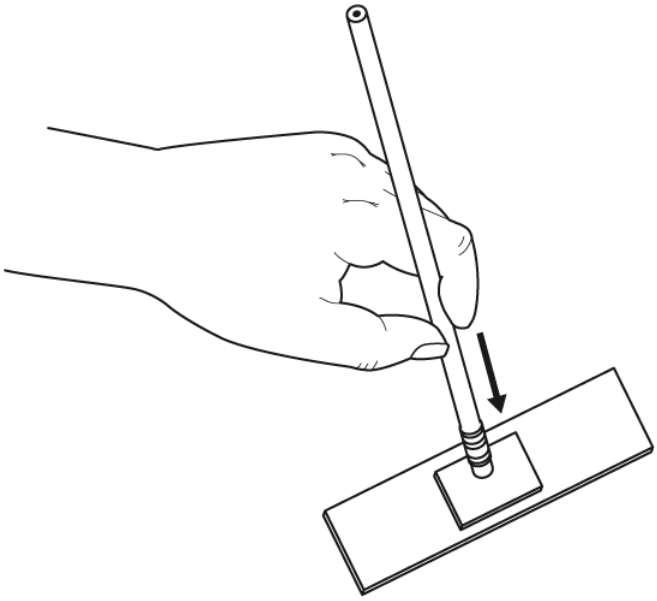
PROCEDURE

1. Your teacher will have set up crosses between wild type and mutant *Sordaria* for you. From the area where the two strains have overlapped, gently scrape the surface agar with a toothpick to collect a sample of fruiting bodies. Be careful not to dig into the agar.
2. Place your sample into a drop of water on a clean microscope slide, and cover it with a coverslip.
3. Put your toothpick into the small paper cup for disposal.
4. Gently press down on the coverslip using the eraser end of a pencil. You are applying pressure to release the asci from the fruiting bodies. Be careful—if you apply too much pressure the asci will break open and the spores will be released; you want to look at the spores *in the sacs.*



5. View the slide under the microscope, starting first with low power. Find asci containing both black and tan spores. Remember that *Sordaria* can also mate with itself, so asci containing spores of only one color will also be found.
6. Look at the arrangement of spores in one ascus. Determine if this is a crossover or a non-crossover ascus.
7. Try to count at least 50 asci, and record the number of non-crossover asci and crossover asci in the Data Table. Since the asci will likely be arrayed in a radial fashion (like spokes on a wheel), it is best to work your way around the slide in a circle while classifying each ascus. Stop counting once you have come back to your starting point, so that you don't count an ascus more than once. You may have to make a number of slides before you see 50 asci with black and tan spores.
8. When you are finished with a slide, place it into the large paper cup for disposal.

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DATA TABLE: NON-CROSSOVER AND CROSSOVER ASCI

Non-crossover asci		**Total:**
Crossover asci		**Total:**

ANALYZE

1. Did the asci in your samples contain all black or all tan spores? How were these asci generated?

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2. If you were to take samples from an area of the plate where the strains did not overlap, how would the spores in the asci appear?

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3. Draw the appearance of the asci on your slides.

4. How many asci were crossover asci?

_____

5. What percentage of total asci (non-crossover and crossover) were the crossover asci?

$$\frac{\text{crossover asci}}{\text{total asci}} \times 100 =$$

EXTEND

For every individual chromosome, maps can be made that show the relative position of genes. Crossing over can be investigated and used to generate these maps, based on the principle that crossing over between two positions on a chromosome will occur more often as the distance between these positions increases. The distance between the color gene and the centromere of a *Sordaria* chromosome (in *map units,* an arbitrary unit of measure) is determined by finding the frequency of crossing over and multiplying by 100. (The frequency of crossing over is calculated by dividing the number of crossover asci by two times the total number of crossover and non-crossover asci.) Use your data to find this distance.