

Meiosis and Crossing Over in *Sordaria*

Background Information:

<http://alpha.furman.edu/~lthomps/bgy30/30sorda.htm>

<Http://www.lehigh.edu/~jas0/102-Module%203-fungi.PDF>

Observing *Sordaria* Life Cycle Stages:

Examine the plates (SEE IMAGE ON *SORDARIA* GENETICS PPT) on each bench containing colonies of *Sordaria fimicola*. The plates were inoculated by placing small pieces of agar containing mycelia from both wild and tan-spored parental strains at four positions on the plate. Note the fuzzy appearance on the plate surface; this is the fungal mycelium composed of highly branched filaments containing haploid nuclei. Because the spore color genes affect melanin production and deposition in the cell walls of the mycelium as well as the spores, you should be able to see a difference in the color of the mycelia on different regions of each plate. Look for many small black objects on the surface of the agar. These are the perithecia or spore-dispersal organs of the fungus. Each contains numerous asci, in various stages of development. Note a dusty coating on the lid in areas immediately above regions on the plate where perithecia are common. These are deposits of ascospores that have been ejected from perithecia.

Observing Outcomes of the Cross:

On these plates, mycelia from two genetically distinct strains are growing together, mating and producing perithecia, each containing numerous asci. This cross is symbolized as.....t x t+ where t = tan spores and t+ = wild (black) spores:

The ordering of the ascospores within this ascus reflects the arrangement of homologous chromosomes during metaphase 1, and will reveal when crossing over between the locus of these alleles and the centromere occurred.

Squashing Perithecia

(SEE IMAGES OF SQUASHED PERITHECIA ON *SORDARIA* GENETICS PPT)

NOTE: THIS IS THE TECHNIQUE FOR SQUASHING PERITHECIA. WE WILL NOT ACTUALLY BE DOING THIS, BUT RATHER OBSERVING COLORED MICROGRAPHS OF SQUASHED PERITHECIA ON THE *SORDARIA* GENETICS PPT

1. Place a drop of distilled water on a clean microscope slide.
2. Remove the petri dish lid, and, remove 10 to 15 perithecia by scraping teasing needle over the surface of the agar. Do not dig into the agar.
3. Place the perithecia into the drop on the slide and uniformly distribute the perithecia throughout the drop.
 - a. Place a cover slip over the perithecia and water.
 - b. Place a tissue over your finger (to avoid putting a fingerprint on the cover slip) and push down gently, but a little firmly, on the cover slips. If you rub the cover slip back and forth (very slightly) over the slide, you will spread the asci better.
4. View the slide with the compound microscope first with the 10x objective in place. (SEE IMAGES ON *SORDARIA* GENETICS PPT)
5. Select for study only those perithecia that contain asci with more than one spore phenotype.
6. Identify the phenotypes of the spores in each ascus with the 40x objective in place.

Data Collection

Properly squashed perithecia will eject their asci in a radial arrangement, like the spokes of a wheel. In collecting data it is best to start at one point, systematically moving in a clockwise or counterclockwise fashion, classifying each ascus that can be clearly seen until a full circle has been completed. You will need to focus carefully to see all the spores in an ascus. Note that the ascus narrows on the end that connects it to the fungal mycelium. Locating the narrowed, broken end will allow you to properly classify any isolated asci that may be found on your slides. Your lab instructor will display several images showing tetrad asci so you can start to learn the two different spore phenotypes. To be most successful at identifying spore phenotypes be sure to...

- Keep light intensity low so you can see color differences better.
- Focus up and down carefully to see all eight ascospores.
- Avoid immature asci (their spores will have a granular appearance and very little color).
- Seek your instructor's help until you become confident in your ability to identify the phenotypes.

FOLLOW THIS PROCEDURE WHEN VIEWING THE MICROGRAPHS ON THE *SORDARIA* GENETICS PPT:

1. As you classify asci, record the sequence of spores within each ascus on the tally sheet
 - a. Each GROUP should classify at least 100 asci to insure an adequate sample size for the class data.
2. Add your count to the class data sheet.

Tetrad Analysis Tally Sheet		
Genotype	tally	count
bbbb		
tttt		
bbtt		
ttbb		
btbt		
tbtb		
tbbt		
bttb		

Genotype	Group 1 count	Group 2 Count	Group 3 Count	Group 4 Count	Group 5 Count	Class Total
bbtt						
ttbb						
btbt						
tbtb						
tbbt						
bttb						
Total Asci Counted →						

Calculations:

Ratio of bbtb:ttbb (all students) _____

$$\text{Percent crossover asci} = \frac{\text{bttb} + \text{tbtt} + \text{tbtb} + \text{ttbt}}{\text{Total Interstrain Ascii}} \times 100 = \underline{\hspace{2cm}}$$

$$\text{Distance between Centromere and Locus of Tan Gene} = \frac{\% \text{ Crossover}}{2} = \underline{\hspace{2cm}}$$

1. Is this distance accurate? If not what other factor needs to be considered?
2. Published results indicate that the map distance from the centromere of the gene for spore color in *S. fimicola* is 26 map units (corresponds to 52% crossover frequency)
3. How close are your results to the published results?
4. Calculate your percent error