

Topic 11. Meiosis

I. The Process of Meiosis

Meiosis is a process of two sequenced nuclear divisions neither of which is identical to mitosis. Meiosis always begins with a nucleus with two sets of chromosomes (a diploid nucleus) where each chromosome has two chromatids. Meiosis produces four nuclei each with one set of chromosomes (haploid nuclei) in which each chromosome has one chromatid. Meiosis together with syngamy constitutes sexuality in eukaryotes. Sexual reproduction generates individuals with new and unique genotypic combinations each generation.

Meiosis, by itself, can generate new combinations of genetic material. It does so in two ways.

- through the independent assortment of different homologous pairs of chromosomes
- and**
- through crossing over between homologous pairs of chromosomes.

Pop Beads as Models of Chromosomes

The shuffling and recombination of genetic material can be modeled using pop beads. Your TA will illustrate the process of meiosis for you using pop beads.....

1. with one pair of homologs without consideration of crossing over to simply demonstrate the differences between meiosis and mitosis.
2. with two pairs of homologs without consideration of crossing to demonstrate the independent movement of differently pairs of homologs.
3. with one pair of homologs showing crossing over to demonstrate how the genetic information is exchanged between homologous chromosomes.

You should then work with a partner to recreate these scenarios, and to do a fourth with two sets of homologs and with crossing over.

Note, we have a lesson on the web page that clearly explains how we use pop beads to model chromosomes in meiosis. You will need to be able to interpret questions about the process of meiosis that requires both an understanding of meiosis, and also of how pop beads are used in lab to represent chromosomes in the various stages of meiosis.

II. Microsporogenesis in Lily

In plants, meiosis results in the formation of spores. In flowering plants, spores (specifically microspores) are produced in the anthers of flowers. These spores mature into pollen grains. Meiosis in the anthers of lily is outlined in lessons, available on the computer on the side bench, and also on the web page. You should recognize each stage of meiosis and also be able to answer questions relating to the events occurring in each stage.

III. Analysis of the Process of Meiosis Using Asci of *Sordaria*

The consequences of independent assortment and crossing over are generally only apparent through analyzing patterns of inheritance over a number of generations. In the case of the fungus, *Sordaria*, however, the products of meiosis, the spores, are aligned in the narrow mother cell wall. Hence, for the trait “spore color”, the consequences of crossing over and of independent assortment are immediately apparent. The following activity was adapted from one written by Jon Glase at Cornell University.

Tetrad Analysis and Gene Mapping in the Fungus *Sordaria fimicola*

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Introduction: The Genetics of Fungi.

Because of the existence of dominance-recessive relationships among alleles, one of the problems inherent in genetic studies of diploid organisms, like Mendel’s pea plants, is the difficulty of inferring genotypes from phenotypes. For example, in peas, homozygous dominant individuals (TT) and heterozygous individuals (Tt) are phenotypically the same, both tall. In contrast, haploid organisms have only one chromosome for each of the chromosome types, so they have only one allele for each trait. This simplifies the interpretation of genetic crosses considerably because each allele is expressed in the phenotype. For this reason, and another discussed shortly, geneticists have long favored haploid organisms like the fungi as subjects for their studies.

Early genetic studies with the fruit fly, *Drosophila melanogaster*, suggested that a mechanism must exist to allow exchange of genetic material between homologous chromosomes. Microscopic studies of meiosis show that this exchange, called **crossing over**, takes place during prophase I when homologous chromosomes are in **synapsis**. During crossing over, breakage-refusion points called **chiasmata** develop between synapsed chromosomes. These chiasmata result from pieces of the two homologues being switched in an equal and reciprocal fashion. Crossing over combines genetic material that had previously been on separate homologues and produces individuals with new genetic combinations. Geneticists also came to realize that crossing over could be used as an important tool for discerning the physical location of genes on chromosomes. They reasoned that if chiasmata can form at any point between two homologous chromosomes, then the frequency of crossing over in the region between two different genes on a chromosome should vary directly with the physical distance between the genes. When this hypothesis was confirmed it was possible to begin mapping the positions of genes on chromosomes.

In most genetic studies, a **cross**, or mating, is made between parents whose genotypes may be partially known. These parents contain gametes that have resulted from many meiotic divisions within their gamete-producing structures. Each

meiotic division produces four haploid nuclei collectively called a **tetrad**. In most organisms the products of each meiotic division are not kept separate but become part of a “pool” of meiotic products (gametes). The mating activities of the parents combine these meiotic products in a random fashion to produce the next generation. Thus, in most organisms, it is impossible to examine the assortment of alleles in an individual meiotic division. However, such a genetic description of an individual meiotic division would be particularly advantageous in studying the occurrence and frequency of crossing over, **and** for demonstrating the random assortment of the chromosomes to the daughter nuclei during meiosis I.

In certain fungi such as the pink bread mold, *Neurospora crassa*, and *Sordaria fimicola* (the organism you will study during this lab), meiosis occurs within a structure called an **ascus**, which isolates each tetrad. The four products of meiosis occur within the ascus in the order in which they arose during meiosis. With these organisms a special type of genetic analysis called **tetrad analysis** can be used. In tetrad analysis, the genetic make-up of each cell of a tetrad can be studied with respect to a particular trait, and this information can be related to the meiotic division that produced the tetrad. Tetrad analysis makes it possible to determine the alternate directions in which homologous chromosomes move during anaphase of meiosis I. Further it makes it possible to determine when crossing over has occurred. This information can be used to map the locations of genes on chromosomes relative to a chromosomes centromere and to each other.

Fertilization consists of two separate, sequential events. First, fusion of the contents of two cells, called **plasmogamy**, forms one cell with two haploid nuclei. Second, fusion of the haploid nuclei, called **karyogamy**, forms a zygote with a diploid nucleus. In most organisms, karyogamy immediately follows plasmogamy. In the fungi, cells with two nuclei (called **dikaryotic cells**) remain for extended periods of time and can undergo cytokinesis to form new dikaryotic cells. Only at a later time does karyogamy occur to form a diploid zygote. In most fungi, the zygote is the only diploid stage in the life cycle.

In *Sordaria fimicola*, the fungal body is composed of haploid cells arranged in long filaments called **hyphae**; the whole network of filaments is called a **mycelium**. If conditions are right, the fungus forms sex organs containing numerous haploid nuclei. These sex organs will conjugate and nuclei will migrate from one organ to the other where they pair up. Hyphae with cells containing two different nuclei will grow from these junctures. Karyogamy occurs within a cell called an ascus primordium to form a diploid zygote. Each zygote immediately undergoes meiosis, producing four haploid nuclei, and each of these divide once, mitotically, yielding a total of eight haploid cells. Each cell develops a resistant cell wall and is called an **ascospore**. All of these events occur within an elongate sac called an **ascus**. At maturity an ascus contains eight ascospores arranged in a precise manner. Clusters of asci develop within a special spore dispersal structures called a **fruiting bodies**. As the spores within each ascus mature, the ascus tip ruptures, and hydrostatic pressure within the ascus propels the spores outward. The entire life cycle from spore germination to production of new spores in perithecia takes about 10 days.

One important feature of ascus-forming fungi is their spore formation process. The meiotic and mitotic divisions producing eight ascospores from one zygote are subject to the physical limitations imposed by the shape of the ascus. The long, thin

ascus causes both meiotic spindles and the mitotic spindles to be aligned during spore formation. As a result, the positioning of the ascospores directly reflects the arrangement of homologous chromosome pairs during metaphase I of meiosis. Also, since ascospores are haploid, all alleles are phenotypically expressed.

The equivalence of the genotype with the phenotype in the haploid cells; the short generation time, and the preservation of the sequenced events of meiosis through the arrangement of the spores in each ascus, make *Sordaria* a useful subject for study by geneticists.

The Genetic Determination of Spore Coloration

The spore color of the normal (wild type) *Sordaria* is black. The black coloration of wild type *Sordaria* spores is due to the production of the pigment melanin and its deposition in the cell walls. Several different genes are involved in producing this phenotype and each gene has two possible allelic forms. We will be concerned with the **tan spore gene** which has two forms: a wild type allele (t⁺) and a mutant allele (t). Normal black spores are produced only if the wild type allele is present. Thus, black ascospores have the genotype t⁺ (remember, spores are haploid). Those with the genotype t ascospores are tan.

Melanin is produced within the cell by a series of sequenced, coupled reactions called a **biosynthetic pathway**. You need to consider this pathway in order to better understand the inheritance of spore coloration in *Sordaria*.

In a hypothetical biosynthetic pathway for melanin production, each reaction is catalyzed by a different enzyme. The wild type allele produces a normal enzyme for one reaction in the biosynthetic pathway for melanin biosynthesis. The mutant allele for tan spore gene (t), does not produce a normal enzyme for that stage of the pathway. The enzyme produced is defective, blocking the pathway resulting in the accumulation of a tan-colored precursor and the formation of tan colored spores.

Crossing Over in *Sordaria*

Observing *Sordaria* Life Cycle Stages. Examine the plates 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 fruiting bodies. 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 fruiting bodies.

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 each ascus will reflect the arrangement of homologous chromosomes during metaphase 1, and will also reveal if crossing over occurred between the locus of the spore color gene and the centromere.

Modeling *Sordaria* Crosses

Work with the preconfigured popit beads and meiotic diagrams on each bench. Note that these beads represent the DNA that make up each chromosome. The magnets to which the beads are attached represent the centromere of a chromosome. Also note that the chromosome contributed from one strain is represented by the red strand, and the other by the yellow. The fact that these are homologous is indicated by their identical shape. The locus for spore color is occupied by beads of a different color. On one strand is a purple bead symbolizing the wild color gene (**t+**) on the other is a green bead symbolizing the tan color gene (**t**).

1. Start your simulation with karyogamy. Bring a single-stranded “red” chromosome together with a single stranded “yellow” chromosome into the top circle. The contents of this circle now represents the karyotype of a zygote.
2. Next simulate DNA replication in the zygote by attaching identical strands together at each centromere. The karyotype of the zygote is now represented by two chromosomes and four chromatids.
3. Now proceed through the process of meiosis as earlier demonstrated by your TA, without crossing over. Note that, depending on the direction of the movement of the chromosomes during anaphase of meiosis I, you can derive two different types of asci which are mirror images of one another. In each case the phenotypes observed are:

t= tan and
b= black

and your derived asci are

ttttbbbb (can be simplified as **ttbb**), and
bbbbtttt (can be simplified as **bbtt**).

Modeling Crossover Tetrads: You should now derive the other types of asci possible which are possible through crossing over. These are:

tbtb **tbbt**
btbt **bttb**

Decide what type of crossover will be required to produce each ascus and break and re-couple bead strands to reflect the crossover. Note that to produce a crossover ascus the crossover must occur between the gene’s locus and the centromere.

Crossovers on the opposite arm of the chromosome or on the same arm but beyond the gene's locus do not produce crossover asci.

Squashing Fruiting Bodies

Each fruiting body is derived from one cell containing two nuclei that formed from plasmogamy. Unlike many fungi, *Sordaria* is self-fertile and some of the fruiting bodies you will examine have resulted from intrastrain matings. Fruiting bodies resulting from self-mating will contain asci with all tan or all black ascospores. Since an ascus must contain at least two different phenotypes in order to detect a crossover, you will be concerned exclusively with fruiting bodies that have resulted from interstrain matings. Fruiting bodies containing interstrain asci (with spores of at least two different phenotypes) should be most abundant in the areas of the dish where the two strains grew together. These cultures will not be used after this lab, so you need not use sterile technique to obtain fruiting bodies.

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

Data Collection

Properly squashed fruiting bodies 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.

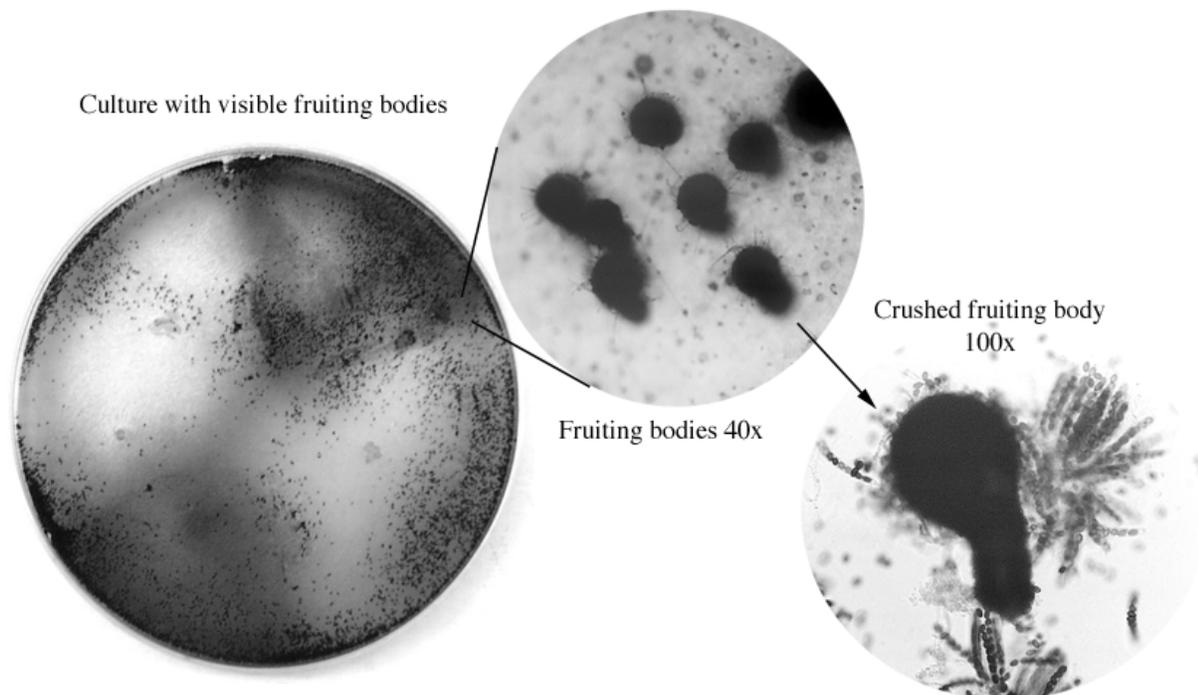
Your lab instructor will display several images showing tetatype asci so you can start to learn the two different spore phenotypes. To be most successful at identifying spore phenotypes be sure to...

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

As you classify asci, record the sequence of spores within each ascus on the tally sheet (attached).

Each individual should classify at least 30 asci to insure an adequate sample size for the class data.

Add your count to the class data sheet. Next week record the counts for all students from a master data sheet to be posted.



Tetrad Analysis Tally Sheet

Intrastrain Ascii

 <p style="text-align: center;">tttt</p>	 <p style="text-align: center;">bbbb</p>
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Interstrain Ascii (Non-crossover)

 <p style="text-align: center;">ttbb</p>	 <p style="text-align: center;">bbtt</p>
Tally:	Tally:

Interstrain Ascii (Crossover)

 <p style="text-align: center;">btbt</p>	 <p style="text-align: center;">tbtb</p>
Tally:	Tally:
 <p style="text-align: center;">tbbt</p>	 <p style="text-align: center;">bttb</p>
Tally:	Tally:

Data Sheet

	Your Total Count	Section Total Count	All Students
bbtt			
ttbb			
btbt			
tbtb			
tbbt			
bttb			

Ratio of bttt:ttbb (all students) _____

$$\text{Percent crossover asci} = \frac{\text{bttb} + \text{tbbt} + \text{tbtb} + \text{btbt}}{\text{Total Interstrain Asci}} \times 100$$

= _____

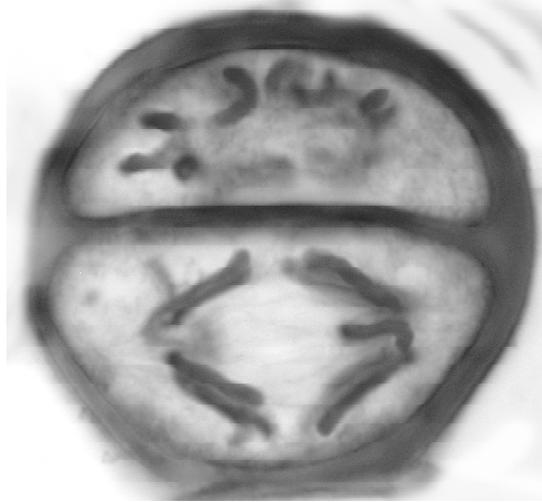
$$\text{Crossover frequency} = \frac{\text{bttb} + \text{tbbt} + \text{tbtb} + \text{btbt}}{2 \times \text{Total Interstrain Asci}} \times 100$$

= _____

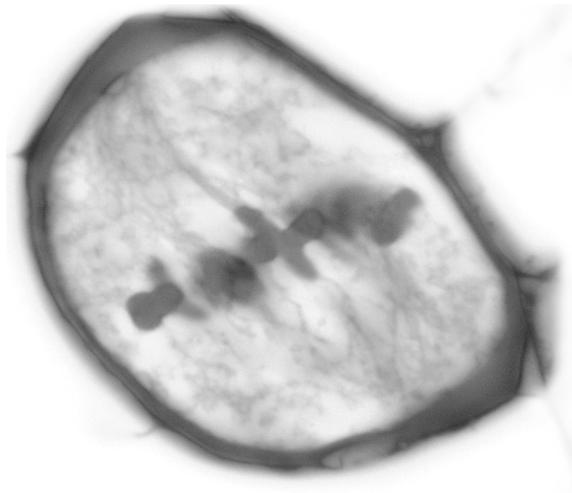
Calculated Distance Between Centromere and Locus of Tan Gene:

Is this distance accurate? If not what other factor needs to be considered?

Three Stages of Meiosis in Lily



Metaphase II



Metaphase I



Telophase II