MEIOSIS AND RECOMBINATION IN SORDARIA FIMICOLA

Introduction:

In ascomycete fungi, a form of meiosis occurs in which the products of meiosis order themselves within a fruiting body according to the physical separation and segregation of chromatids during the meiotic process. This is covered in some detail on pages 150-152 (including Figures 4.26 and 4.27) in Hartl and Jones, *Essential Genetics*. You should study these pages before beginning this module. As described, ordered tetrad analysis provides a way to measure the genetic map distance between a gene and the centromere of the chromosome on which that gene resides. That is what you will do over the next two weeks in this laboratory.

I. Natural history and Life Cycle of Sordaria fimicola

Sordaria fimicola is an ascomycete fungi that can be found growing in rotting vegetation and animal dung (in fact, the name *Sordaria fimicola* means "filthy dung dweller"). *Sordaria* and another ascomycete, the common bread fungus *Neurospora crassa* (Fig. 4.26), have been used as model systems for studying the process of chromosome exchange (crossing-over) because of their reproductive characteristics.

The life cycle of *Sordaria* is representative of the ascomycetes (although there are substantial differences in the details among species). The individual fungus begins as a haploid ascospore. The ascospore germinates to form hyphae (singular = hypha), which are long filaments comprised of haploid cells. These hyphae grow and extend throughout the nutrient source (dung or rotting vegetation in nature, nutrient medium in the laboratory situation) and digest it by means of enzymes secreted by the cells. Nutrients are then absorbed into the cells.

When the hyphae of different individuals come in contact, cells in the hyphae can fuse to form single cells with two nuclei, one derived from each individual. Such a cell is called a dikaryon (literally "double nucleus") or heterokaryon (literally "different nucleus"). The dikaryon has a N+N genotype; it is not actually diploid, because it has two separate nuclei, each of which is haploid. After many mitotic divisions, (forming a large mass of cells, the nuclei in some of the dikaryon cells fuse to form a single diploid nucleus. Such cells may be properly referred to as zygotes. The diploid zygotes then undergo normal meiosis to form four haploid nuclei. Individual cells form around these eight nuclei, and these cells become the ascospores. All eight ascospores are held within a single membranous sac called the perithecium. In nature, the perithecium eventually ruptures and releases thousands of ascospores into the wind. Thus, the life cycle is completed. As you can see, the great majority of the life cycle of this species is haploid.

In some ascomycetes, the ascospores are arranged randomly within the ascus, but in *Sordaria*, *Neurospora* and many others, they are arranged in a way that reflects the events that took place during the first and second meiotic divisions. The four products of meiosis (in any organism) are called a tetrad. When an additional mitotic division occurs, as in ascomycetes, the tetrad becomes an octad. However, since we are really only interested in the products of the meiotic events, we generally view the ascospores as four pairs. Hence, we perform tetrad analysis (rather than "octad analysis"), as shown in Figure 4.27 in Hartl and Jones.

II. Strains and Matings:

Sordaria, like other organisms, exhibits genetic variants that result in phenotypes that are qualitatively different from the common (wild-type) phenotype. One character that exhibits several different variant types is the color of the ascospores. The common wild-type has black ascospores, but strains with red, pink, gray and tan ascospores also exist. For this experiment, we will use one strain (G) that has gray ascospores, and another (T) that has tan ascospores. The different colors are the result of simple allelic differences from the wild type state. However, since several loci have the potential to determine ascospore color, you cannot assume that the gene that causes tan color is allelic to the one that causes grey color. It may be that they are variants at totally different gene loci. You will make this determination in this experiment.

Procedure:

First Week – Start the Cross, Watch the Hyphae Grow, See the Perithecia

You will have access to corn meal agar plates on which the three strains, B (common wild-type, ascospores are black), G (gray ascospores), and T (tan ascospores), have been growing for about a week at room temperature.

1. Using sterile technique, cut some small agar plugs from each plate (squares of about 6-7 mm x 6-7 mm). Onto each "mating plate", place a total of four plugs upside down near the center of the plate, as described by your instructor, one plug in each quartile of the plate. For example, place two plugs of strain B about 2-3 cm apart and two plugs of strain G also about 2-3 cm apart, oriented 90 degrees from the B plugs. That is, the plugs should be at the ends of a 2 cm "+" sign in the middle of the plate. Do two plates for a BxG mating and two for a BxT mating.

2. Observe the plates throughout the next seven days, as you keep them at room temperature and out of direct sunlight. Record the appearance of the plates each day. On one of the first two days, observe the hyphae tips under the microscope. Can you detect growth while you watch? When do the hyphae completely cover the agar surface? When do the perithecia start to become visible? What do the plates look like one week after plating?

Second Week - Visualizing and Scoring the Asci

Inside the perithecia are the asci with their sets of 8 ordered ascospores. The job now is to gently rupture the perithecia to release the asci, but not to rupture the asci in the process.

1. Use a toothpick, dissecting needle, forceps or spatula to pick off some of the mature perithecia from the strain "boundaries" near the edge of the plate. Place the perithecia in a small drop of water on a microscope slide. Add a cover slip and blot away any excess water. View under low power to see the intact perithecia. Then, use an eraser or similar soft object to push down gently on the cover slip. The goal is to rupture the perithecia and release the asci intact. Too little pressure will not rupture the perithecia, while too much pressure will also burst the asci and release the ascospores. You may need to do this step several times to get a good preparation, *i.e.*, lots of intact asci visible under the microscope.

2. Use low power to find good asci, and then higher power to examine the colors and arrangements within the asci. You are looking for <u>mixed-color asci</u>; only these are the result of mating between the two different strains on the plate. For each mating, count a minimum of 100 mixed-color asci. Record how many of these have a 4:4 arrangement of ascospores (these are the non-recombinants), and how many have a 2:4:2 or 2:2:2:2 arrangement (these recombinant arrangements arise only when crossing over has occurred between the "color" allele and the centromere of the chromosome on which this gene resides).

Data Analysis:

We want to determine the frequency of crossing over between a particular gene controlling ascospore color and the centromere of the particular chromosome on which the gene resides. From this information we can estimate the linkage (genetic map units) between the gene and the centromere of the chromosome it is on.

1. Calculate the recombination (crossing-over) frequencies.

	# recombinant asci		
Recombination (Crossing over) Frequency =		Х	0.5
	total # two-color asci		

[The multiplication of the frequency by 0.5 is necessary because a "recombinant ascus" actually contains four non-recombinant as well as four recombinant versions of the chromosome on which the gene of interest resides. That is, a bivalent in which a single cross over occurs ("50% recombination") gives rise to an ascus that will be counted as being recombinant].

2. From the recombination frequencies, estimate the genetic map distance (in cM) from the centromere of the chromosome to the "gray" allele and to the "tan" allele. You can make use of Figure 4.17 from Hartl and Jones, assuming an intermediate amount of interference. Also, pay attention to the text on page 152.

3. Do you have enough information to determine whether the "tan" and "gray" alleles are variations of the same gene or two different genes? On the same or different chromosomes? Explain your answer.

4. What further experiments could you do to map these genes in more detail? Explain.