Demonstration of crossing-over during meiosis in *Sordaria fimicola*

Introduction

       Sexual reproduction is a special case of cell reproduction in which the genes of two individuals are "shuffled." In this way it is unlike asexual reproduction, in which all the daughter cells (and organisms) are genetically identical to the parent cell. The result of asexual reproduction is a series of clones (rather like the bacterial colonies we observed last week). In contrast, sexual reproduction involves the fusion of two cells called gametes, one typically understood as male, the other as female (sperm and eggs in humans). Gametes have half the typical number of chromosomes. For example, if the species typically has 46 chromosomes, like humans, then the gametes will have 23. The reduction in the number of chromosomes occurs during a specialized cell division called meiosis.

       The choice of which chromosomes (those originally inherited from the father or from the mother, paternal or maternal, respectively) are found in which gamete is a random event. Thus depending on **N**, the total number of kinds of chromosomes in the genome, there are 2^N different combinations of paternal and maternal chromosomes that can arise due solely to the divisions of meiosis. Additional variation arises from two other sources: mutation and recombination. We have already had some (disappointing) evidence of the rarity of mutation (i.e. our failure to detect any ampicillin-resistant mutations in a total of 0.3 mL of a population containing about 3×10^9 *E. coli* cells per mL.) Today we will discover a system that allows us to estimate recombination, and indirectly a means of mapping the chromosome.

       To interpret our data, we need to become comfortable with the process of meiosis, a series of cell divisions that only a special subset of the cells of higher plants and animals can undertake. These cells, the germ cells, are reserved in specialized structures (called gonads in animals) at a very early time in the development of the organism. In humans, for example, the ovaries are set aside and endowed with germ cells in prenatal life, so that a female infant already has her full complement of potential ova (eggs) by the time she is born. In fact, each of those ova has already begun the process of meiosis, but that process will not be completed until that particular ovum is released from the ovary, beginning at sexual maturity 11 to 14 years later and concluding at menopause another three to four decades after that.

       Single-celled organisms, like fungi (molds and yeast), can also carry out meiosis. In such organisms, cells equivalent to sperm and eggs (haploid cells) grow attached to one another by mitotic divisions. The structures known as hyphae connect in a multiply branching mat known as a mycelium. At the tips of the mycelium, individual spores form, called conidia. Since the divisions are mitotic, all the nuclei generated are equivalent to one another. When nutrient supplies are low or other stresses appear in the environment, conidia from one mating type (usually called + or A) fuse with hyphae of the opposite mating type (- or a). In fact, one can imagine that under precisely those conditions of environmental stress, it is advantageous to try out new combinations of genes. The hypha now contains two distinct nuclei, one from each mating type; it forms a sac-like structure called an ascus, and within this structure the two nuclei fuse. The resulting nucleus is diploid. Almost immediately, the nucleus enters meiosis, and proceeds through the two typical meiotic divisions: first, a reduction division, and second, an equational division.

As the reduction division begins, homologous chromosomes from each parent find one another and engage in close pairing, occasionally exchanging partners by physically breaking opposite one another and rejoining across the paired structure. This "crossing over" event occurs in all meioses, with a frequency that depends on the genetic distance between observable "loci," the locations of particular genes whose effects on the organism are visible. Once the division has commenced, the centromeres of the homologous pairs separate to opposite sides of the mitotic spindle, and the resulting nuclei are once again haploid. The equational division separates the sister chromatids from one another, just as in mitosis, and the four products of meiosis result.

In most organisms it is impossible to determine which cells arose in the first division and which in the second, because the products are in an indeterminate order. In fungi of the family Ascomycetes, however, the divisions take place in a sac, the ascus, that is long and narrow, so the meiotic spindle can align only along the axis of the sac. The result is that the first division gives two products, one at the base of the sac and the other toward the tip. The equational division now separates the sister chromatids, and the four products are in order: the lower two from the first division, the upper two from the second. Now a mitotic division occurs, after replication of the nuclear DNA, and each of the four nuclei become eight. The eight mature into ascospores, encased in the ascus, and a large number of asci are contained within a fruiting body, the perithecium, connected at their bases. In nature, the mature perithecium will burst releasing the ascospores to disseminate and carry their genes far and wide. In the laboratory, we try to time our experiment to happen just before they burst. The perithecia are easily ruptured, so they can be placed on a microscope slide and burst to reveal the asci within.

Why is this important? It turns out that certain genetic properties of the nuclei are expressed in the ascospores. Some strains have black, some brown, and some tan spores. Here *Sordaria* display another helpful property: they are self-fertile (homothallic). Each strain's conidia can fuse with its own hyphae. If a black strain mates with itself, all the spores will be the same color. But if a strain of one color mates with one of another color, the spores will express one color or the other: there will be two colors in the ascus. Not only will two colors be seen in a single ascus, the color distribution of the spores in the ascus represent the three meiotic divisions in order. If no crossing over occurs, 4 spores of one color will be at one end and 4 of the other color at the other end. But if crossing over occurred, the two sister chromatids on a single centromere will no longer be equivalent. When the centromeres separate at the first meiotic division, they will carry with them two non-identical arms, and those arms won't separate until the second division. The result will be an ascus with 2 + 2 + 2 + 2 color distribution or one with 2 + 4 + 2. This is hard to visualize. Refer to the illustration for assistance.

Today we will examine asci from a cross that I set up 11 days ago. According to what I have read, the spores should still be enclosed in asci in their perithecia, but meiosis should be complete and the spores mature to the point that their color is visible.

<u>Procedure</u>

On Friday, June 27, I planted four agar squares of *Sordaria* mycelium onto plates made of corn meal agar, which induces them to mate. The mycelia planted at two positions on the plate were from the tan strain (labeled -) and at opposite positions they were of the black strain (+). The marker lines on the bottom of the plate designate the quadrants where each strain was planted. The agar blocks on which the mycelia were introduced can still be seen.

As the mycelium grew out from each block, it gradually comes into contact with that from the other strain. Fusion takes place and then meiosis and maturation of the asci. If you look under the dissecting microscope, you will see the little black perithecia, each shaped sort of like a blob with a curlicue at one end. One source I read compared it to a Hershey's Kiss.

The task today is to collect perithecia, mount them on a microscope slide, gently rupture them and then assess their contents. You have been provided with the following tools:
- a microscope
- a dish of *Sordaria*
- a bottle of water
- glass microscope slides
- cover slips
- tissue paper
- toothpicks
- bibulous paper (blotting paper works too)

Put a drop of water on the microscope slide. Use the toothpick to remove a few mature perithecia from the area near the junction of the two mycelia and put them in the drop of water. Gently add a cover slip and press straight down with the eraser end of a pencil or your thumb. Avoid sideways movement of the cover slip, since that may cause the asci to burst, and once the spores are released, you cannot tell what order they were in. It might help to put the slide with cover slip between the leaves of a book of bibulous paper.

Use the microscope to examine the slide with the 10x objective lens (overall magnification of 100x). Each perithecium should have released its asci in a sort of rosette, anchored at one end to the perithecium. Ignore rosettes that are all dark (black strain self-crossed or all light gray or clear (tan strain self-crossed). Pay close attention to rosettes that have both colors in single asci.

Now rotate the 40x objective lens into place (400x magnification). For each perithecium <u>count</u> the number of asci where the order of the colors is 4:4, the numbers that are 2:2:2:2, and the numbers that are 2:4:2. If you find any color hard to assess or believe you have a different distribution of colors than the three above, put parentheses around that value and let me see it too. That may be a sign of a rare phenomenon called "gene conversion."

Depending on the quality of your mount, you should be able to get 10 to 20 asci from each perithecium. Don't give yourself a headache, but try to get data from at least 5 perithecia. Keep the results of each perithecium on a separate line in your data tablet. Convenient headings for the columns would be

Number of 4:4 asci	Number of 2:2:2:2 or 2:4:2 asci	Total number of asci
These are from meioses with no cross-overs	These are from meioses in which a cross-over event occurred between the centromere and the color gene	

Use your data to calculate the percent of the total number of asci in each perithecium that are of the two types of spore distribution. Enter your data on the class data sheet as well.

Data analysis

In constructing a genetic map we consider the unit of genetic distance along the map as defined by the percent of recombination: a distance that causes 1% of the gametes to be recombinant types = 1 map unit. Be careful: each recombination generates two recombinant and two parental chromosomes.

Thus the map distance = $\dfrac{\text{(number of recombinant octads)}}{2 * \text{(total number of octads)}}$

By that definition, how far is the color gene from the centromere? Can you think of any reason that this estimate might be systematically faulty?

Calibration of the microscope field of view

Most people think of the microscope as an instrument of description, whose output is an image. In fact, though, it can be a measuring tool as well. When you examine a specimen under the microscope, it occupies a circular "field of view," the light gathering dimension of the particular lens you are using. As the magnification of the lens increases, the field of view decreases, and that decrease is proportional. You can calibrate your microscope using the transparent ruler.

Place the ruler on the stage so the millimeter markings are under the 4x lens. Bring the markings into focus. Now count the number of divisions that are seen in one diameter of the field. Record that number. Now rotate the 10x lens into the light column. How many divisions are there across the diameter of the field of view now? What is the relationship between the actual physical diameter of the field of view and the magnification of the lens? Can you use that relationship to predict the measure of the 40x field of view?

With these calibrations, you can measure the size of any specimen. Use the 40x objective lens to examine an ascus. How much of the field diameter does the ascus length occupy? How much does its width occupy? What is the diameter of a spore in units of field diameter? Now translate those measures into millimeters using your calibrations. Neat, huh?