Chapter 1

A Study of Gene Linkage and Mapping Using Tetrad Analysis in the Fungus *Sordaria fimicola*

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Contents

Introduction

The laboratory presented here has been used in the introductory course for biology majors at Cornell University for the past 15 years. It was designed to accomplish the following objectives:

1. to reinforce knowledge of meiosis and its importance in transmission genetics,
2. to introduce the concepts of gene linkage and mapping,
3. to provide experience with fungal life cycle stages and with sterile methods for manipulating microorganisms,
4. to illustrate the use of the chi-square statistical test for selecting between different genetic models, and
5. to show application of the probability laws to the study of biological problems.

The fungi, especially those ascomycetes that produce isolated, ordered tetrads, have been fundamentally important to the teaching of genetics (Cassell and Mertens, 1968; Fincham, 1971). We selected the genus *Sordaria* over *Neurospora* for this study because of the absence of asexual, conidiospores in *Sordaria*. This reduces considerably the problem of accidental contamination of cultures. *S. fimicola* is a species that has been extensively studied (El-Ani et al., 1961; El-Ani and Olive, 1975) and for which numerous genetic markers are available. Spore color genes that have been studied in this species (Olive, 1956) are especially useful for teaching purposes because they allow tetrad analysis of intact asci and direct observation of the effects of crossing over. We chose *S. fimicola* over *S. brevicollis*, another species in this genus that has been used for teaching purposes, because *S. fimicola* does not undergo the spindle overlap during the second meiotic division that occurs in *S. brevicollis* (Chen and Olive, 1965). Spindle overlap makes tetrad analysis less direct and reduces its pedagogical effectiveness in illustrating the genetic implications of meiosis. However, since *S. brevicollis* is a heterothallic species, all of its perithecia contain asci resulting from interstrain mating. This makes locating asci suitable for tetrad analysis easier than in *S. fimicola*, which is homothallic.

The version of the laboratory described here involves examining the results of mating between mutant strains for two spore color genes (tan spore and gray spore) in order to map these genes and determine if they are linked. The laboratory extends over 3 weeks. Students set up the cross during the first week. Asci mature during the second week and are held in the refrigerator until the third week when tetrad analysis occurs and data are shared among the class. Included in the Appendix is a set of study questions (and answers) designed to help students with the analysis and interpretation of these data. This laboratory can be accomplished in one 3-hour laboratory if the genetic cross is set up for the students about 10 days ahead of time.

An alternate, simpler approach that requires more laboratory time is to have students begin with tetrad analysis of the wild type-mutant crosses (wild type × gray spore; wild type × tan spore). These data allow each gene to be mapped in isolation of the other gene. This is then followed by

tetrad analysis of the mutant-mutant cross and these data are only used to determine linkage. The analysis of the mutant-mutant cross included here is more complex because students are using data to both map genes and determine if they are linked. Though more complex, we think that this form of analysis is more educationally effective because it requires application of both of Mendel's principles of inheritance and a knowledge of meiosis with each ascus examined.

This study can be extended in a number of ways for use in upper-level biology courses, such as genetics. The genetic model most emphasized in this study is the unlinked model, but students could use the same approach to develop detailed predictions for the linked model (with genes on opposite or on the same side of the centromere). All models presented here consider only single crossovers. The effect of multiple crossovers and the use of mapping functions and the Poisson distribution to estimate real map distances from recombination map distances can also be included. Finally, a study of aberrant asci in *Sordaria fimicola* (Fincham, 1971) can lead to a consideration of such modern topics as gene conversion and the Holliday model for recombination by crossing over. For more information, see Chapter 6 on mapping functions and Chapter 18 on gene conversion and the Holliday model in the text by Suzuki et al. (1989).

Materials

Compound microscopes, 10X and 40X objectives, binocular heads if possible (1 per student)
Inoculating loops (1 per student)
Alcohol lamps or equivalent (1 per pair of students)
Bottles of alcohol for disinfecting bench tops (1 per pair of students)
Microscope slides and coverslips (4 boxes per lab for making wet mounts)
Boxes of kimwipes (1 per pair of students)
Dropper bottles filled with water (1 per pair of students)
Stock plates of gray spore and tan spore *Sordaria* strains (1 each per four students, used to set up crosses)
Sterile agar plates for making crosses (2 per pair of students)
Permanent markers (1 per student)
Lens paper for cleaning microscope lenses
Matches for lighting burners

Notes for the Instructor

1. Media preparation: The media we use for *Sordaria* plates is made from the following recipe: 17 g corn meal agar, 10 g sucrose, 7 g glucose, 1 g yeast extract, 0.1 g KH_2PO_4, and 1 liter water. Autoclave media at 15 psi for 20–30 minutes. Let the plates age for 2–3 days before plating.

2. Stock plates can be purchased from Carolina Biological Supply Co. (gray spore, #15-6293; tan spore, #15-6295). Inoculate your stock plates about 7 days before you want students to set up the crosses.

3. Mating plates: After the crosses have been made store in a 25°C incubator. Invert the plates after 1–2 days. About 7 days after the crosses are set up, start to monitor the plates for spore release. Spores will appear as fine dust on the inner surface of the petri dish lid. When spores are seen, store the plates in a refrigerator until the students need them. You can also make test squashes to directly observe the asci. Immature spores have little color and are granular in appearance. Over-ripe asci break easily and release their spores.

Student Outline

Introduction

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 double-chromatid, 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 increased genetic variation. Geneticists also came to realize that crossing over could be used as an important tool for learning more about the 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.

In certain fungi, such as the pink bread mold, *Neurospora crassa*, and *Sordaria fimicola*, 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 when crossing over has occurred, and to use this information to determine if genes are linked and to map the locations of genes on chromosomes.

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. Only at a later time when karyogamy occurs is a diploid zygote formed. In most fungi, the zygote is the only diploid stage in the life cycle.

In *Sordaria fimicola* (Figure 1.1), the multicellular 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. Nuclei then migrate from one organ to the other where they pair up. Budding off forms dikaryotic hyphae. Karyogamy occurs within an ascus primordium to form a diploid zygote. Each zygote immediately undergoes meiosis, producing four haploid nuclei which each 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*, so that at maturity an ascus contains eight ascospores arranged in a precise manner. Clusters of asci develop within a special spore

dispersal structure called a *perithecium*. As the spores within each ascus mature, the ascus tip ruptures, and hydrostatic pressure within the ascus propels the spores out of the perithecium. The entire life cycle from spore germination to production of new spores in perithecia takes about 10 days.

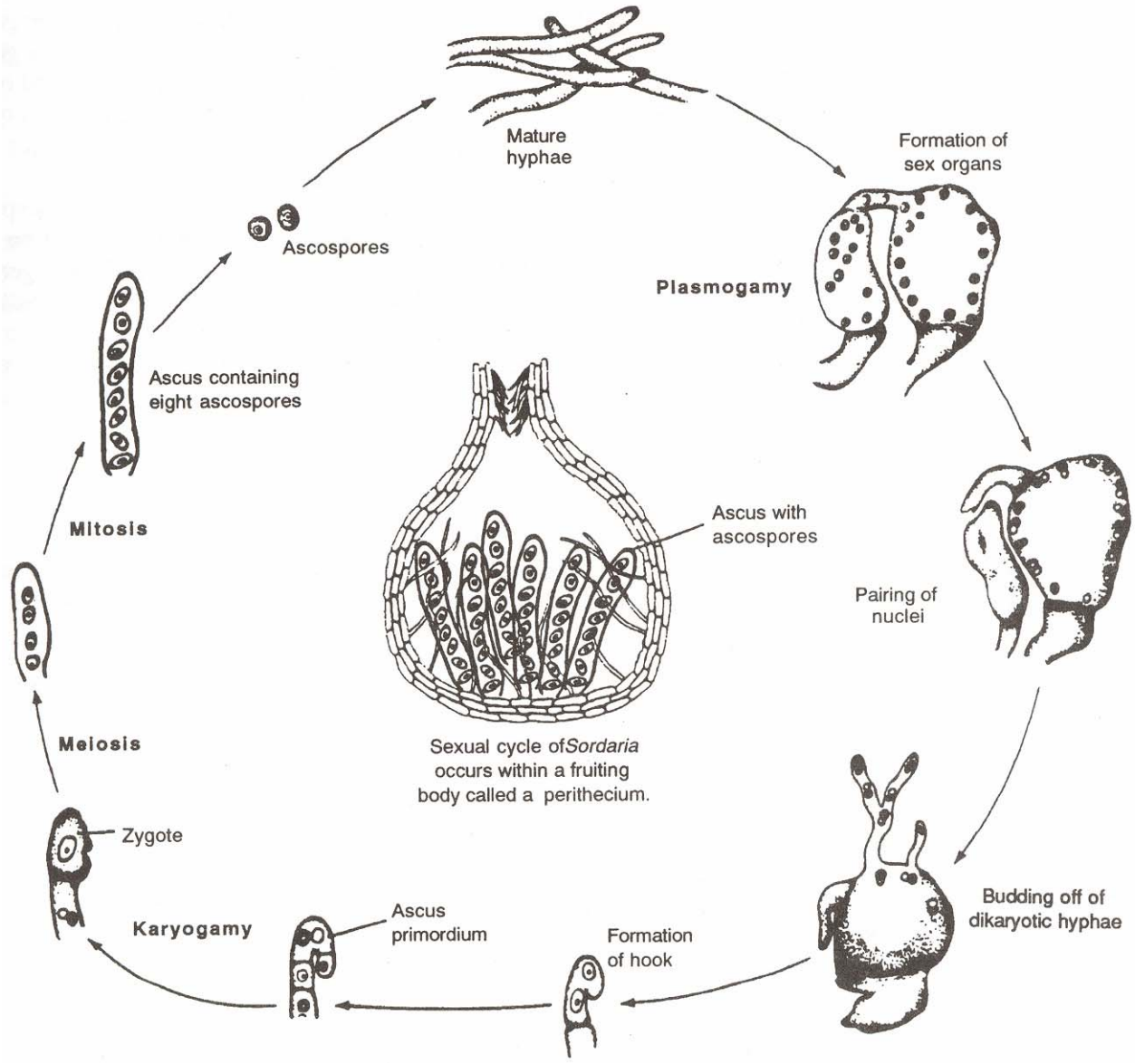

Figure 1.1. The life cycle of the fungus *Sordaria fimicola* (modified from Rushforth, 1976).

One important feature of ascus-forming fungi is their special 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 genotype and phenotype in their haploid cells and their short generation time are two additional reasons for the extensive use of these fungi in genetics.

The spore color of the normal (wild type) *Sordaria* is black. This phenotype is due to the production of the pigment melanin and its deposition in the cell walls. Several different genes are involved in the control of the melanin biosynthetic pathway and each gene has two possible allelic forms. The *gray spore gene* has two allelic forms: the wild type allele (g^+) and a mutant allele (g). The *tan spore gene* also has two forms: a wild type allele (t^+) and a mutant allele (t). Normal black spores are produced only if both wild type alleles are present at the loci of both genes. Thus, black ascospores have the genotype g^+t^+ (remember, spores are haploid). Those with the genotype $g\ t^+$ are gray, while g^+t ascospores are tan. Ascospores that are $g\ t$ show a cumulative effect of the two mutations and are colorless.

Microscopic study shows that *Sordaria* has seven different chromosomes (n = 7). Are the gray spore and tan spore genes located on the same or on different chromosomes? Genes that are located on the same chromosome are called *linked* genes. Genes on different chromosomes are called *unlinked* genes. Today you will initiate a cross between the gray ascospore mutant strain and the tan ascospore mutant strain of *Sordaria fimicola*. In two weeks, you will use tetrad analysis to collect data from this cross and you will apply a knowledge of meiosis and Mendel's two principles of inheritance to determine if the tan spore and gray spore genes are linked or unlinked and to map the location of each gene on its chromosome.

Setting Up the Gray Spore × Tan Spore Cross

1. Each pair of students should obtain two petri dishes containing cornmeal-glucose agar supplemented with 0.1% yeast extract. In addition, the class will be sharing several stock culture plates of the two mutants.
2. Disinfect the work surface where you will be setting up the genetic crosses by thoroughly wiping the bench top with an ethanol saturated paper towel.
3. With a marking pen, divide the bottoms of your petri dishes into fourths. Label each quadrant with a g^+t or $g\ t^+$ as shown in Figure 1.2.

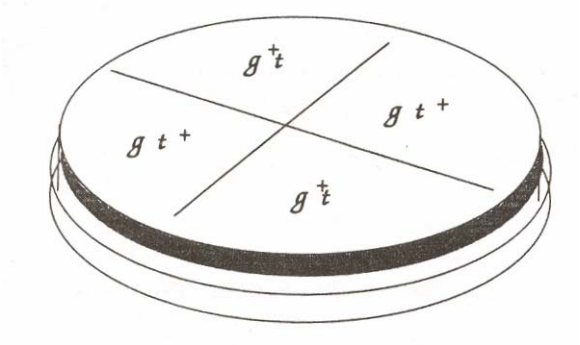

Figure 1.2. Appearance of petri dish bottom for receiving inocula to make the gray spore ($g\ t^+$) × tan spore (g^+t) cross.

4. Flame an inoculating loop with your alcohol burner. Lift the lid of one of the stock culture plates slightly and cool the inoculating loop by touching it to the lid's sterile inner surface. Use the loop to cut a small piece of agar (about 3 mm square) containing abundant mycelial growth.
5. Quickly transfer this inoculum to the center of the appropriate quadrant of one of your petri plates. Lift the lid of this plate just enough to allow insertion of the loop.

6. Reflame your loop and repeat this procedure with both mutant stock cultures until all quadrants are appropriately inoculated. Flame the loop after your last transfer of inoculum.
7. Carefully label each petri plate with your name and date of inoculation. Using two small pieces of tape, tape the plate closed and give it to your laboratory instructor.
8. When you are finished, disinfect the lab bench again using an ethanol saturated paper towel.

Your plates will be placed in an incubator at 25°C. When the perithecia that result from these genetic crosses are mature (about 8–10 days), the dishes will be placed in a refrigerator and returned to you two weeks from the day you performed the cross. The following section provides information to help you understand the results obtained from this cross.

Predictions And Data Collection

Two weeks ago, you set up a cross between the gray spore mutant strain and the tan spore mutant strain of *Sordaria fimicola*.

gray spore strain		*tan spore strain*
$g\ t^+$	×	g^+t

Because the parental strains have different alleles for both genes, you will be able to observe the interaction of both genes in determining spore color. Recall that black spores have a wild type allele for each gene (g^+t^+) and colorless ascospores have a mutant allele for each gene ($g\ t$).

How will you collect data and interpret the results from this cross in order to decide if the two genes are linked? The answer to this question can be simply derived if you return to the process of meiosis and Mendel's principle of independent assortment.

According to the *Principle of Independent Assortment*, if two genes are unlinked they should assort independently of each other at metaphase I of meiosis. Independent assortment of the tan and gray spore genes will lead to certain predictable results in the cross involving the two mutant strains. Linked genes will not assort independently, and, if this is the case, the results from the mutant-mutant cross will be quite different from those in the unlinked situation. The criterion, then, for deciding if two genes are linked or unlinked is whether they show independent assortment. Let us consider these two situations and examine the predictions that each makes for the cross you will initiate today.

Unlinked Gene Hypothesis

Consider Figure 1.3. Because pairs of homologous chromosomes line up independently of each other at metaphase I of meiosis, two possible arrangements of two pairs of homologous chromosomes (Pair 1 and Pair 2) are possible. Assume that stipple chromosomes for both pairs came from one parent, and the black chromosomes came from the other parent. In a large number of cells undergoing meiosis, one-half of the cells will have both black chromosomes on one side of the spindle's equator and both stipple chromosomes on the other side (Arrangement A). In the other one-half of the cells, the chromosomes will have Arrangement B.

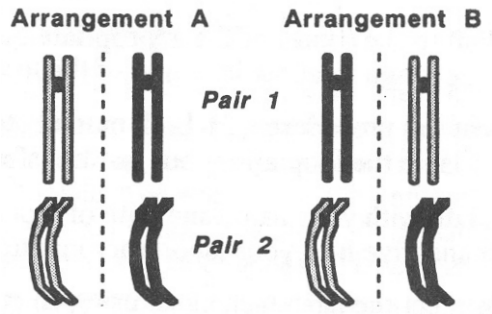

Figure 1.3. The independent arrangement of two pairs of homologous chromosomes (Pair 1 and 2) on the spindle's equator at metaphase I of meiosis. Black and stipple show parental origin of chromosomes.

In Figure 1.4, the upper homologous pair bears the alleles for the tan spore gene and the lower pair bears the alleles for the gray spore gene. If we assume that the stipple chromosomes for both pairs came from the tan spore parent (g^+t) then the alleles present on the stipple chromosomes would be g^+ and t. If the black chromosomes come from the gray spore parent ($g\ t^+$), then the alleles present should be g and t^+. Figure 1.4A and B show the two possible arrangements of these chromosomes, due to independent assortment, and the resulting ascus types. Both kinds of asci shown in Figure 1.4 are the type produced by a meiotic division in which *no* crossing-over occurred; only two genotypes of spores are present in each ascus and they are arranged in a 4:4 pattern. In Figure 1.4A, one-half of the spores have a g^+t genotype like the tan parent; the other half have a $g\ t^+$ genotype like the gray parent. This ascus arrangement is called a *Parental Ditype (PD)* ascus: *ditype* because only *two* genotypes are represented in the ascus and *parental* because both genotypes are like those of the parents. In Figure 1.4B there are also only two genotypes present (g^+t^+ and $g\ t$) but neither is like the parents' genotypes. This ascus arrangement is called a *Non-Parental Ditype (NPD)* ascus: *ditype*, again, because only two genotypes are represented and *non-parental* because both genotypes differ from the parents.

Situations C and D of Figure 1.4 show two examples of single crossovers occurring between the centromere and the tan locus (C), and the centromere and the gray locus (D). Recall that there are four different ways a crossover can occur between the non-sister chromatids for each pair of homologous chromosomes. In all such cases, an ascus results which contains spores of all *four* genotypes, arranged in a 2:2:2:2 sequence. It is also possible for both chromosomes to be involved in single crossovers. Some of these will produce asci with four spore genotypes in a 2:2:2:2 arrangement (Figure 1.4E); others (Figure 1.4F) will yield asci with only two spore genotypes, but arranged in a 2:4:2 pattern. All of these *non-4:4 asci* are called *Tetratype (T)* asci.

Situation G and H in Figure 1.4 show how some multiple crossovers involving the chromatids of a single chromosome type can lead to asci that are PD or NPD even though crossover did occur. We shall later discuss how the occurrence of these additional, crossover-produced PD and NPD asci will be a source of error in attempts to map the locations of these genes. However, since these types of crossovers produce equal numbers of PD and NPD asci, their existence does not change our expectations for the tan spore × gray spore cross. In summary, we can state the prediction of the *unlinked gene hypothesis* in the following deductive logic format:

If the gray spore and tan spore genes are unlinked, then the two genes should assort independently and the frequency of PD and NPD asci should be equal.

☐ In the space provided below each ascus, fill in the genotypes for the four pairs of spores for each ascus shown in Figure 1.4. Several have been done for you.

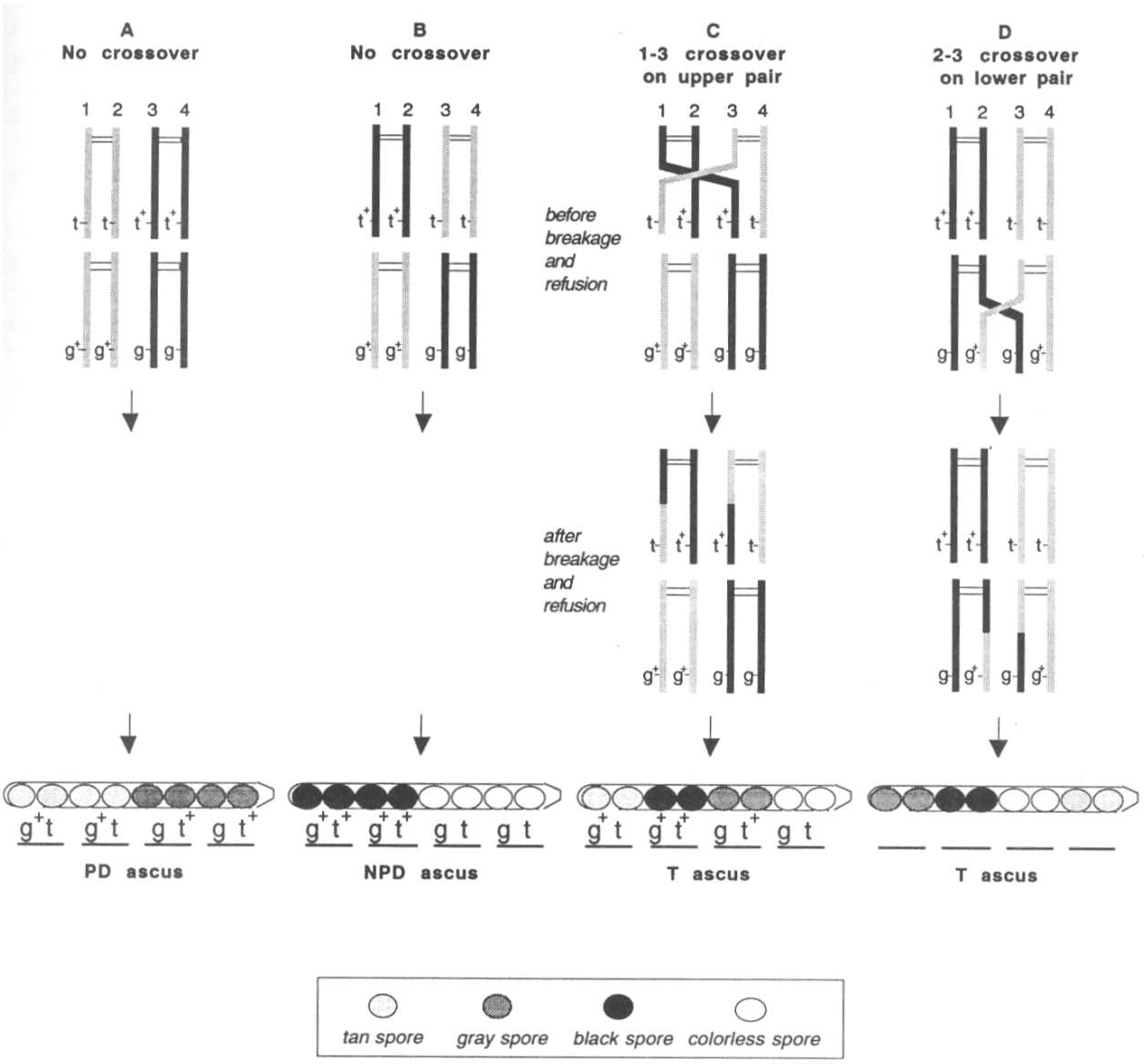

Figure 1.4. Predicted ascus types from the unlinked gene hypothesis for the tan and gray ascospore color genes. *Note:* Black homologues come from the gray spore strain; stipple homologues come from the tan spore strain. A-B show independent assortment with no crossing over; C-D show examples of crossovers involving a single pair of chromosomes. Situations E to H are shown on the next page.

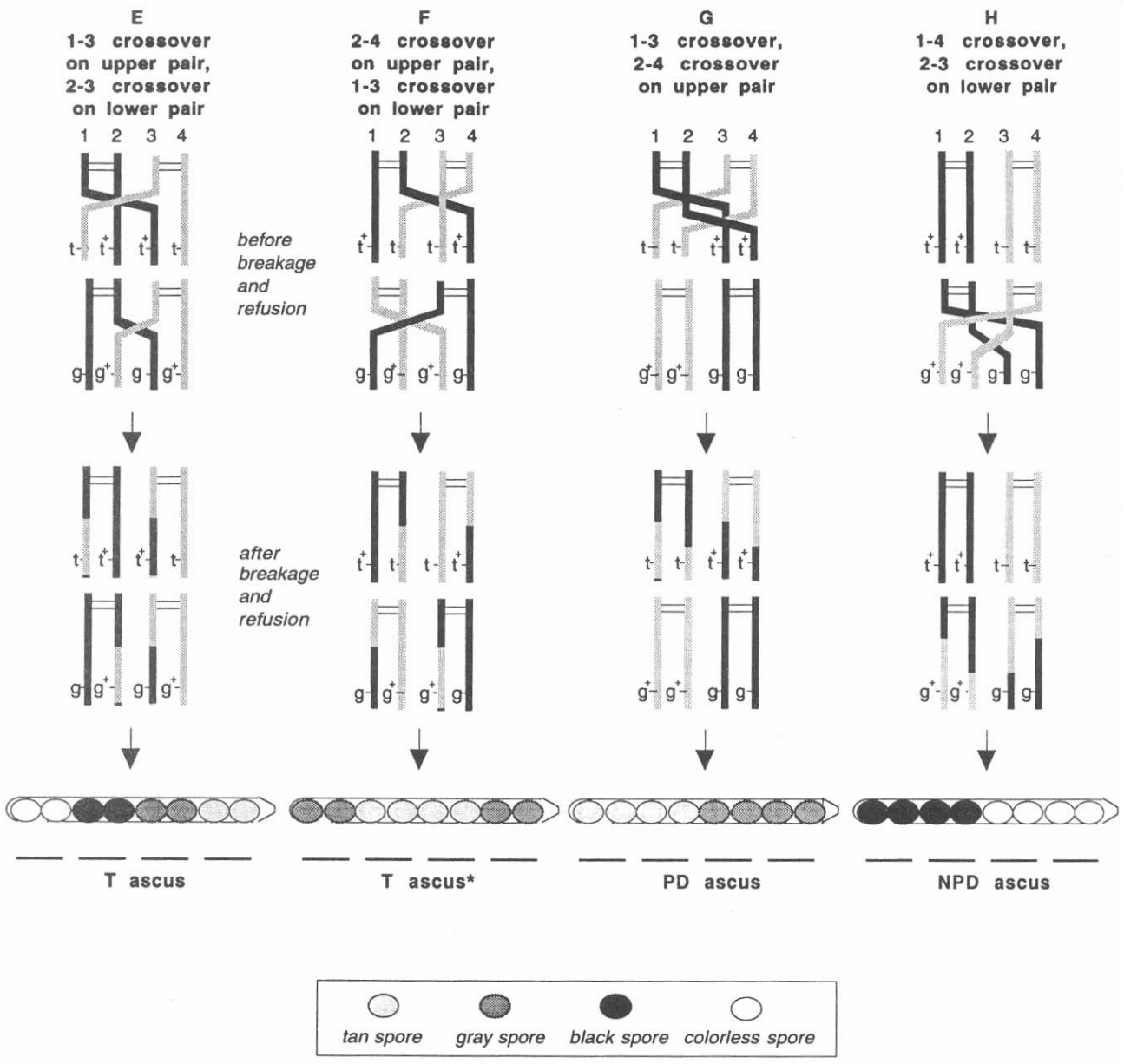

Figure 1.4 (continued). Predicted ascus types from the unlinked gene hypothesis for the tan and gray ascospore color genes. *Note:* Black homologues come from the gray spore strain; stipple homologues come from the tan spore strain. E-F show examples of crossovers involving both pairs of chromosomes and G-H shows examples of double crossovers between one pair of homologous chromosomes. (* Even though the ascus in F has only two spore-color phenotypes and they are the parental phenotypes, this is still classified as a tetratype ascus because the spores are in a non-4:4 arrangement.)

Linked Gene Hypothesis

If both spore-coloration genes are located on the same pair of homologous chromosomes, they will be inherited together because they are physically linked together. In Figure 1.5, the right-hand homologue came from the tan spore parent (it has a *t* and g^+ allele) while the left-hand homologue came from the gray spore parent (it has a t^+ and *g* allele). If the two genes are linked and no crossing over occurs (Figure 1.5A), only PD asci will result. In Figure 1.5B, a crossover occurs between the tan spore gene locus and its centromere. In Figure 1.5C, the crossover is between the two gene loci. Both produce T asci. *Note:* The placement of the gray and tan gene loci in this diagram is arbitrary and does not imply anything about their true locations.

Obviously, if a single chiasma can form between two chromatids, several chiasmata can also form, resulting from double, triple or higher order crossovers. Also, since there are two sets of chromatids available, it is possible for more than two chromatids to be involved in crossovers at the same time. A two-chromatid, double crossover is shown in Figure 1.5E. Figure 1.5D and F show four-chromatid double crossovers. Notice that some multiple crossovers (Figure 1.5E and F) are not genetically detectable, because they produce PD or NPD asci. These types of multiple crossover cause us to underestimate map unit distances. Notice also that the NPD ascus produced in Figure 1.5F could be interpreted as evidence for non-linkage. However, because the probability of multiple crossovers is low and because multiple crossovers produce, on average, equal numbers of PD and NPD asci, this complication does not change our expectations for the tan spore × gray spore cross.

In summary, if two genes are linked, we would expect mostly PD and T asci, but only a small proportion of NPD asci. We can state the prediction of the *linked gene hypothesis* in the following deductive logic format:

If the gray spore and tan spore genes are linked, then the two genes should *not* assort independently and the PD asci frequency should be greater than the NPD asci frequency.

☐ In the space provided below each ascus, fill in the genotypes for the four pairs of spores for each ascus shown in Figure 1.5. Several have been done for you.

Mapping Genes on Chromosomes

The exchange of genetic material between homologous chromosomes which occurs during crossing over creates a major exception to Mendel's principle of segregation. Recall that the segregation of alleles from the two parents occurs during anaphase I of meiosis, that is, during the first division of meiosis. If crossing over occurs, however, the alleles rearranged by the crossover are not segregated until anaphase II of meiosis, that is, during the second division of meiosis. Thus, it is said that crossing over leads to *second division segregation* of the alleles involved in the crossover. Gene mapping became possible when it was realized that the frequency of second division segregation was related to the physical distance separating the genes involved.

In Figure 1.4C, notice that only four of the eight ascospores are genetically changed due to crossover; the two tan spores with copies of chromatid 1 and the two gray spores with copies of chromatid 3. These spores are called *recombinant* ascospores because they contain chromosomes that have been changed by the crossover, and are now a combination of the parental chromosomes. The other four spores that bear chromosomes not affected by the crossover and are still like the parental chromosomes, are called *non-recombinant* ascospores.

☐ Study Figure 1.4D and identify the recombinant and non-recombinant spores resulting from that crossover.

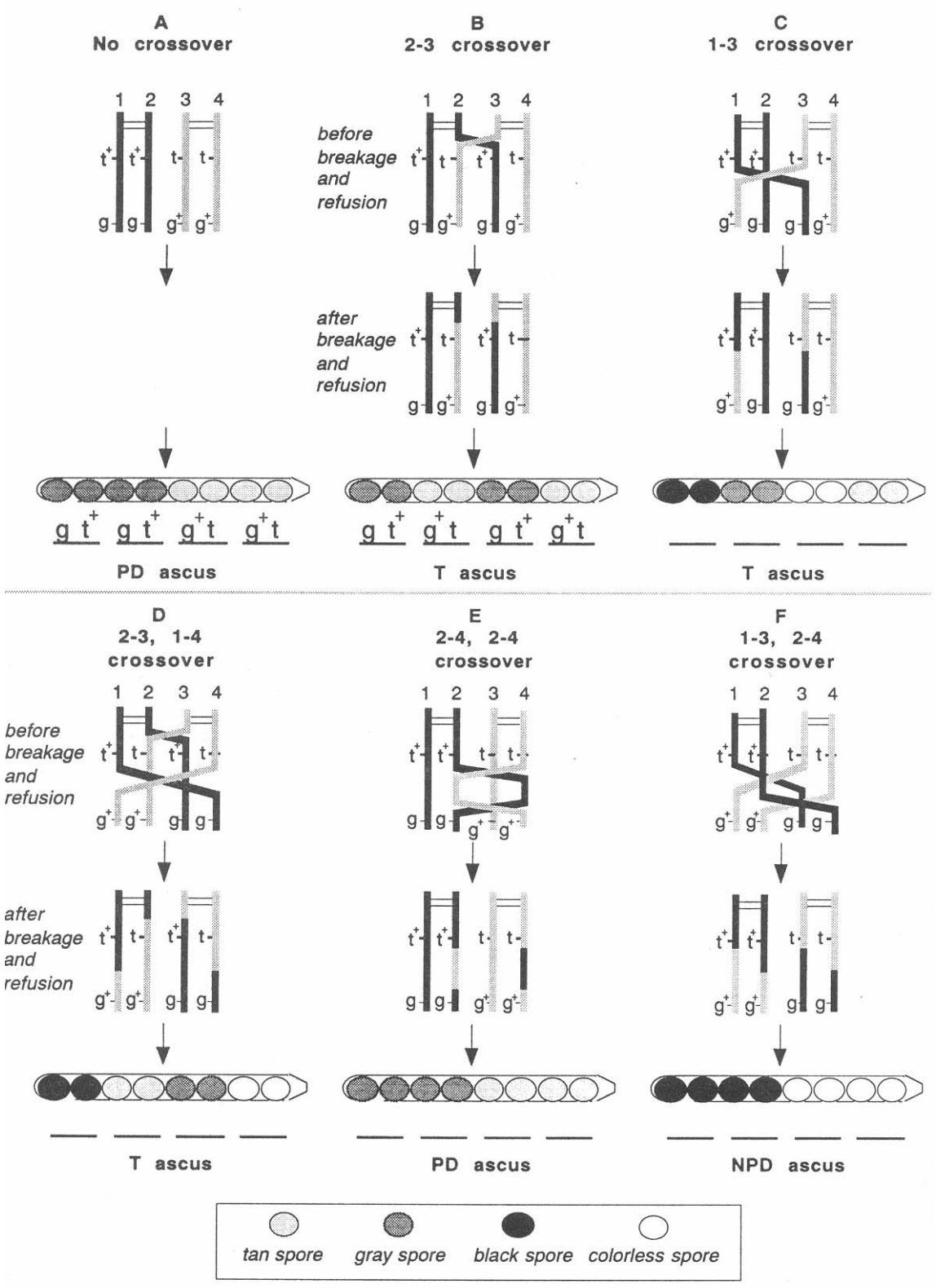

Figure 1.5. Predicted ascus types from the linked gene hypothesis for the tan and gray spore coloration genes. Black homologue comes from the gray spored strain; stipple homologue comes from the tan spored strain.

Some thought should convince you that only crossing over between the spore color gene locus and its centromere will result in a T ascus. Crossover beyond the gene locus or on the other arm of the chromosome will not affect the gene under consideration. If we assume that crossing over can occur at any point along a chromosome, it is logical that the probability of a crossover occurring between a gene locus and the centromere will be proportional to the locus-centromere distance. Therefore, we can use the frequency (proportion) of crossover-produced recombinant ascospores as a measure of the relative distance separating the gene locus and the centromere. Geneticists define a crossover *map unit* as the distance on a chromosome that produces one recombinant post-meiotic product per 100 post-meiotic products. Here, the number of map units would be equal to the number of recombinant ascospores per 100 total ascospores (both recombinant and non-recombinant).

$$\textit{map units} = \frac{\textit{recombinant spores}}{\textit{Total spores (recomb + non-recomb)}} \quad \textit{x 100}$$

Given that map units express the % recombinant spores resulting from crossovers and each single crossover produces 4 recombinant spores and 4 non-recombinant spores, the map unit distance is always one half the frequency of crossing over for the gene.

Notice that if you are using tetrad analysis to map genes in a cross involving two genes, you must differentiate between tetratype asci that result from single crossovers affecting only one gene, such as those shown in Figure 1.4C and 1.4D, and tetratype asci that result from double crossovers affecting both genes, such as those shown in Figure 1.4E and F. We shall return to this idea later in this study.

Using tetrad analysis, geneticists have been able to obtain genetic maps of chromosomes of many organisms. These maps indicate the sequence of genes on chromosomes and the relative locations of these genes. However, because a genetic map is based on crossover frequencies, the relative distances between genes do not correspond to real, physical distances. That is, although the sequence of genes is correct, some genes may be closer together and others farther apart than genetic maps indicate. This is because some regions of chromosomes have a greater, or lesser, tendency to form crossovers than other regions. For example, the centromere seems to inhibit crossing over and genes located close to it do not crossover as much as they should based solely on their physical location.

Squashing Perithecia

Unlike many fungi, *Sordaria* is self-fertile and some of the perithecia you will examine have resulted from *intrastrain* matings. Perithecia resulting from self-mating will contain asci with all tan or all gray ascospores. Since an ascus must contain at least two different phenotypes in order to detect a crossover, you will be concerned exclusively with perithecia that have resulted from *interstrain* matings. Perithecia 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.

1. Each pair of students should assemble the following equipment: inoculating loop, microscope slides and coverslips, dropper bottle with water, two petri dishes with the gray spore ($g\ t^+$) × tan spore (g^+t) cross, and a box of kimwipes.
2. Place a drop of water on a clean microscope slide.
3. Remove the petri dish lid.

4. Now, remove 10 to 15 perithecia by scraping the loop's tip back and forth over the *surface* of the agar. *Do not dig into the agar*.
5. Place the perithecia into the drop on the slide and uniformly distribute the perithecia throughout the drop. Place a cover slip over the perithecia and water.
6. Place a kimwipe over your finger (to avoid putting a fingerprint on the cover slip) and push 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.
7. View the slide with the compound microscope first with the 10X objective in place.
8. Select for study only those perithecia that contain asci with more than one spore phenotype. Identify the phenotypes of the spores in each ascus with the 40X objective in place. Figure 1.6 shows two properly squashed perithecia.

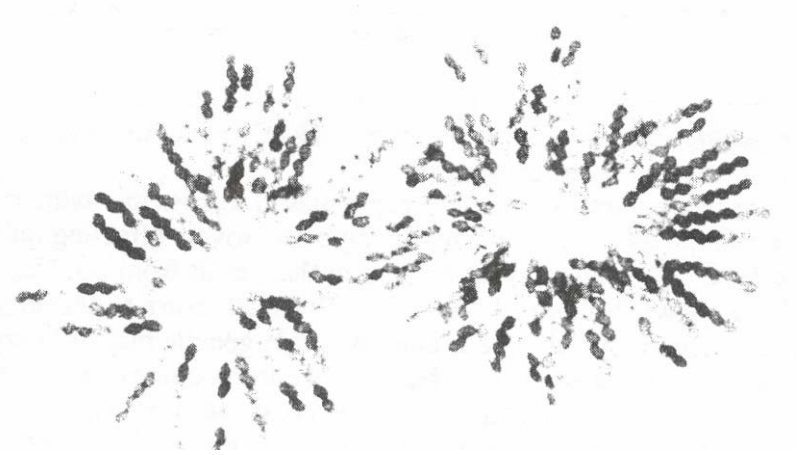

Figure 1.6. Photomicrograph of *Sordaria fimicola* showing two well-spread perithecia suitable for tetrad analysis.

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, categorizing 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.

1. To be most successful at identifying spore phenotypes as you use the microscope to examine perithecial squashes be sure to:

(a) keep light intensity low so you can see color differences better,

(b) focus up and down carefully to see all eight ascospores,

(c) avoid immature asci (their spores will have a *granular* appearance and very little color), and

(d) seek your instructor's help until you become confident in your ability to identify the phenotypes.

2. As you classify asci, write down in abbreviated form the sequence of spores within each ascus. For example, the ascus in Figure 1.4E could be represented as CCBBGGTT (from left to right)

or, since all spore phenotypes are in duplicate due to the mitotic division after meiosis, more simply CBGT.

3. Each individual should classify at least 30 asci to insure an adequate sample size for the class data. Enter individual data in Table 1.1.

In addition to PD and NPD asci, you will see a variety of T asci, some with all four spore phenotypes and some with only two phenotypes in a 2:2:2:2 or a 2:4:2 arrangement. However, there are some restrictions on the types of two-phenotype asci that are possible. Black and gray only or black and tan only are not possible (either in a 4:4 or non-4:4 arrangement) because these arrangements require more wild type alleles than are available in a mutant × mutant cross. Likewise, colorless and gray only or colorless and tan only are not possible (either in a 4:4 or non-4:4 arrangement) because these arrangements require more mutant alleles than are available. In other words, two-phenotype asci can only include the phenotypes tan and gray or colorless and black.

4. Go back through your asci descriptions and identify each ascus as either PD, NPD, or T in Table 1.1. *Remember:* (1) *PD* asci are 4 gray:4 tan or reverse; (2) *NPD* asci are 4 colorless:4 black or reverse; and (3) *T* asci are *all* others (all four phenotypes or two phenotypes in a 2:2:2:2 or 2:4:2 arrangement, but the phenotypes must be gray and tan or colorless and black).

Data Analysis And Transformation

Your class will collaborate in analysis and transformation of data collected in this study. Be sure to obtain a complete copy of all of the class data; you will need these data when you address the study questions associated with this chapter. (*Note:* You will need to use the chi-square test as part of the data analysis of this study.) The following sections discuss each of the major objectives of this study.

Linkage or Non-Linkage

The criterion to be used in deciding if the two genes are linked or not is whether they assort independently; unlinked genes do, linked genes do not. As discussed earlier, if the tan and gray spore genes are unlinked, we expect equal numbers of parental ditype asci (PD) and non-parental ditype (NPD) asci. Determine the total number of PD and NPD asci observed by the whole class and use the chi square test to compare these numbers with the expected values assuming the genes are unlinked (see study question 1).

☐ Complete Table 1.2 using the class data assembled from individual Tables 1.1.

Gene Mapping

Tetratype (T) asci result from crossing over between homologous chromosomes. However, as shown in Figures 1.4 and 1.5, some crossovers affect just one gene, while others affect both genes. In using tetrad analysis data to map the gray and tan spore genes, you must determine whether the alleles for one or both genes were switched by the crossover that produced each tetratype ascus you observed. Using the information that follows, each pair of students should analyze the tetratype asci they recorded in Table

Table 1.1. Data collected from a genetic cross between the gray and tan spore strains of *Sordaria fimicola*. (*Note:* C = colorless, B = black, T = tan, G = gray spore.)

Phenotypes (i.e. TGBC)	PD, NPD or T?	Genotypes	Gray Spore Gene		Tan Spore Gene	
			Recomb.	Non-Recomb.	Recomb.	Non-Recomb
		- - -				
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PD Asci = ________ Spore Totals = ________ ________ ________ ________

NPD Asci = ________ Crossover Asci = ________ ________

T Asci = ________ Non-crossover Asci = ________ ________

Total Asci = ________ ________ ________

1.1 and record the correct number of recombinant and non-recombinant spores for each gene in that table. You will then combine individual data to estimate map locations for the two genes based on all of the class data.

We must assume that NPD and PD asci contain only non-recombinant ascospores (even though some of these asci resulted from multiple crossing over, as we shall discuss shortly). For each NPD and PD ascus you recorded in Table 1.1, place a 0 in the *Recomb. spores* and an 8 in the *Non-recomb. spores* column of *both* the tan and gray spore gene categories. In the case of the T asci, to determine which genes were affected by crossing over you must determine which genes show second division segregation. Examine the genotypes for the spores in the ascus shown in Figure 1.4C. If there is no crossover between a gene's locus and the centromere then according to Mendel's principle of segregation the gene's alleles will show first-division segregation. First division segregation produces a 2:2 arrangement of genotypes within the ascus for that gene (recognizing that adjacent spores are genetically identical). A crossover between the gene's locus and the centromere will lead to second division segregation and the arrangement of genotypes within the ascus will be 1:1:1:1 or 1:2:1 for that gene. In Figure 1.4C, notice that the arrangement for the gray spore gene is $g^+ : g^+ : g : g$, but $t : t^+ : t^+ : t$ for the tan spore gene. This observation indicates that during the meiotic division that produced this ascus a crossover occurred for the tan spore gene but not the gray spore gene. Had you observed this ascus, you would know that only the chromosome with the tan spore gene was changed due to the crossover and that the four recombinant ascospores resulting from that crossover should be associated with the tan spore gene. Therefore, in Table 1.1 you would place a 4 into the *Recomb. spores* and *Non-recomb. spores* columns for the tan spore category and a 0 in the *Recomb. spores* and an 8 into the *Non-recomb. spores* column for the gray spore category.

Look at the pattern of genotypes shown for the ascus shown in Figure 1.4D. Which gene is showing second division segregation? Had you observed this ascus, you would know that only the chromosome with the gray spore gene was changed due to the crossover and that the four recombinant ascospores resulting from that crossover should be associated with the gray spore gene. Therefore, in Table 1.1 you would place a 4 into the *Recomb. spores* and *Non-recomb. spores* columns for the gray spore category and a 0 in the *Recomb. spores* and an 8 into the *Non-recomb. spores* column for the tan spore category.

Look at the pattern of genotypes shown for the asci shown in Figure 1.4E and F. Which genes show second division segregation? Notice that all eight ascospores in these asci are recombinant: four are recombinant for the one gene, and four for the other gene. When you observe asci of these types, you would place a 4 in the *Recomb.* and *Non-recomb. spores* columns of *both* gene categories.

This same approach is used whether one assumes that the genes are unlinked or linked. For example, in the ascus in Figure 1.5B, the pattern of genotypes for the two genes indicate that both genes were affected by the crossover. This would occur if the gray and tan genes are located on the same side of the chromosome relative to the centromere and a crossover had occurred between the centromere and the gene locus nearest the centromere. If you had observed this ascus, you would place a 4 in the *Recomb. spores* and a 4 in the *Non-recomb. spores* column for *both* gene categories, since both genes were affected by the crossover.

If you had observed the pattern of genotypes for the ascus shown in Figure 1.5C, you would place a 4 in the *Recomb. spores* and the *Non-recomb. spores* column for the gray spore gene and a 0 in the *Recomb. spores* and an 8 in the *Non-recomb. spores* column for the tan spore gene, because only alleles of the gray gene are showing second division segregation. Verify that this is true.

1. For each ascus you recorded in Table 1.1, enter the four spore genotypes in the space provided and determine the numbers of recombinant and non-recombinant spores for each gene.

2. Complete Table 1.3 using the class data assembled from individual Tables 1.1.

3. To estimate the map locations of the two genes, whether unlinked or linked, simply determine the total number of recombinant spores for each gene and divide by total spores observed (see study question 2). *Note:* A check on your computations would be to ascertain that total spores observed for both genes (non-recombinant spores + recombinant spores) is the same number.

Comparison with Published Map Locations

Published gene-centromere map locations for the two genes are approximately 27 map units for the tan spore gene and 33 map units for the gray spore gene (Olive, 1956). You should use the chi square test to determine if your estimated map locations differ significantly from these values. Since map units are really the percent of all the spores that are recombinant spores, you might be inclined to use *non-recombinant spores* and *recombinant spores* as the categories in the chi square test. However, since the observed values in your chi square categories represent your sample size, you need to select categories that accurately reflect the amount of information you have collected. Since the number of spores, whether non-recombinant or recombinant, is really a function of the number of asci you observed, you must use crossover and non-crossover asci as categories in the test. So, for example, if a gene is 27 map units from its centromere, recognizing that each crossover produces one-half recombinant and one-half non-recombinant spores, you would expect 54% crossover asci and 46% non-crossover asci . Your observed values will be the actual *number* of crossover asci and non-crossover asci for that gene. The expected values would be the total *number* of asci seen by the class times 0.54 for the expected crossover asci and times 0.46 for the expected non-crossover asci (see study question 3).

☐ Complete Table 1.4 using the class data assembled from individual Tables 1.1.

Table 1.2. Class data showing the number of Parental Ditype (PD), Non-Parental Ditype (NPD), and Tetratype (T) asci from a genetic cross between the tan and gray spore strains of *Sordaria fimicola* .

Ascus type	Number counted
Parental Ditype (PD)	
Non-Parental Ditype (NPD)	
Tetratype (T)	

Table 1.3. Class data showing recombinant and non-recombinant spores for the tan and gray spore genes of *Sordaria fimicola* from a genetic cross between the two mutant strains.

Spores	Gray Spore Gene	Tan Spore Gene
Recombinant		
Non-recombinant		
Total		

Table 1.4. Class data showing observed and expected crossover and non-crossover asci for the tan and gray spore genes of *Sordaria fimicola* from a genetic cross between the two mutant strains.

Asci	Tan Spore Gene	Gray Spore Gene
Observed Crossover[1]		
Observed Non-crossover[2]		
Expected Crossover[3]		
Expected Non-crossover[4]		

1. T asci with recombinant spores for gene
2. PD and NPD asci and T asci without recombinant spores for gene
3. (Total asci examined × 0.54) for the tan spore gene or (total asci examined × 0.66) for the gray spore gene
4. (Total asci examined × 0.46) for the tan spore gene or (total asci examined × 0.34) for the gray spore gene

Testing a Complete Genetic Model

You will now use the published gene-centromere map distances to predict the frequencies of PD, NPD, and T asci assuming that the tan and gray spore genes are unlinked. Consult Figure 1.7 with respect to the following discussion.

If the distance between the tan locus and its centromere is 27 map units, then the frequency of meiotic divisions with a single crossover between the tan locus and its centromere is equal to 0.54. Therefore, the frequency of meiotic divisions with no crossover for that gene will be 0.46 (1.00 – 0.54). If the distance between the gray locus and its centromere is 33 map units, then the frequency of meiotic divisions with a single crossover between the gray locus and its centromere is equal to 0.66. In turn, the frequency of meiotic divisions with no crossovers for the gray gene would be 0.34 (1.00 – 0.66). The predicted frequency of asci resulting from *no* crossovers in meiosis (i.e., *PD and NPD asci*) would be as follows:

Frequency of meiotic divisions with no crossover between *tan* gene and its centromere	×	Frequency of meiotic divisions with no crossover between *gray* gene and its centromere	
(0.46)	×	(0.34)	= 0.16

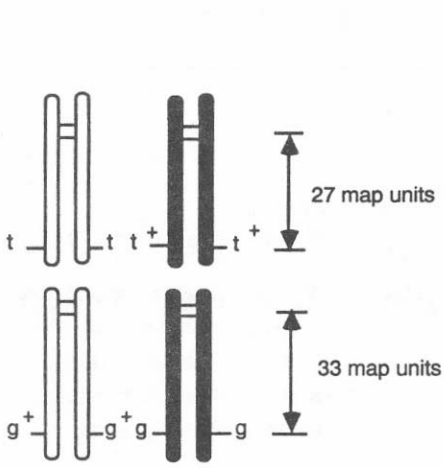
Since unlinked genes show independent assortment, half of the asci resulting from no crossover would be PD, and the other half NPD. Therefore, (Predicted frequency of PD asci) = (Predicted frequency of NPD asci) = 0.16/2 = 0.08.

A single crossover between *either* gene and its centromere or a simultaneous single crossover between both genes and their centromeres would all produce T asci. Therefore, the predicted frequency of T asci would be the sum of the products of the independent probabilities of all three of these events, or

Predicted T asci frequency = (0.54×0.34) + (0.66×0.46) + (0.54×0.66) = 0.84

Tan Crossover only — gray crossover only — tan and gray crossovers

☐ Test this genetic model by performing the chi square test described in study question 4.



TOTAL MEIOTIC DIVISIONS

	Expected Frequency of Crossovers	Expected Frequency of Non-crossovers
27 map units	0.54	0.46
33 map units	0.66	0.34

Figure 1.7. The relationship between map unit distance and the expected frequencies of meiotic divisions with and without single crossovers for two unlinked genes

Multiple Crossover as an Error Source in Tetrad Analysis

After you have completed this analysis you should consider again the occurrence of multiple crossing over and its influence on your mapping attempts. We have assumed that all PD and NPD asci result from meiotic divisions without crossover and that all spores in PD and NPD asci are non-recombinant. Yet, as shown in Figures 1.4 and 1.5, some multiple crossovers produce PD and NPD asci. Did your chi square analysis in question 4 of the observed frequencies of the three ascus types (PD, NPD, and T) show significantly more PD and NPD and proportionally fewer T asci than expected based on single crossovers?

Because of the production of PD and NPD asci by multiple crossing over some of the spores in your *Non-recomb. spores* columns of Table 1.1 are really recombinant spores. Also, some T asci contain eight recombinant spores due to multiple crossovers, but only show second division segregation for a single gene (see Figure 1.5D). Since map units are % recombinant spores, multiple crossovers cause us to *underestimate* map units.

Because each crossover is an independent event, the probability of two crossovers occurring together is the product of their independent probabilities. Thus, the probability of multiple crossing over is small if genes are close together, but increases rapidly as genes are further apart. Geneticists minimize the problem of multiple crossing over by only mapping genes that are close together. The gene-centromere map units for the gray and tan genes cited above were obtained by adding together map units for other linked genes located between these genes and their centromeres. Thus, you may find that your estimates may differ significantly from these values.

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APPENDIX A
Sample Data and Study Questions With Answers

Table 1.2. Class data showing the number of Parental Ditype (PD), Non-Parental Ditype (NPD), and Tetratype (T) asci from a genetic cross between the tan and gray spore strains of *Sordaria fimicola* .

Ascus type	Number counted
Parental Ditype (PD)	40
Non-Parental Ditype (NPD)	34
Tetratype (T)	254
Total	328

Table 1.3. Class data showing recombinant and non-recombinant spores for the tan and gray spore genes of *Sordaria fimicola* from a genetic cross between the two mutant strains.

Spores	Gray Spore Gene	Tan Spore Gene
Recombinant	756	816
Non-recombinant	1868	1808
Total	2624	2624

Table 1.4. Class data showing observed and expected crossover and non-crossover asci for the tan and gray spore genes of *Sordaria fimicola* from a genetic cross between the two mutant strains.

Asci	Tan Spore Gene	Gray Spore Gene
Observed Crossover	189	204
Observed Non-crossover	139	124
Expected Crossover	(0.54 × 328) = 177	(0.66 × 328) = 216
Expected Non-crossover	(0.46 × 328) = 151	(0.34 × 328) = 112

1. Are the tan spore and gray spore genes linked or unlinked? Use the data included in Table 1.2 and the chi-square test to compare the numbers of PD and NPD asci.

Categories	Observed	Expected
Parental Ditype (PD)	40	37 (74 × 0.5)
Non-Parental Ditype (NPD)	34	37 (74 × 0.5)

$X^2 = 0.48$. The tan spore and gray spore genes are unlinked (there is no significant difference between the number of PD and NPD asci).

2. Using the data included in Table 1.3, estimate below the gene centromere-map unit distances for the tan and gray spore genes.

Tan spore gene: gene-centromere map distance = (756/2624) × 100 = 28.8 map units

Gray spore gene: gene-centromere map distance = (816/2624) × 100 = 31.1 map units

3. Use the data included in Table 1.4 and the chi-square test to determine if the gene-centromere map units estimated from the class data for each gene differ significantly from the published map distances. The categories to use in these tests are crossover and non-crossover asci.

Tan Spore Gene

Categories	Observed	Expected
Crossover asci	189	177.1 (328 × 0.54)
Non-crossover asci	139	150.9 (328 × 0.46)

$X^2 = 1.74$. (No significant difference between published and estimated map distance.)

Gray Spore Gene

Categories	Observed	Expected
Crossover asci	204	216.5 (328 × 0.66)
Non-crossover asci	124	111.5 (328 × 0.34)

$X^2 = 2.12$. (No significant difference between published and estimated map distance.)

4. Assuming that the tan and gray spore genes are unlinked, perform a chi square test comparing the observed numbers of PD, NPD, and T asci (Table 1.2) with the expected numbers based on the published gene-centromere map distances.

Categories	Observed	Expected
Parental Ditype (PD)	40	26.2 (328 × 0.08)
Non-Parental Ditype (NPD)	34	26.2 (328 × 0.08)
Tetratype (T)	254	275.6 (328 × 0.84)

$X^2 = 11.19$. (Significant differences at $\alpha = 0.01$ between observed and expected PD, NPD, and T asci. Multiple crossing-over produces more PD and NPD asci then expected based on the single crossover model.)

5. (a) What is the probability of a single crossover between the tan spore gene locus and the centromere?

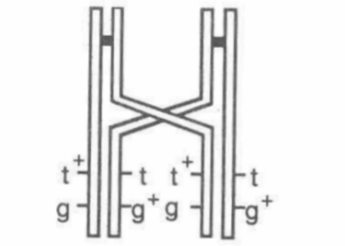
   • 27 map units = 27% recombinant spores for gene = 54% crossover asci for gene = 54% single crossovers = 0.54 probability

   (b) What is the probability of a *double* crossover between the tan and gray spore gene loci?

   • 6 map units = 12% single crossovers = 0.12 probability
   • Probability of a double crossover (two single crossovers) = 0.12 × 0.12 = 0.014

   (c) Diagram a crossover that would produce the ascus shown below starting with the chromosome arrangement shown above.

   • A 2-3 crossover is required.



2 gray:2 tan:2 gray:2 tan