LECTURE 6: TETRAD ANALYSIS

Reading: Ch. 5, p. 132-140

Problems: Ch. 5, solved problem III, 5-20, 5-24 – 5-27, 5-30 (mislabeled as the 2nd 5-29), 5-31

---------First we went over "interference" (see notes from last lecture)--------

TETRAD ANALYSIS IN FUNGI

In the diploid organisms that we've considered so far, each individual represents only one of four potential gametes that are produced from each parent in a single meiotic event. We don't know which of the other progeny in the cross are created by gametes produced by the same meiotic event. So we have to analyze large numbers of progeny and use statistics to establish linkage (or lack thereof) and to do mapping. By contrast, some yeast species house all four products of a single meiosis in a sac called an **ascus**. The haploid cells are called **ascospores** (or **haplospores**) and can germinate and live in the haploid state, growing and dividing by mitosis. Thus, phenotype equals genotype in these haploid cells! We will review tetrad analysis in Baker's yeast, *Saccharomyces cerevisiae*; you should review ordered tetrad analysis in *Neurospora crassa* on your own.

Life cycle of *Saccharomyces cerevisiae*. The collection of four products of meiosis found in each ascus is called a **tetrad**.

Nomenclature conventions of *Saccharomyces cerevisiae*:

Capital letters indicate dominant alleles (e.g. *HIS4*, or sometimes just +)
Lower case letters indicate recessive alleles (e.g. *his4*)
Usually, the wild-type allele is the dominant one (e.g. $HIS4^{+}$)

We characterize tetrads based upon the number of parental and recombinant spores they contain. Consider a cross between an a-mating type haploid yeast strain of genotype *HIS4 trp1* with an α-mating type haploid yeast strain of the opposite genotype *his4 TRP1*. These two genes are unlinked. What are the possible kinds of tetrads that result when the resulting diploid strain undergoes meiosis?

Parental ditype (PD): A tetrad containing 4 haploid cells of the parental class.
Nonparental ditype (NPD): A tetrad containing 4 recombinant haploid cells (the two parental classes have recombined to form the reciprocal nonparental combination of alleles).
Tetratype (T): A tetrad containing four kinds of haploid cells, two different parental class spores and two different recombinant class spores. In crosses involving 2 unlinked genes, tetratypes arise when a crossover occurs between one of the two genes and its centromere.

If PD = NPD, then the two genes are unlinked (either they are located on different chromosomes, or they are far apart on the same chromosome). Think independent assortment here (50% recombination frequency). Since all Ts (no matter how many) are 50% parental and 50% recombinant, then the only way that 50% of the <u>total</u> number of haploid progeny spores can be 50% recombinant is if the number of PDs (100% parental class) equals the number of NPDs (100% recombinant).

If PD >> NPD, then the two genes are linked. When PD >> NPD, the haploid spores of the parental class significantly outnumber the haploids of the recombinant class, a sign of linkage. Consider and example of two linked genes, *ARG3* and *URA2*.
P: *arg3 ura2* x *ARG3 URA2*
Diploid: *arg3 ura2* / *ARG3 URA2*

Meiotic products:
PD (*arg3 ura2*; *arg3 ura2*; *ARG3 URA2*; *ARG3 URA2*) = 127
NPD (*arg3 URA2*; *arg3 URA2*; *ARG3 ura2*; *ARG3 ura2*) = 3
T: (*arg3 ura2*; *arg3 URA2*; *ARG3 ura2*; *ARG3 URA2*) = 70

Recombination frequency, RF = [(NPD + 1/2T) / total number of tetrads] x 100. In our example, this is ([3 + 1/2(70)]/200) x 100 = 19 map units. We will modify the equation to obtain a better estimate of map distance. If you draw out the possible crossover events between two linked genes, you can see the different tetrads that result; see Fig. 5.19.

No crossovers --------> PD
Single crossover --------> T
Double crossover (2-strand) --------> PD
Double crossover (3-strand) --------> T
Double crossover (3-strand) --------> T
Double crossover (4-strand) --------> NPD

You can see how we can modify the equation to make it more accurate. Remember that half (2/4) the strands recombine if there is a single crossover event and that 4 strands recombine if there is a double crossover event (even if all of the strands don't participate, some participate more than once.)

Map distance = (total rec. events / total tetrads) x 100 = [(1/2[SCO] + DCO) / total tetrads] x 100
Map distance = ([1/2 (T – 2 NPD) + 4 NPD]/ total tetrads) x 100
Map distance = (1/2 T + 3 NPD) / total tetrads x 100
For our example above, map distance = ([1/2 (70) + 3 (3)] / 200) x 100 = 22 map units

This modified equation makes 2 assumptions: (1) there are no more than two crossovers in the interval and (2) there is no chromosomal interference (all types of DCOs occur with equal frequency.

----------We will cover the material below nest time----------

ORDERED TETRADS AND GENE-CENTROMERE DISTANCE

In *Neurospora crassa*, meiosis occurs within the tight confines of a narrow ascus, resulting in the formation of **ordered tetrads**. Because of the precise positioning of each meiotic product within the ascus, one can infer the arrangement (and segregation) of each chromatid of homologous chromosomes during Meiosis I and II. This gives information about the distance between the gene and its centromere. (Meiosis II is followed by mitosis; each pair of genetically identical daugthers sits adjacent to one another. Each ascus is thus made of up 8 haploid ascospores.)

Consider a gene required for ascospore color (ws+ gives black spores and ws gives white spores):

P: ws+ x ws
Diploid: ws+ / ws (immediately undergoes meiosis)

If no recombination between ws gene and the centromere occurs, then the resulting ascospores are arranged in a neat array with black and white spores clearly segregated from one another, each type cleanly segregated to either side of the imaginary line separating the 4th and 5th ascospores. This is called **a first division segregation pattern**. Since the daughters of the mitotic division lie right next to one another, we can simplify the two possible configurations to:

(ws+ ws+ ws ws)
(ws ws ws+ ws+)

If recombination occurs between ws and the centromere, then a **second division segregation pattern** is observed. Now, both types of spores are found on either side of the imaginary line between the 4th and 5th ascospores. Now there are four possible configurations:

(ws+ ws ws+ ws)
(ws ws+ ws+ ws)
(ws+ ws ws ws+)
(ws ws+ ws ws+)

When an ascus shows a second division segregation pattern, we know that half of the chromatids are recombinant and the other half have not participated in crossovers. Thus, we can calculate the distance of a gene from its centromere simply by dividing the percentage of second division octads by 2.

Gene-centromere distance = ([# of second division octads / total octads] x 100) / 2

To examine linkage of two genes in Neurospora, we can use the same formulas as we did for Baker's yeast.