

Analysis of Tetrads from the yeast *Saccaromyces cerevisiae*

When normally haploid yeast cells of two different mating types encounter each other, they fuse to form a diploid zygote (this constitutes a cross), which immediately undergoes meiosis to regenerate four individual haploid spores – a tetrad (the equivalent of an F1 generation). Since each meiotic tetrad is contained in a sac-like structure called an ascus, it can be recovered as an intact group. The spores are not arranged in any particular order in the ascus, so they are called random or unordered spores, rather than ordered spores.

Each tetrad results from the events of meiosis in a *single* cell and genetic analysis of the genotypes in the tetrad can tell us about the events during meiosis in that single cell. Because we can culture many such matings and recover many many tetrads from a specific genetic cross, it is possible to detect rare events and to estimate their frequencies accurately. Remember that the products of meiosis are haploid spores that each germinate to regenerate the vegetative haploid state, and because they are haploid, the genotype is expressed directly in the phenotype.

The tetrad patterns we observe across multiple asci in turn tells us about gene linkage. That is, we can determine whether two genes in the yeast cells are unlinked, or tightly or loosely linked to each other, and whether both genes are tightly or loosely linked to the centromere, or whether one or both genes are not linked to the centromere.

To understand what the four spores in each ascus can tell us, we need to think about the possible events during meiosis, and how those events will determine the genotypes of the four spores.

In division 1 of meiosis, when the two pairs of alleles are segregating, 3 patterns of segregation are possible:

If we cross cells that are AB x ab, there are three possible types of 4-spore asci:

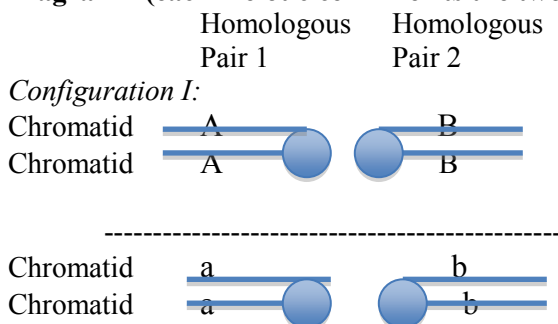
AB AB ab ab	parental ditype PD – two genotypes which match the two parental genotypes
Ab Ab aB aB	nonparental ditype NPD – two genotypes which interchange the parental alleles
AB Ab aB ab	tetratype TT – all four possible genotypes

Let's look at how these three ascus types can arise:

If there is no linkage between the two genes:

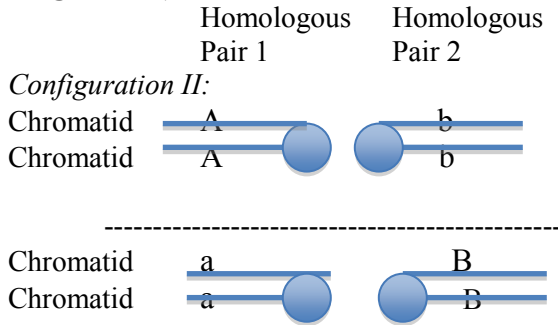
If the genes A/a and B/b are unlinked (A/a are on one homologous chromosome pair & B/b are on another). Continuing our assumption that that the parental genotypes are AB and ab, then there are two possible configurations, depending on how the two chromosome pairs segregate in the first meiotic cell division:

Diagram I (each meiotic cell inherits the two chromosomes from the same parent):



Each spore in the resulting tetrad inherits one chromatid of each of the chromosomes, so if no crossovers occur, after meiosis, this configuration yields a Parental Ditype PD ascus: **AB AB ab ab**

Diagram II (each meiotic cell inherits one chromosome from each parent):



Again, each spore in the resulting tetrad inherits one chromatid of each of the chromosomes, so if no crossovers occur, after meiosis, this configuration yields a Parental Ditype PD ascus: **AB AB ab ab**

To summarize:

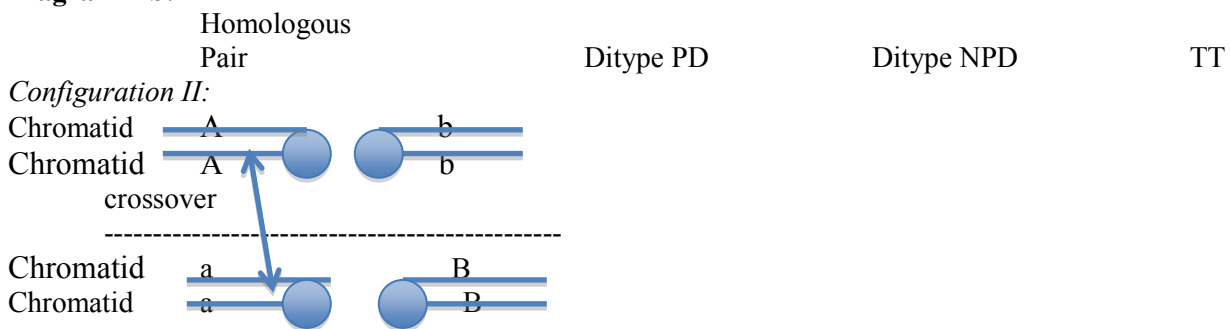
When genes are **unlinked**, the parental ditype tetrads and the nonparental ditype tetrads are expected in equal frequencies (PD = NPD) since the two possible configurations are random and approximately equal.

If there is no crossing over between either gene and its centromere the two chromosomal configurations are equally likely at metaphase I, and so PD = NPD, and TT = 0.



When there is a crossover between either gene and its centromere, a tetratype tetrad results (TT > 0), but this does not change the fact that PD = NPD, which still tells us that the genes are not linked.

Diagram IIb:



A single crossover between the A/a gene and the centromere yields a tetratype tetrad with all four possible genotypes: **Ab ab AB aB**

Therefore, we can summarize as follows:

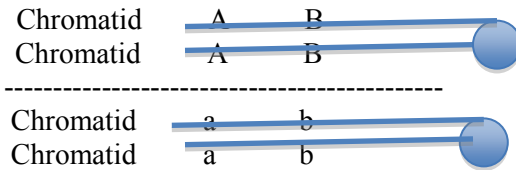
Unlinked gene pairs, and no crossover between either gene and its centromere, yields PD = NPD, TT = 0.

Unlinked gene pairs, with a crossover between either gene and its centromere yields PD = NPD, TT > 0.

If there IS linkage between the two genes:

Consider the case in which two genes are linked (on the same chromosome) as shown here in an early stage of meiosis:

Diagram III: Homologous Chromosome Configuration:



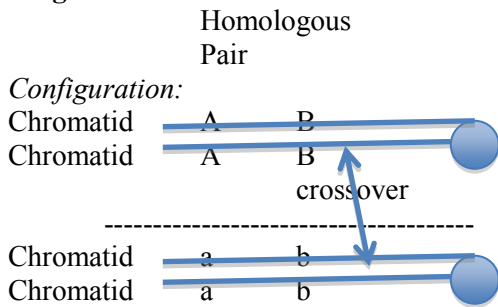
Each spore in the resulting tetrad inherits one of these chromatids, so if no crossovers occur, after meiosis, this configuration yields a Parental Ditype PD ascus: **AB AB ab ab**

Therefore, we can summarize as follows:

Linked gene pairs, and *no crossovers between them*, yields (TT= 0, NPD = 0).

If a single crossover occurs between two linked genes and their centromere:

Diagram IV:



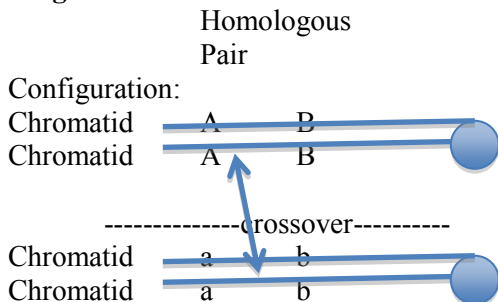
After meiosis, this crossover yields **AB ab AB ab**.

Therefore, we can summarize as follows:

A crossover between linked gene pairs and their centromere is not evident because it yields only PD tetrads as when there is no crossover of this type.

If a single crossover occurs between two linked genes:

Diagram V:



After meiosis, a single crossover yields a tetratype tetrad: **AB Ab aB ab**

Rare double crossovers are equally likely to involve either 2, 3 (2 alternatives) or 4 chromatid strands. The formation of a two-strand, three-strand or four-strand double crossover results in PD, TT or NPD tetrads respectively.

Table I: Tetrad types generated by specific crossover events

No crossover generates	PD only
Single crossover generates	TT only
2-strand double crossover generates	PD only
3-strand double crossover (alt 1) generates	TT only
3-strand double crossover (alt 2) generates	TT only
4-strand double crossover generates	NPD only

With linked genes, PDs always greatly outnumber NPDs, because PDs primarily result from no crossovers, while NPDs result exclusively from very rare double crossovers.

Reasoning and calculating map distance from the data:

We can summarize the possibilities as follows:

- 1.) If NPD is equal in frequency to PD (NPD = PD), the genes are unlinked and no map distance can be calculated.
- 2.) If the most frequent tetrad is PD and NPD is far less frequent (NPD << PD), then the genes are linked and we can compute their map distance.

As Table 1 shows, we cannot directly count the number of single crossovers; while single crossovers always result in TT tetratypes, TT tetratypes also arise in other ways (from 3-strand double crossovers).

Similarly, we cannot directly *count* all the double crossovers, because some double crossovers generate PD and TT tetrads, which can also arise in other ways. But we can *calculate* the total number, because NPDs only arise from double crossovers. As Table 1 shows that each observed NPD represents 1 of every 4 double cross-overs that have occurred. Since the four types of double crossovers are equally likely, the total number of double crossovers = 4 * NPD.

Now we can calculate the number of single crossovers from the number of TTs, correcting for the two types of 3-strand double crossovers that occurred. Since each of the two types is as likely as the NPD double crossovers, the number of TTs that arise from single crossovers = TT - 2*NPD. Since only half of the spores in TTs are recombinant, the number of single crossovers = 1/2 * (TT - 2*NPD).

Penultimately, the frequency of recombination equals (the number of single crossovers plus the number of double crossovers) divided by the total number of tetrads scored:

$$[1/2 * (TT - 2NPD) + 4NPD] / (PD + TT + NPD) = \text{frequency of recombination}$$

or

$$(1/2 * TT + 3NPD) / (PD + TT + NPD) = \text{frequency of recombination}$$

Finally, the map equal the recombination frequency times 100:

$$(1/2 * TT + 3NPD) / (\text{total tetrads}) * 100 = \text{map distance (centimorgans)}$$