Tetrad analysis

Tetrad analysis in genetics refers to analysis of four products formed from meiosis. In organisms like yeast the tetrad contains four spores while in case of Neurospora the ascus in which products of meiosis are held, there are eight ascospores.

Although meiosis occurs in all sexually reproducing organism, in most of them, the products of meiosis are mixed such as in case of pollen grains. However, in few cases the products of a single meiosis are held in a separate body. It is possible to isolate each such spore containing body which can be further dissected to isolate and culture each spore separately. Thus, it is possible to study the phenotype and the genotype of each isolated spore. This analysis is known as tetrad analysis and some organisms in which it is carried out are yeast, Chlamydomonas and Neurospora.

Tetrads in which the spores are arranged according to the order in meiotic division are called ordered tetrads as in Neurospora, while where the order or sequence is not specific are called unordered tetrads as in yeast or Chlamydomonas.

Higher organisms which undergo meiosis are diploids (or polyploids). Some lower organisms which undergo meiosis spend considerable part of life cycle in haploid state. The haploid organisms provide a suitable system since there is only one allele in haploid state and there is no dominance phenomenon.

Life cycle and meiosis in yeast

Fig.1 shows life cycle of yeast and Fig.2 shows meiosis in yeast and asci containing ascospores.
Diploid cells starved for nitrogen or carbon source undergo meiosis. After nuclear division, each prospore membrane closes on itself to enclose a haploid nucleus within two distinct membranes. Spore wall synthesis then begins in the lumen between the two prospore membrane-derived membranes. After spore wall synthesis is complete, the mother cell collapses to form the ascus. Since the spores in the ascus are not arranged in any particular order this is called an unordered tetrad. Normally spores form a tetrahedral shape, some times all spores are in one plane.

**Life cycle and meiosis in Neurospora**

*Neurospora* spends most of its life cycles in the haploid state Fig.3. *N. crassa* which is most commonly studied is heterothallic which means two different mating types are required to undergo sexual reproduction. In the sexual phase, hyphae of different mating types come into contact and nuclei fuse resulting in many transient diploid nuclei inside fruiting bodies called perithecia. Each diploid nucleus undergoes meiosis. The four haploid products of one meiosis stay together in a sac called an ascus. In *N. crassa* each of the four products of meiosis undergoes a further mitotic division, resulting in an octad of eight ascospores within each ascus. Ascospores germinate and produce hyphae resulting in colonies. Ascospores from a single ascus can be individually picked up and inoculated to produce colonies.
Segregation of alleles

From a pair of homologous chromosomes the homologues segregate in the 1st meiotic division. If there is no crossover, the two alleles on the homologous chromosomes thus segregate in the first meiotic division. Even when there is a crossover, the alleles between the centromere and the crossover segregate in the 1st division.

Fig. 4. Segregation of alleles in (A) 1st division or (B) in 2nd division.
meiotic division Fig.4A. When there is a crossover between the centromere and the locus, the alleles of the locus segregate only in the second division of meiosis Fig.4B. In *Neurospora* after meiosis there is a mitotic division which brings the number of spores in each ascus to eight.

**Auxotrophic mutants**

Microorganisms can mutate to produce different types of mutants. Mutants which have lost the ability to produce a particular metabolite and can be made to grow by supplying the same as a nutrient are called auxotrophs. The wild type is a prototroph which means it does not require addition of the metabolite in the medium as a supplement. Auxotrophs for a particular amino acid or a vitamin are commonly used mutants in genetic studies.

**Tetrad analysis in Neurospora**

*Neurospora crassa* produces asci with eight spores which are arranged in an order after meiosis. Hence ordered tetrad analysis is possible. This is particularly useful in studying recombination, estimating linkage and mapping of genes. Crosses are made between strains of known mutations and from the fruiting bodies asci are dissected out, spores are isolated and their phenotypes are determined.

The question if crossover occurs at two strand stage or four strand stage (i.e. if crossover took place and then chromosome doubling occurred or first chromosome doubling occurred and then crossover took

1. If crossover occurs at two strand stage
2. If no crossover occurs at two strand stage
3. If crossover occurs at four strand stage

Since spore arrangement seen in 3 is observed crossover occurs at four strand stage.

**Fig. 5. Spore arrangement shows that crossover takes place at four strand stage.**
place) is answered by looking at the spore arrangement (Fig.5). The sequence of spore arrangement indicates if a crossing over has occurred between the genes under study or the centromere and a gene. Assume a cross between wild type (al+) and albino mutant (al). If there is no cross over between the centromere and the gene, the spore arrangement observed is 4al+:4al and if there is a crossover between the centromere and the gene involving two of the four strands, the spore arrangement is 2al+:2al:2al+:2al (Fig.6).

In such a cross if 129 asci showed 4:4 arrangement and 141 asci showed 2:2:2:2 arrangement, the total number of asci scores is 129+141=270. In the 141 asci which showed recombination, only two out of the four strands took part in crossing over. Hence, recombination% is half of the number of asci showing segregation. The recombination is calculated as \(0.26\) or 26%.

If two genes (P and Q) are on the same side of the centromere, and if at four strand stage two strands are involved in a crossover between the centromere and a gene and the same two strands are involved in a crossover between two genes, the consequence is shown in Fig.7A. However if the first crossover between centromere and gene P is between two strands the second crossover between P and Q the other two strands the consequences are shown in Fig. 7B.
Two genes on two separate chromosomes, two arms of the same chromosome or on the same arm of the same chromosome are different possibilities of location of genes and, no crossover, single crossover between centromere and a gene or single crossover between the two genes, two crossovers one between centromere and the other between the two genes involving the same two strands or involving two different strands each time, two crossovers involving three strands are the different possibilities and correspondingly there are sequence arrangements of the spores.

Detection of linkage

Consider a dihybrid cross between an adenine requiring strain (ad) with A mating type and a wild type strain with a mating type. The cross can be written as

ad A X + a on observing the asci there may be parental combinations (parental ditypes or PD) which are formed as a result of no recombination and nonparental ditypes (NPD) formed as a result of recombination. In addition, recombination also results in tetratypes (T) in which four types of spores are formed.

<table>
<thead>
<tr>
<th>Table1. Types of tetrads in a cross between two strains of Neurospora.</th>
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<tbody>
<tr>
<td>Parental ditypes</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>ad A</td>
</tr>
<tr>
<td>ad A</td>
</tr>
<tr>
<td>+ a</td>
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<tr>
<td>+ a</td>
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<tr>
<td>Number observed: 10</td>
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</table>

The difference between parental ditypes and nonparental ditypes is not significant which indicates independent assortment between the mating type gene and the adenine requiring locus. A significantly higher number of parental ditypes would mean tendency to be inherited together or genetic linkage.

In Neurospora a strain auxotrophic for pyridoxine (pdx) and a strain auxotrophic for pantothenic acid (pan) were crossed. Results of tetrad analysis are shown in Table2.

<table>
<thead>
<tr>
<th>Table2. Types of tetrads and numbers observed in crosses between strain pdx + and + pan.</th>
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<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>pdx</td>
</tr>
<tr>
<td>pnx</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
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<td>15</td>
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</table>

Recombination at pdx is seen in 4, 5 and 6 (1+1+3+2=16), and recombination at pan is seen in 3, 5 and 6 (17+1+3+2=32). This recombination is due to crossover between the centromere and gene. As recombination types are less for pdx, this locus is nearer to the centromere and recombination types are more for pan, the locus is farther.
The recombination at pdx and pan loci can be used to calculate recombination % and draw a linkage map on which the two loci can be localized with respect to the centromere. In calculating recombination percentage, the frequency of tetrads with single crossover is multiplied by $\frac{1}{2}$ since in these cases two of the four strands are involved in crossover while the frequency of double crossovers is used as it is since in double crossover four out of the four strands participate in crossover.

The distance between centromere and pan is obtained as follows

The total number of tetrads scored is $15+1+17+1+13+2=49$. 32 showed recombination at pan due to single crossover and four due to double crossover, therefore

Recombination = $\frac{1}{2}(32/49) + (4/49) = 0.387$ or 38.7%.

Recombination at pdx is calculated as

$\frac{1}{2} (16/49) = 0.163$ or 16.3% (there are no double crossovers between centromere and pan).

Distance between pan and pdx is obtained by subtracting 0.163 from 0.387 which is 0.224.

Distance between pan and pdx can also be obtained as: $\frac{1}{2} (17+1+2)/49 + 1/49 = 0.224$ or 22.4%.

The relative positions of the centromere, pdx and pan are shown in Fig 8.

Unordered tetrads

These occur in many four spored yeasts, some eight-spored euascomycetes and in basidiomycetes. Here the spores resulting from a single meiosis event are held together, however, the arrangement of the spores has no correlation with the order during meiosis. As a result of this the centromere cannot be used as a
marker in genetic studies, linkage distances between two genes have to be determined through their recombination value.

Three types of tetrads result after meiosis parental ditype, nonparental ditype and the tetratype. In case of unlinked markers parental and nonparental ditypes occur in equal proportions. A statistically significant deviation from equal proportion indicates linkage. Information about linkage and the location of the genes can be obtained by comparing the distances between each of the two genes. However, three factor crosses are necessary to establish the order of loci with certainty.

If the previous data in Table 2 are pertaining to unordered tetrads, the crossovers between centromere and pdx will not be considered. Only crossovers between pdx and pan loci will be considered for calculation. The data are then interpreted as shown in Table 3.

| Table 3. Types of tetrads and numbers observed in crosses between strain pdx + and + pan. |
|---|---|---|---|---|---|
| 1  | 2   | 3  | 4  | 5   | 6   |
| pdx | +  | pdx | Pan | pdx | +  | pdx | +  |
| pdx | +  | pdx | Pan | pdx | Pan | +  | +  |
| +  | Pan | +  | +  | +  | +  | pdx | Pan |
| +  | Pan | +  | +  | Pan | +  | +  | +  |

No crossover only parental ditypes
Two crossovers between pdx and pan, all four strand involved
Single crossover between pdx and pan, two strands involved
Single crossover between pdx and pan, two strands involved
No crossover between pdx and pan
Single crossover between pdx and pan, two strands involved

Parental types (no recombination between pdx and pan)= 15+13=28.
Recombinants with one crossover =17+1+2=20.
Recombinants with two crossovers=1.
Total number scored=49.
Recombination % will be \[
\frac{\frac{1}{2}(20)+1}{49} = \frac{11}{49} = 0.224 \text{ or } 22.4%.
\] The value is same as in previous calculation.
G.W. Beadle (1903-1989) and E.L. Tatum (1909-1975)

American geneticists Beadle and Tatum's experiments involved exposing *N. crassa* to x-rays, to create mutations. They showed that mutations caused changes in specific enzymes involved in metabolic pathways. This led them to propose a link between genes and enzymatic reactions, which became known as the “one gene, one enzyme hypothesis”. They were awarded Nobel Prize in Physiology or Medicine in 1958 along with J. Lederberg.

Micromanipulation technique in yeast

A sporulated culture is used for isolation of ascospores. The culture containing asci is digested with zymolyase in a controlled manner. Separation of spores requires use of micromanipulation technique. The treated asci are placed on petridish with medium or on thin agar slabs. The four spored ascus is picked up using the microneedle of the micromanipulator. Each spore is then separated using micromanipulator and the spores are placed on the medium surface for growth as a colony.