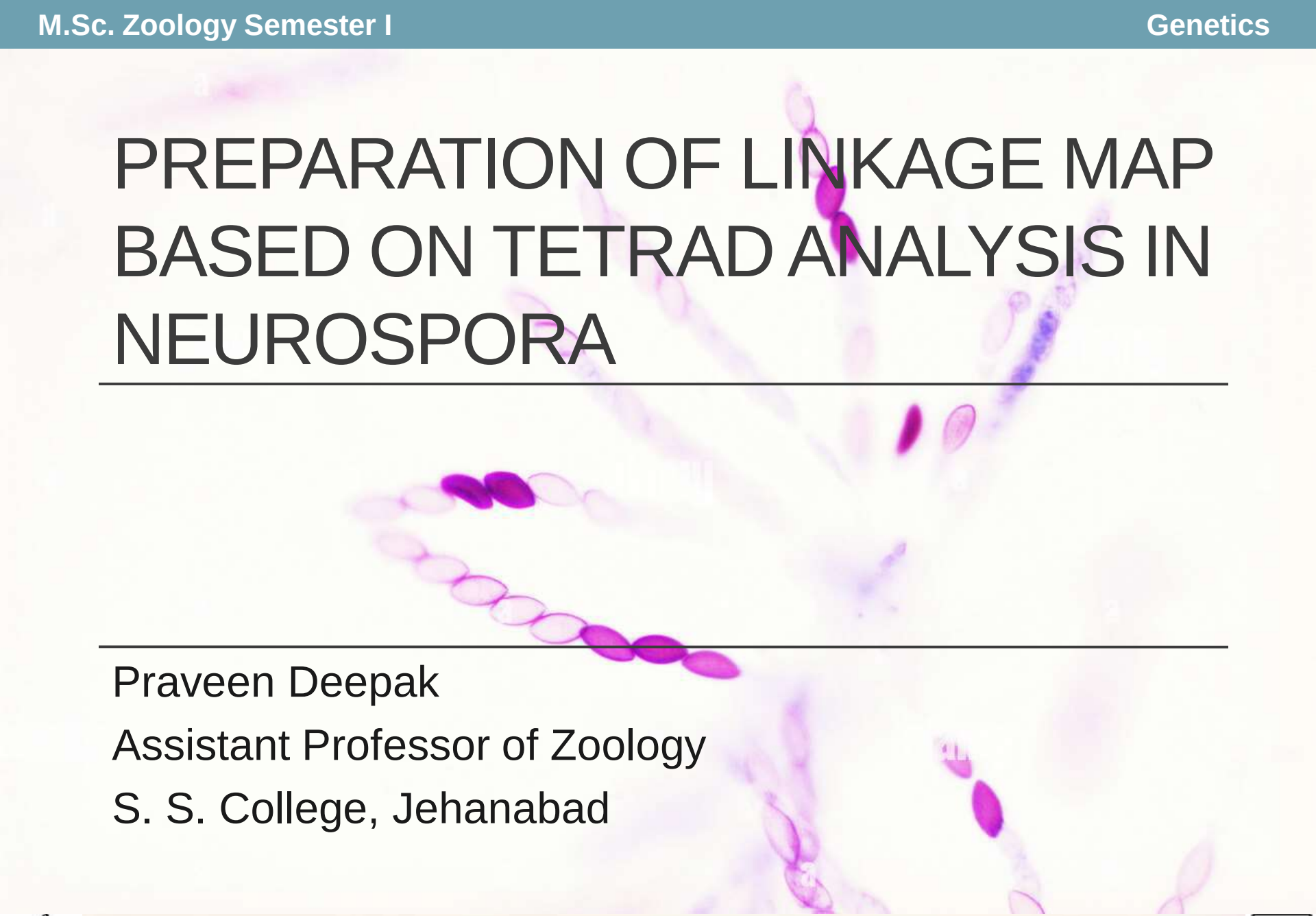
PREPARATION OF LINKAGE MAP BASED ON TETRAD ANALYSIS IN NEUROSPORA



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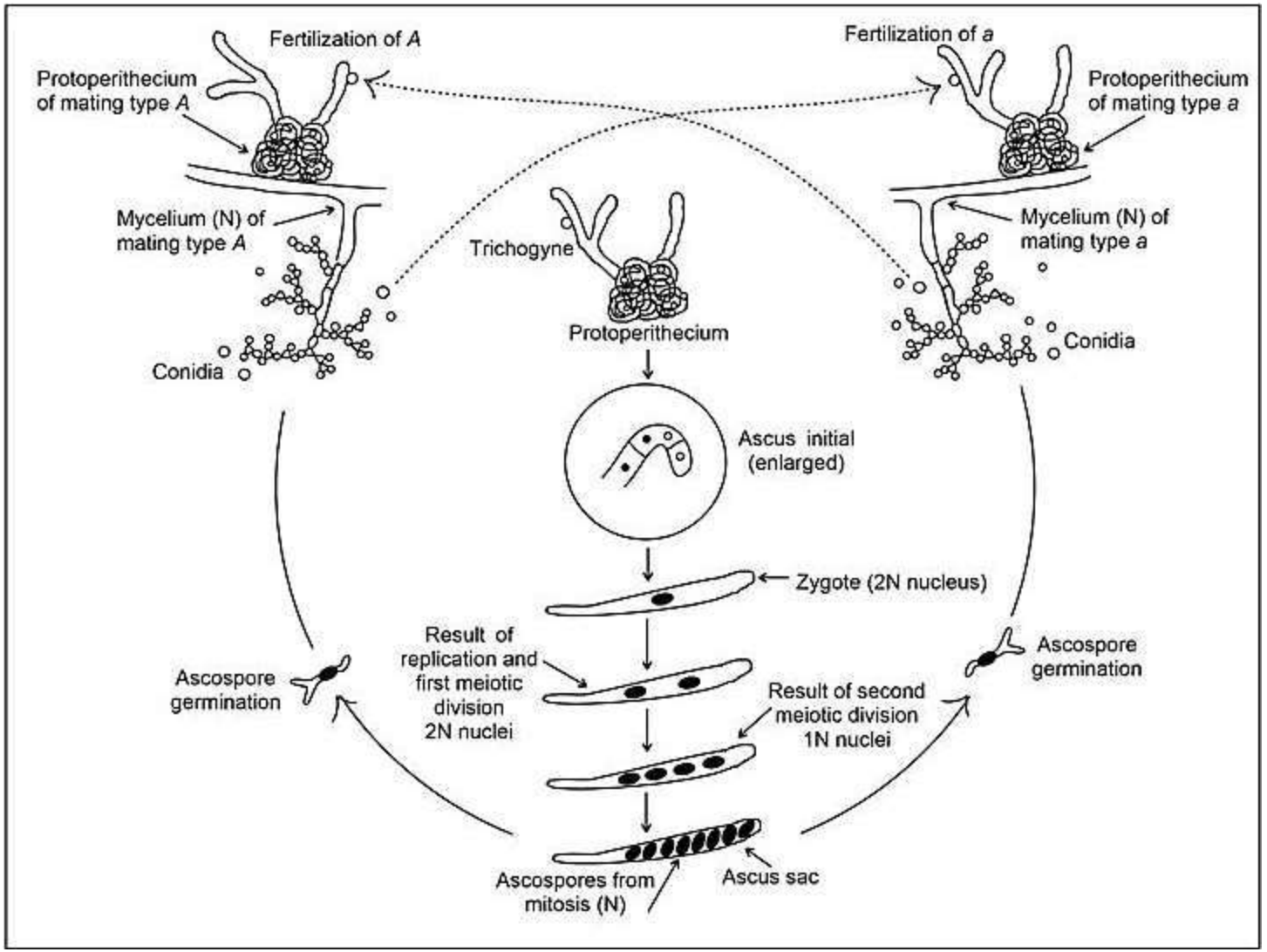
Introduction

- Literal meaning of ‘tetrad’ is ‘a group or set of four’. As the term implied, it deals with the four spores produced after meiosis in the fungi of Division Ascomycota., and Class Sordariomycetes.
- In Ascomycota, these spores are called as ascospore. All ascopores are contained in the sac-like structure, known as ascus.
- The formation of these four ascospores in the fungi is known as tetrad. Further mitosis leads to the formation of 8 haploid cells contained in ascus known as octad.
- For tetrad analysis, lower eukaryotes, such as algae and fungi, especially in Fungi, *Saccharomyces cerevisiae*, *Coprinus lagopus*, *Chlamydomonas reinhardtii* (Tetrad), *Neurospora crassa* (Octads), and *Aspergillus nidulans* (Octads) are extensively used.

Why Ascomycota?

- Haploid in nature.
- Produce very large number of progeny.
- The life cycles of these organisms tend to be very short.
- Potential for analyzing all the products from each meiotic division.
- Easy to make direct observations on the behavior of genes during meiosis. Examination of cross overs is possible.
- Mapping of centromeres can be possible.

Life cycle of *Neurospora crassa*



Formation of Ascus in the *Neusospora crassa*

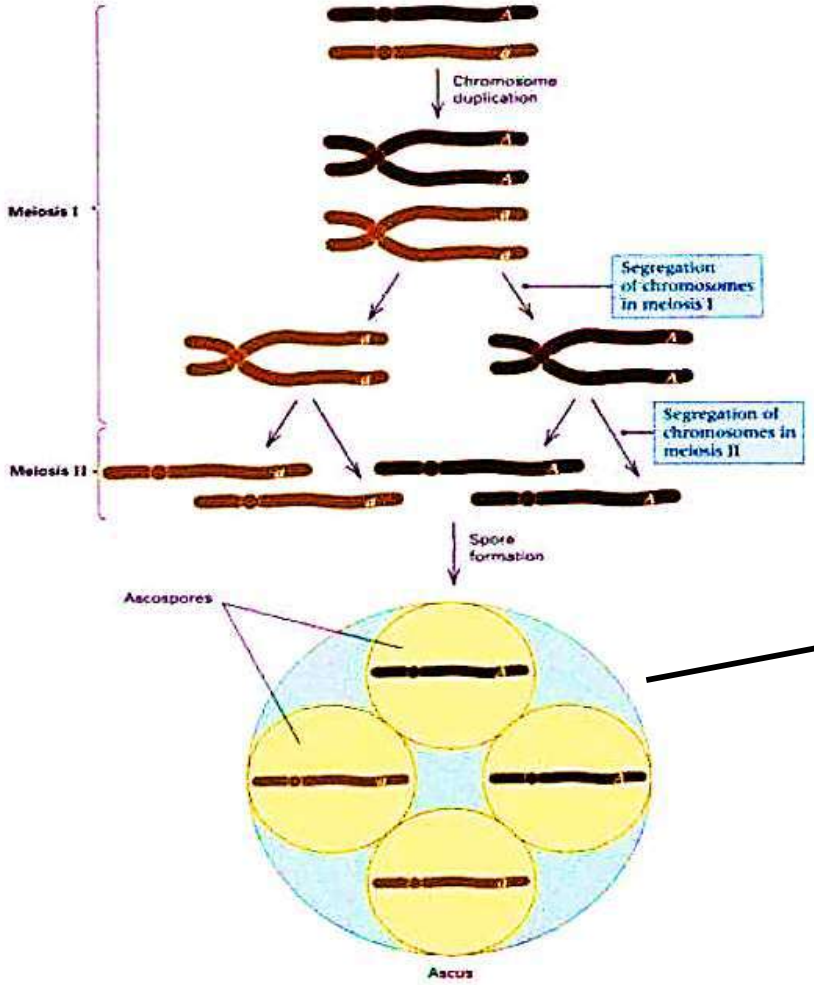


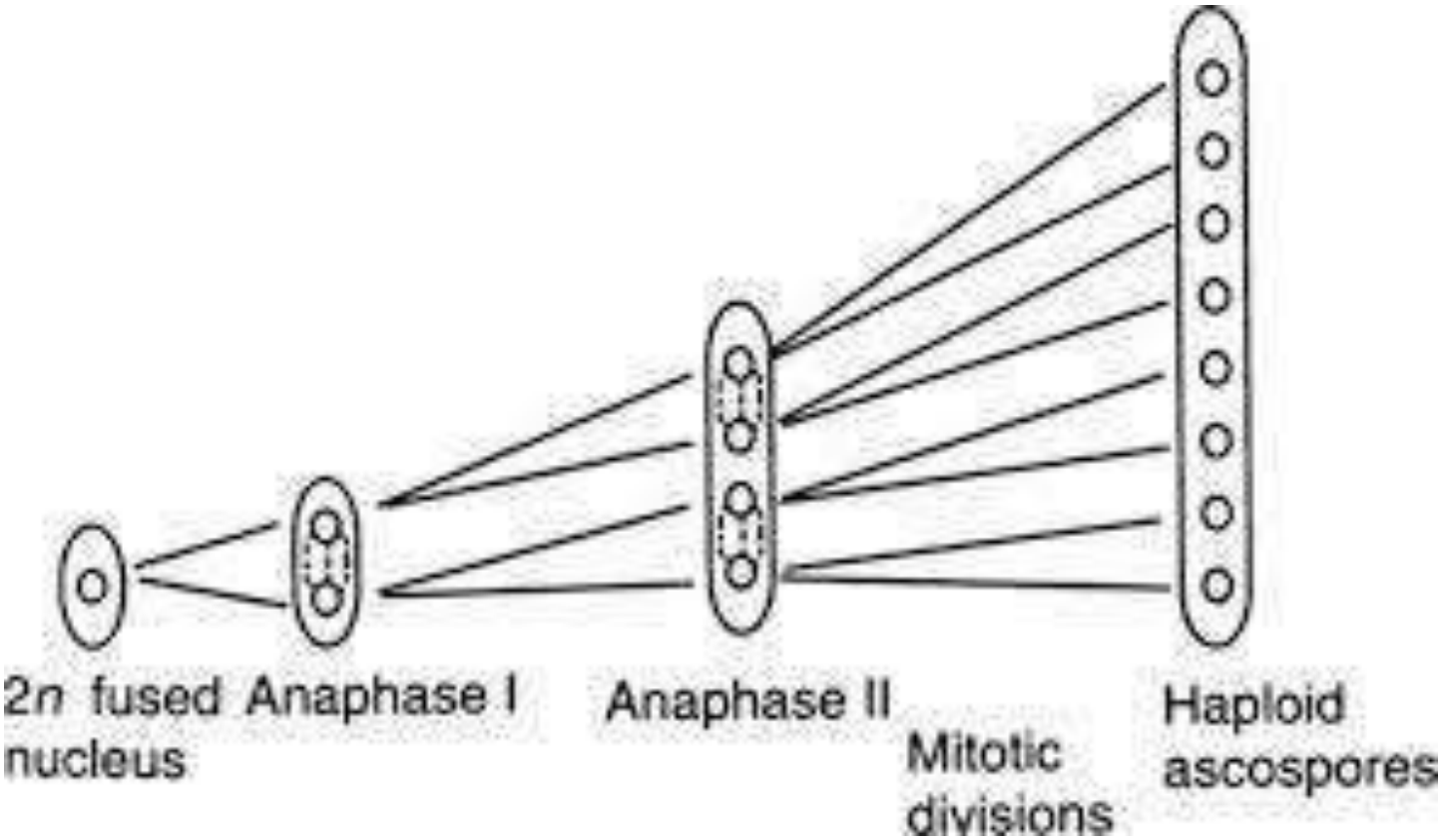
Figure 4.21
Formation of an ascus containing all of the four products of a single meiosis. Each product of meiosis forms a reproductive cell called an ascospore; these cells are held together in the ascus. Segregation of one chromosome pair is shown.

The 'tetrad' can be unordered tetrad or ordered tetrad. *Neurospora* gets ordered tatrads.

Tetratypes

- A tetrad containing four kinds of haploid cells, two different parental class spores and two different recombinant class spores.
- It represents single cross over.
- In crosses involving 2 unlinked genes, tetratypes arise when a crossover occurs between one of the two genes and its centromere.
- It is of two kinds; ordered and unordered tetrads.
  - ***Ordered tetrad –*** Tight Ascus is produced wich prevents spores from randomly moving around, e.g. *Neurospora crass*.
  - ***Unordered tetrad:*** Ascus povides enough space for tetrads to randomly mix together, e.g. *Sachharomyces cerevisiae*.

Ascus formation in Neurospora crassa through meiosis



Tetrad analysis in *Neurospora crass*

- *Neurospora crassa* produces ordered tetrads.
- It is a type of tight ascus produced by *Neuropora* mycelium .
- Tightness of ascus prevents ascospores or spores from randomly moving around and assures fixing of spores to a particular arrangement.

$$Map\ distance = \frac{\frac{1}{2} number\ of\ SDS\ asci}{Total\ number\ of\ asci} \times 100$$

SDS is second division segregation. It is comparable to recombinant in Mendelian cross.

Tetrad analysis in *Neurospora crass*

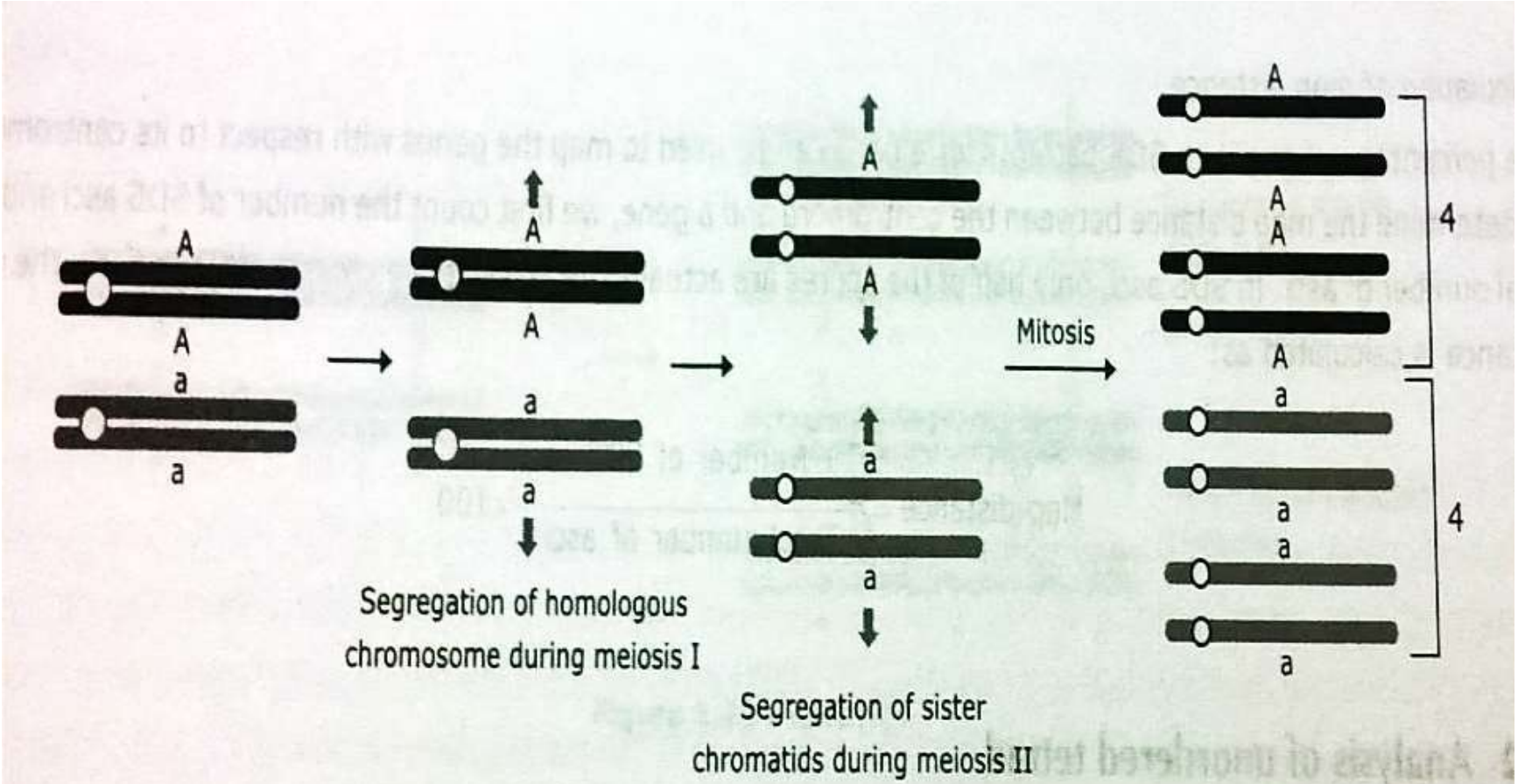


Figure 1.18 First Division Segregation (FDS) : No crossing over produces a 4 : 4 arrangement.

Tetrad analysis in *Neurospora crass*

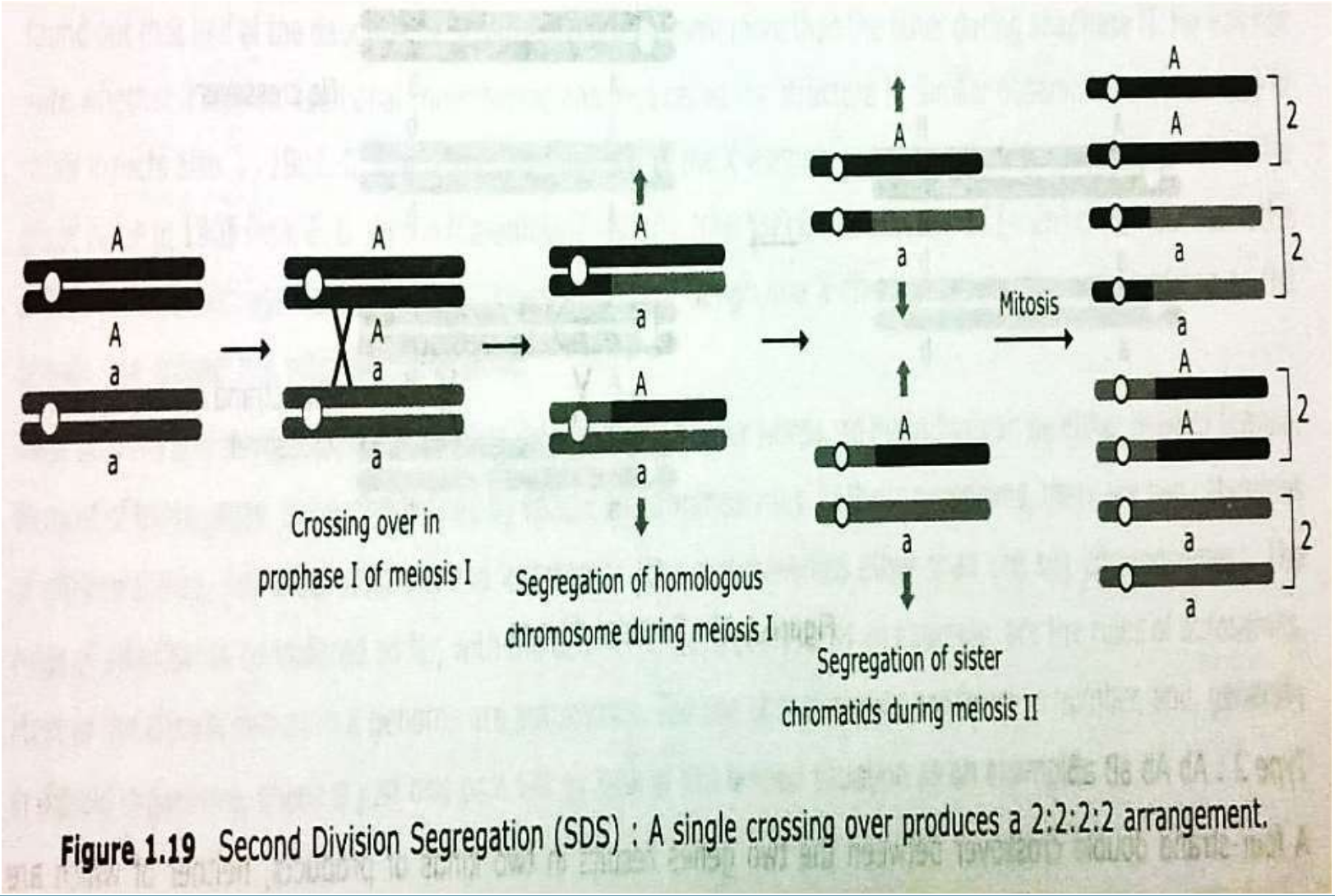


Figure 1.19 Second Division Segregation (SDS) : A single crossing over produces a 2:2:2:2 arrangement.

Tetrad analysis in *Neurospora crass*

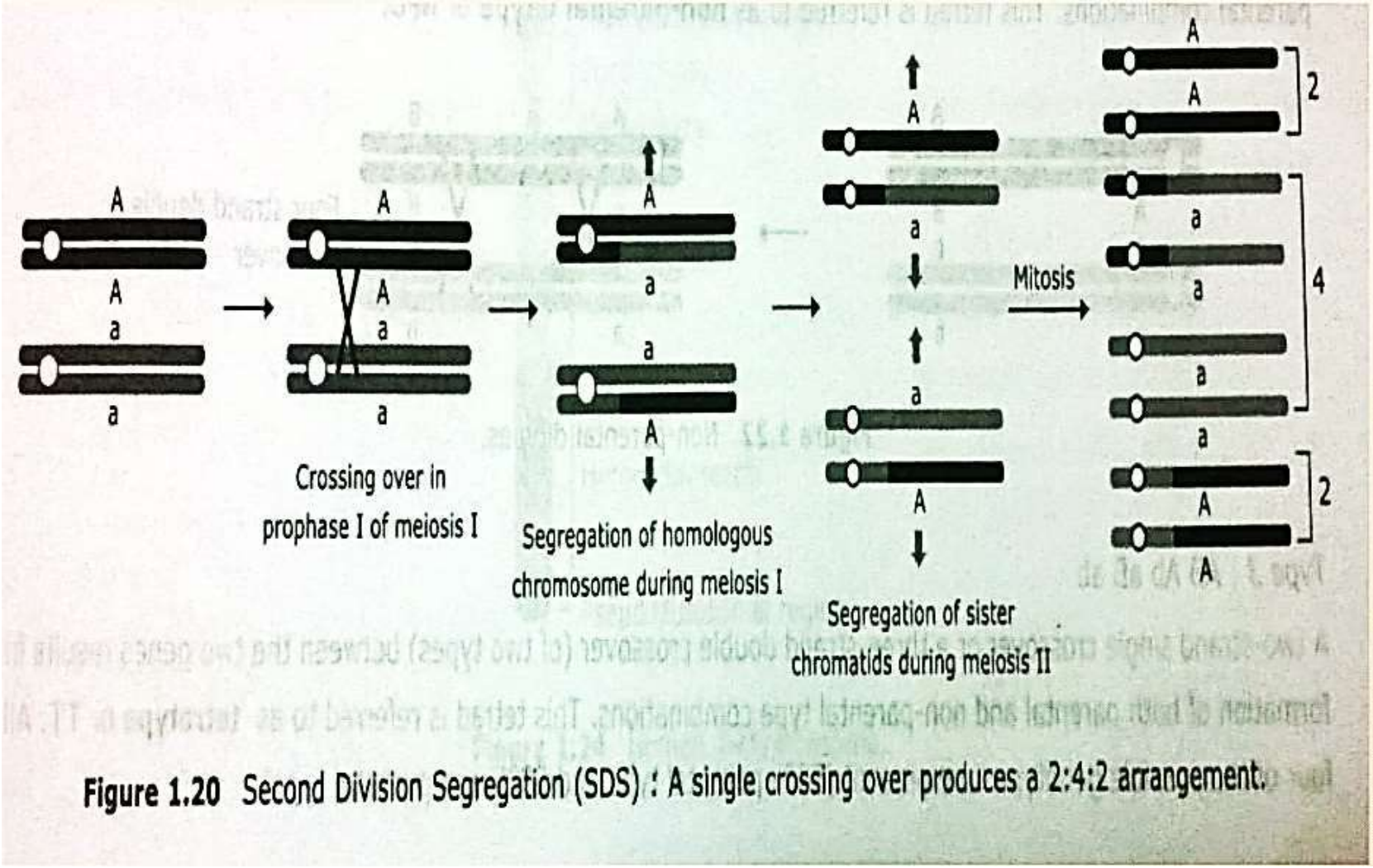


Figure 1.20 Second Division Segregation (SDS) : A single crossing over produces a 2:4:2 arrangement.

Laboratory practice on tetrad analysis and linkage map

Objective

To determine whether the genes are linked or not and to construct the genetic map of genes on the chromosomes of *Neurospora crassa* by applying the knowledge of meiosis and Mendelian inheritance.

Principle

Like higher organisms, sexual phase of Neurospopra crassa chromosomes synapse during gametogenesis in meiotic cell division and crossing over between homologues occurs. Crossing over combines genetic material that had previously been on separate homologues and produces individuals with increased genetic variation. These recombinant gamete can be visualized easily due to color. Due to genetic variation, location of the gene can be possible to derive and with the help of position of genes, a linkage map can be produced.

Laboratory practice on tetrad analysis and linkage map

Materials

- Compound microscope
- Inoculating loops
- Alcohol lamps
- 70% alcohol
- Slides and coverslips
- Dropper bottles filled with water
- Stock plate of wild *Neurospora crassa*
- Sterile agar plate for making crosses
- Permanent markers
- Bloating paper and tissue paper or lens paper
- Matches for lighting burners

Media preparartion

17g corn meal agar, 10g sucrose, 7g glucose, 1g yeast extract, 0.1g KH_2PO_4, and 1 liter water. Autoclave media at 15 psi for 20-30 min. Plate is left for 2 to 3 days for aging.

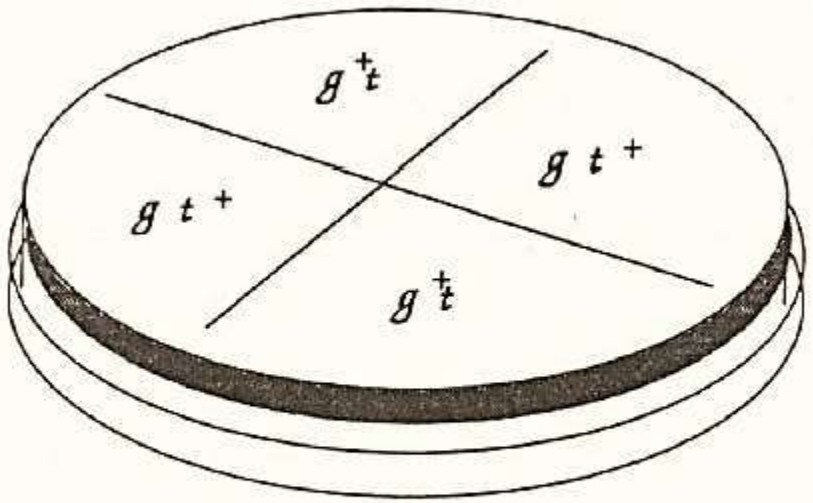
Laboratory practice on tetrad analysis and linkage map

Crossing of the spore

- Two mutants of *Neurospora crassa* are taken, such as grey spore and tan spore in a separate agar plate supplemented with 0.1% yeast extract.
- With a marking pen, divide the bottoms of Petri dishes into four quadrant and labeled with g^+t or gt^+
- Inoculating loop is flamed with alcohol burner, and cooled by touching to stock culture plates. Then, a small piece of agar (~3mm^2) containg fungal growth is taken with the help of inoculating loop.
- The inoculum is quickly transferred to appropriate quadrant labeled in the Petri dish.
- Inoculating loop is reflamed and above procedure is repeated until all quadrants are appropriately inoculated.
- The Petri plate is carefully labeled and plate is closed with tape.
- Then Petri plate is placed in an incubator at 25°C.
- After 8-10 days, perithecia are matured, the Petri plates are placed in a refrigerator and examined after 4 or 5 days.

Laboratory practice on tetrad analysis and linkage map

Crossing of the spore



Labeling of bottom of Petri plate and inoculation of mutrant fungal spores

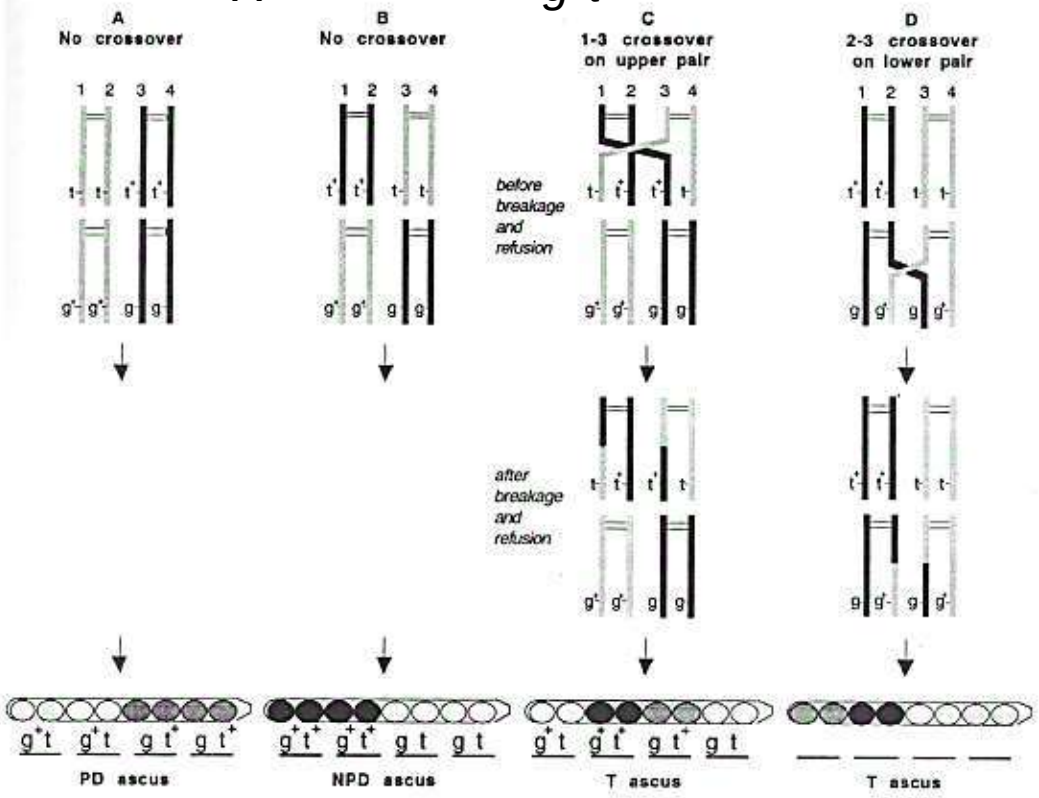
Figure 1.2. Appearance of petri dish bottom for receiving inocula to make the gray spore ($g\ t^+$) × tan spore (g^+t) cross.

Laboratory practice on tetrad analysis and linkage map

Predictions and Data Analysis

Grey spore strain gt^+ X *tan spore strain* g^+t

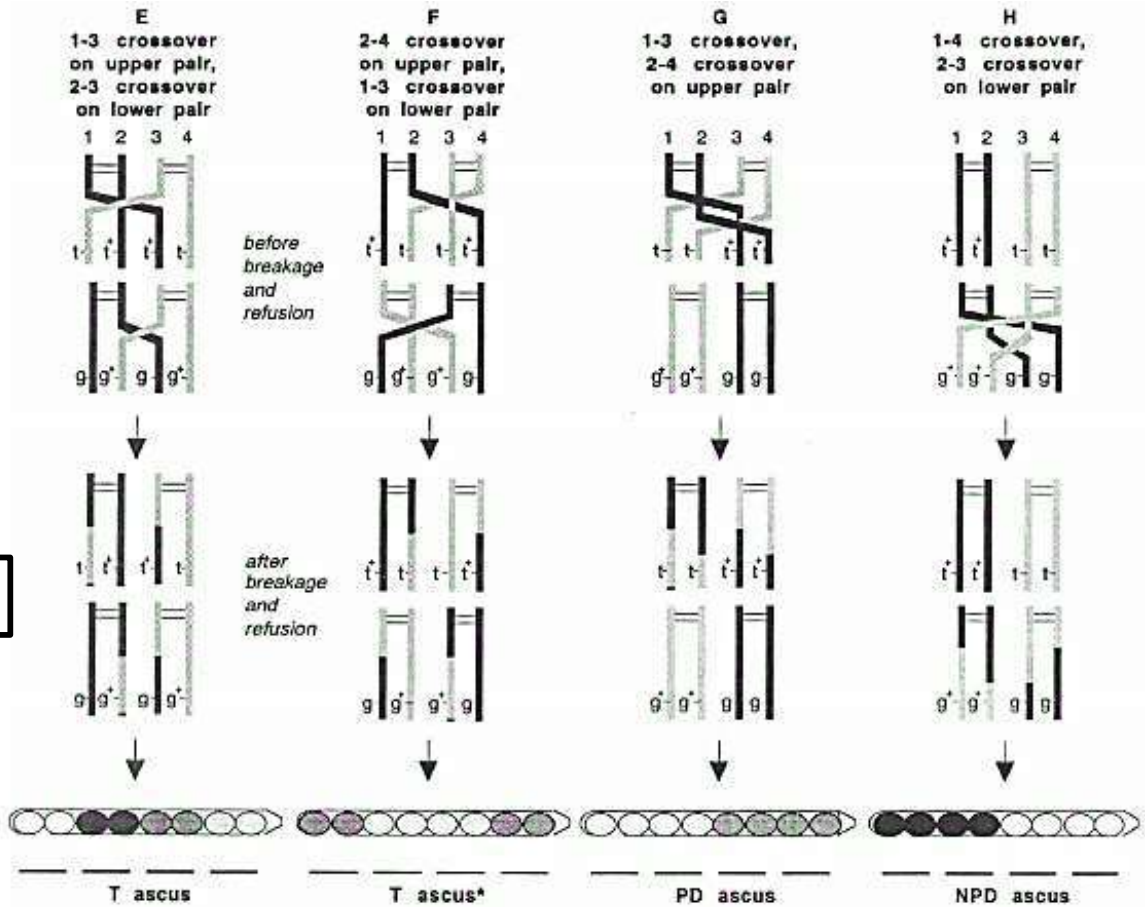
Gens are unlinked



Laboratory practice on tetrad analysis and linkage map

Predictions and Data Analysis

Gens are unlinked



Laboratory practice on tetrad analysis and linkage map

Data

collection

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 Recomb.	Gray Spore Gene Non-Recomb.	Tan Spore Gene Recomb.	Tan Spore Gene Non-Recomb
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PD Asci = ______ Spore Totals = ______ ______ ______ ______

NPD Asci = ______ Crossover Asci = ______ ______

T Asci = ______ Non-crossover Asci = ______ ______

Total Asci =

Laboratory practice on tetrad analysis and linkage map

Calculating recombination frequency

$$\text{map units} = \frac{\text{recombinant spores}}{\text{Total spores (recomb + non-recomb)}} \times 100$$

By getting map units, a genetic map is prepared.

Sample problem

Two strains of *Neurospora*, one mutant for gene a, the other mutant for gene b are crossed. Determine the linkage relationships between these two genes and the map distance.

	Spores				
	1 + 2	3 + 4	5 + 6	7 + 8	Number of Asci
1	a+	a+	+b	+b	79
2	a+	++	ab	+b	14
3	a+	ab	++	+b	6
4	a+	+b	a+	+b	1


SUCCESS ISN'T ABOUT GREATNESS. IT'S ABOUT CONSISTENCY. CONSISTENT HARD WORK LEADS TO SUCCESS. GREATNESS WILL COME