Student name: **Code number:**

GENETICS

Lab 3

(2014 - 2015)

Objectives:

Upon completion of this lab, the students should be able to:

- Understand the different stages of meiosis.
- Describe the events during each phase of meiosis (number and structure).
- Put the stages of meiosis (I and II) in the proper order.
- review the events in meiosis that result in the independent assortment of genes, and the phenomenon of crossing over
- know how meiotic recombination and independent assortment contribute to variation
- examined the relationship between gene segregation and meiosis.
- learn how the frequency at which they become unlinked can be used to construct a genetic map of each chromosome.
- understand how and why alleles from different genes can be inherited together ‘linked’.
- discuss how some genes may be completely linked and not assort independently
- know how genes become separted by crossing over during prophase I
- understand how crossover frequency can be used to map genes
- describe the relationship of the distance between genes on the same chromosome and the frequency of crossing over between the genes
- explain how the position of genes on a chromosome can affect the recombination frequency
- use the recombination frequency between genes to map genes to a chromosome
- calculate the recombination frequency between linked genes based upon the frequency of offspring phenotypes

Please read and make sure you understand the following instructions and knowledge before you go on.

Revised from Lecture 2:

- Meiosis
- Meiosis vs Mendel

Revised from Lecture 3:

- Recomination frequency
- Parental and recombination ratios of gametes
- Crossover vs recombination
- Mapping of genes according to Morgan

1. MEIOSIS

Meiosis is a special type of cell division necessary for sexual reproduction in eukaryotes, such as animals, plants and fungi. The number of sets of chromosomes in the cell undergoing meiosis is reduced to half the original number, typically from two sets (diploid) to one set (haploid) known as gametes.

Meiosis takes place in two stages, Meiosis I where reduction in chromosome numbers and crossing-over occurs; and Meiosis II, which is similar to Mitotic cell division and reduction in chromosome structures occurs. Meiosis I and Meiosis II have four phases each. They are:

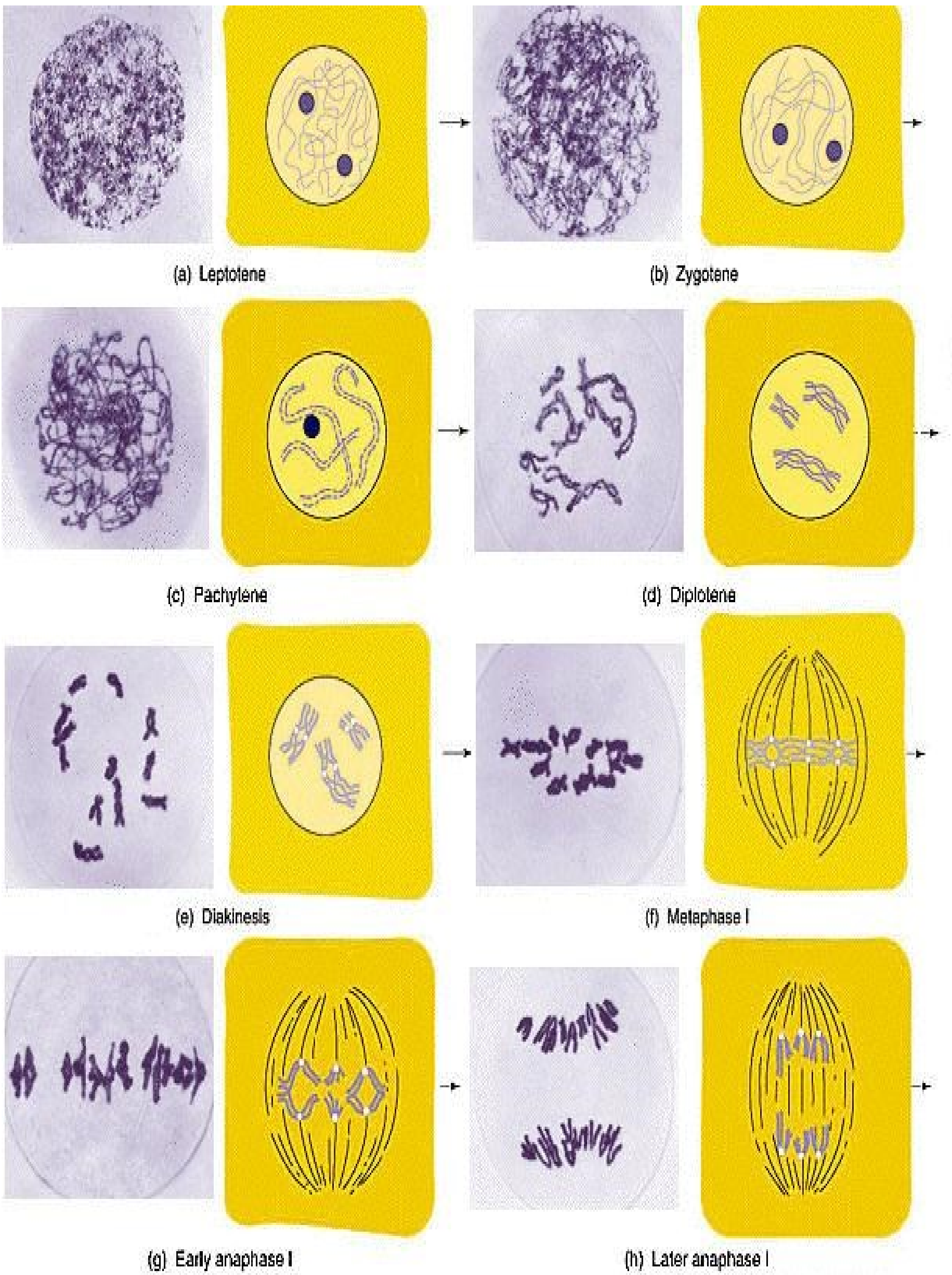
Meiosis I: prophase 1, metaphase 1, anaphase 1, and telophase 1 and
Meiosis II: prophase 2, metaphase 2, anaphase 2, and telophase 2.

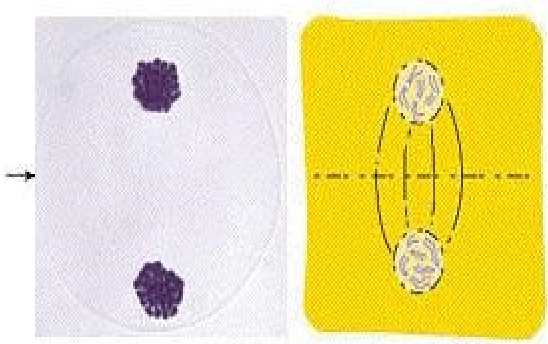
Meiosis I starts with a complete Interphase in which the monad becomes dyad. Meiosis II in contrary has no complete interphase (interkinesis) as there is no S-phase and dyad becomes monad.

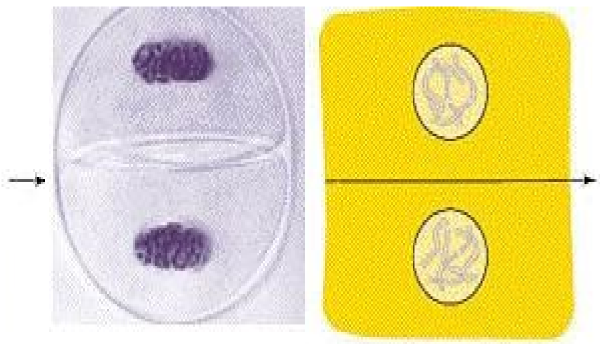
Interphase I	→	Meiosis I	→	Interkinesis	→	Meiosis II.
Monad to dyad		Dyad		Dyad		Dyad to monad
2n		2n to n		n		n

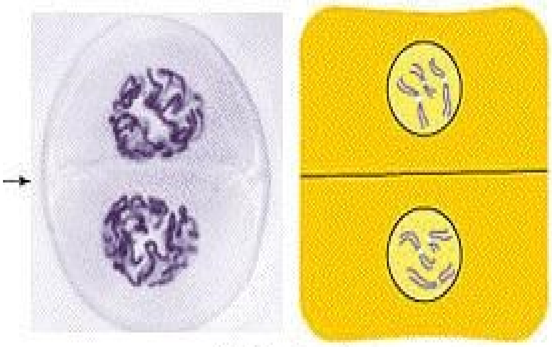
Crossover occurs in Prophase I. it consist of 5 critical substages: leptotene, zygotene, pachytene, diplotene and diaknesis.

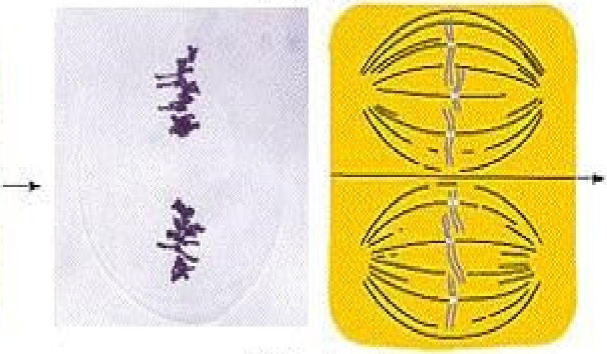
Anaphase I and II are the stages in which reduction in number or structure happens.

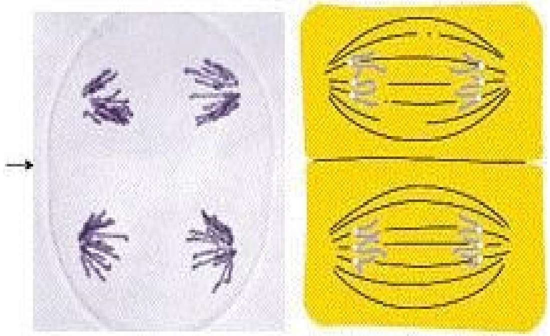

(a) Leptotene
(b) Zygotene
(c) Pachytene
(d) Diplotene
(e) Diakinesis
(f) Metaphase I
(g) Early anaphase I
(h) Later anaphase I

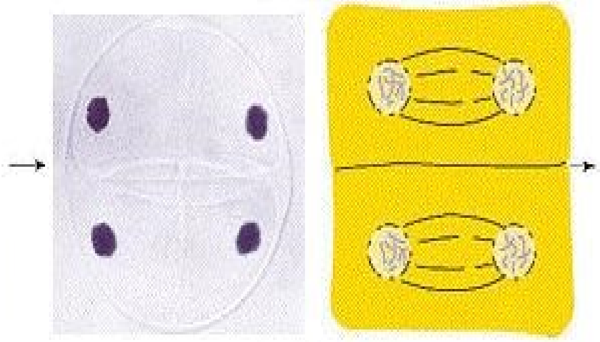

(i) Telophase I

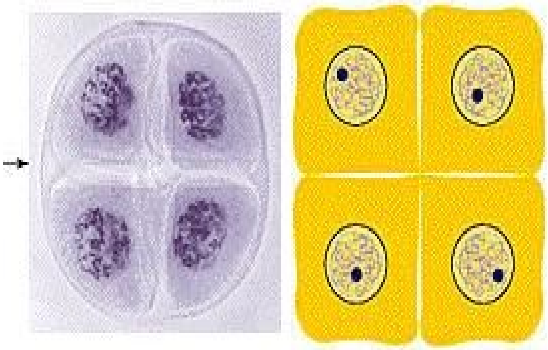

(j) Interphase

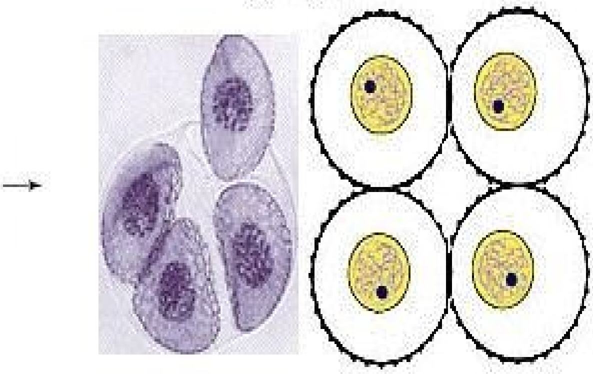

(k) Prophase II

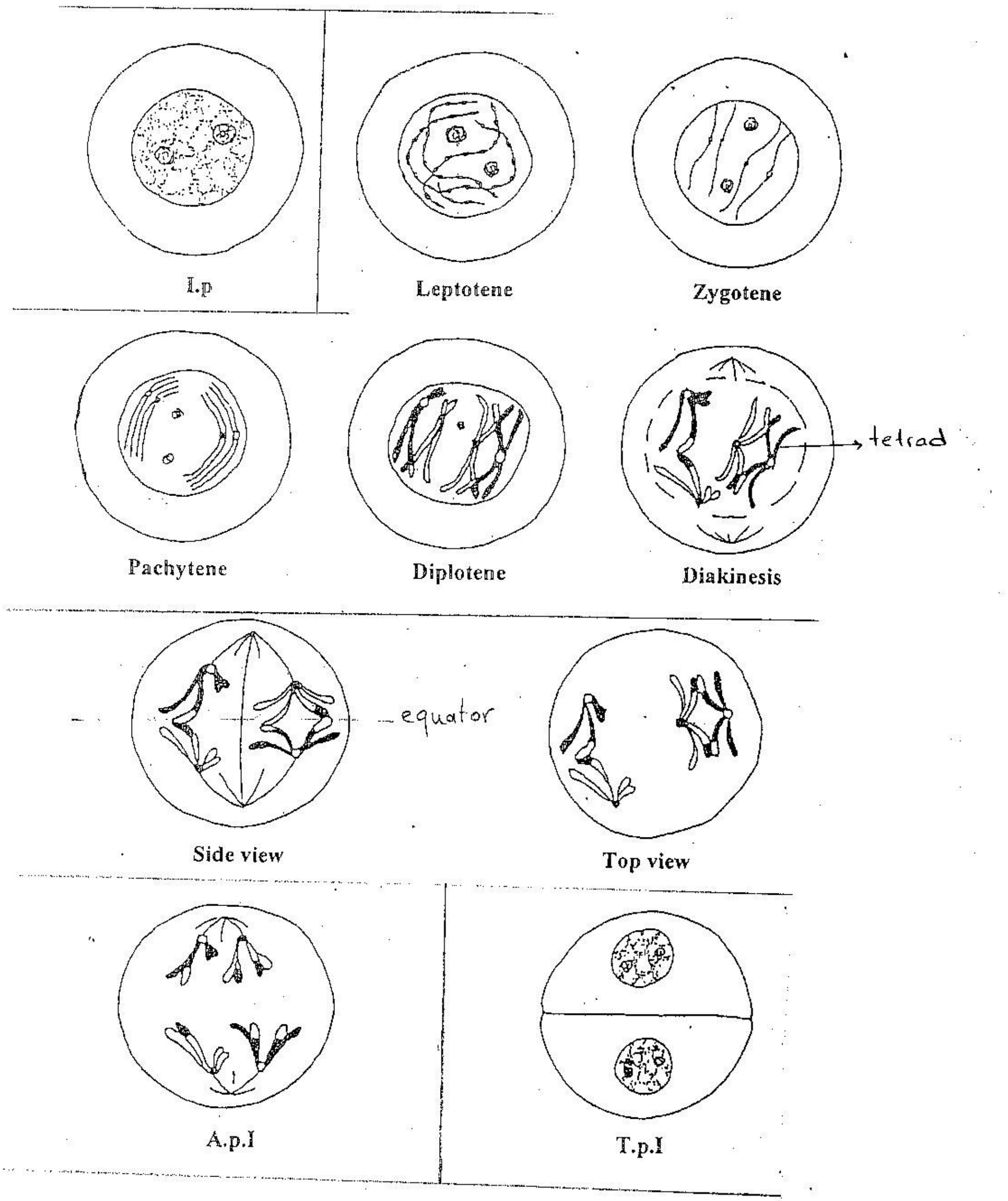

(l) Metaphase II


(m) Anaphase II

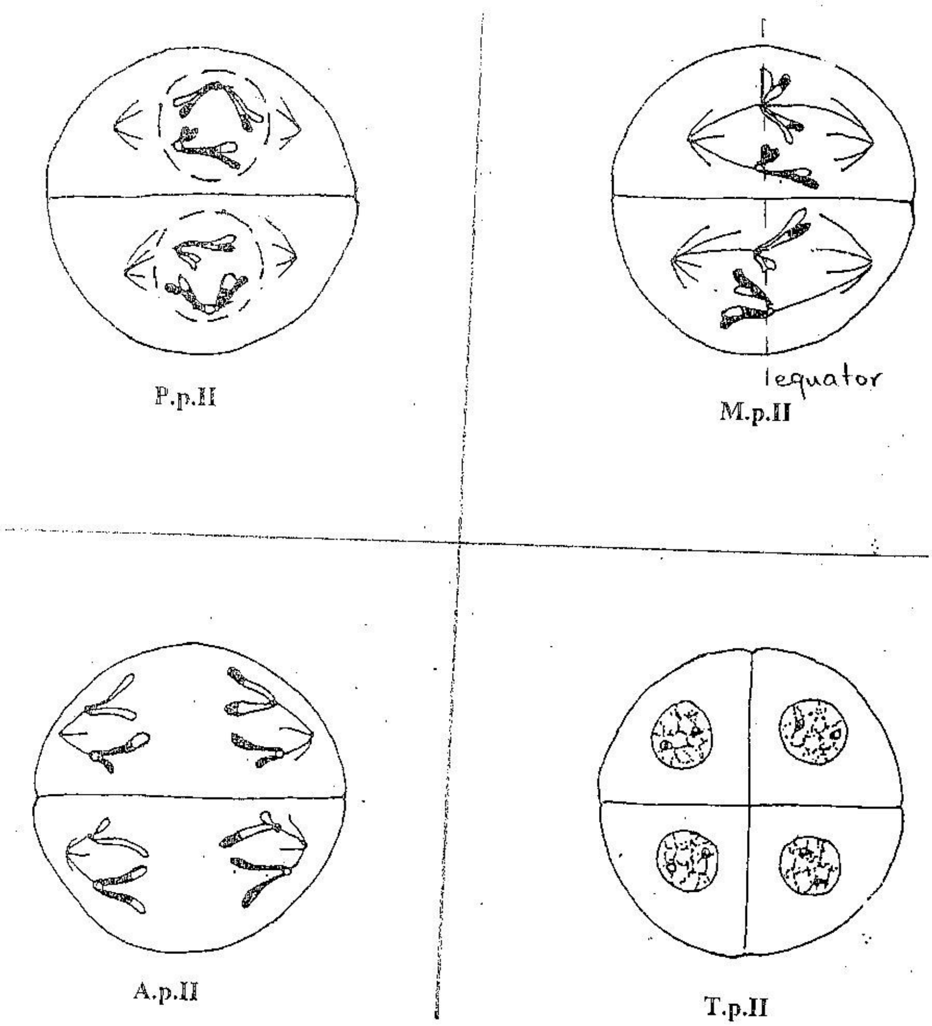

(n) Telophase II


(o) The tetrad


(p) Young pollen grains



Meiosis, stages of the first division.



Meiosis, stages of the second division.

Student name: **Code number:**

STUDENT'S ASSINMENT

Give it to your laboratory instructor

1. Choose the correct answer of the following:

- The formation of a cell plate is beginning across the middle of a cell and nuclei are reforming at opposite ends of the cell. What kind of a cell is this?
  a. An animal cell in metaphase
  b. An animal cell in telophase
  c. An animal cell undergoing cytokinesis
  d. A plant cell in metaphase
  e. A plant cell in metaphase
  f. A plant cell undergoing cytokinesis

- Synapsis and crossing over occurs during which of the following phases of meiosis
  a. Interphase
  b. Prophase I
  c. Anaphase I
  d. Prophase II
  e. Telophase II

- If there are 12 chromosomes in a plant cell in the G1 stage of the cell cycle, what is the diploid number of chromosomes for this organism?
  a. 6
  b. 12
  c. 24
  d. 36

e. 48

- Which of the following is false in comparing prophase I of meiosis and prophase of mitosis?
  a. The chromosomes condense in both.
  b. Tetrads form in both
  c. The nuclear envelope disassembles in both
  d. A spindle forms in both
  e. Each chromosome has two chromatids in both

- The process of male gamete formation is called

A. oogenesis. B. spermatogenesis. C. cytokinesis.

- The chromatids of a tetrad wrap around each other, break, and rejoin resulting in the exchange of genes. This is called

A.synapsis. B. mitosis. C. crossing-over. D. meiosis.

- What is needed for fertilization?

A. sperm only B. egg only C. both sperm and egg

- A cell produced by meiosis has the ____ number of chromosomes.
  a. diploid
  b. haploid
  c. triploid
  d. double

- In plants, meiosis occurs within the special reproductive structures called

a. flowers.
b. gonads.
c. hospitals.
d. Punnett squares.

- Complex organisms produce sex cells that unite during fertilization, which forms a single cell known as
    - A. a gonad.
    - B. a zygote.
    - C. an embryo.
    - D. a gamete.

2. True or False

____ Meiosis occurs in animals but does not occur in plants.

____ Meiosis reduces the chromosome number from diploid to haploid.

____Four chromatids of a homologous chromosome pair are a tetrad.

____Meiosis occurs in animals but does not occur in plants.

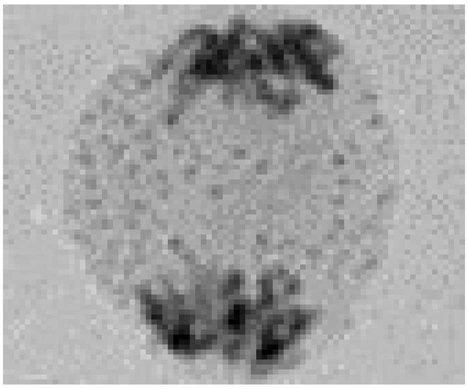
____Meiosis reduces the chromosome number from diploid to haploid.

3. At the end of Meiosis I, are the cells haploid or diploid?

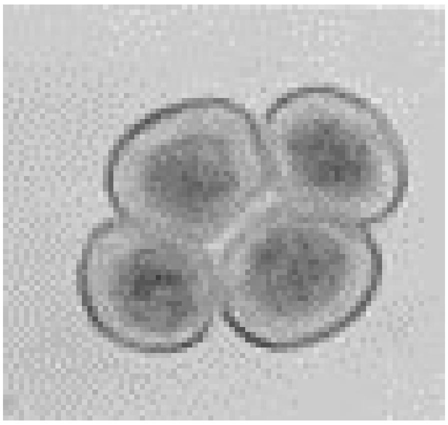
………………………………………………………………………………

4. Circle the correct option to identify the phases of meiosis depicted below.

a. Prophase I
b. Anaphase II
c. Telophase I
d. Metaphase I



a. Prophase I
b. Metaphase I
c. Anaphase I
d. Telophase II



a. Prophase II
b. Metaphase II
c. Anaphase II
d. Telophase II

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5. What happens to the chromosome number during meiosis?
   a. It doubles
   b. It stays the same
   c. It halves
   d. It becomes diploid

LINKAGE AND CHROMOSOME MAPPING IN EUKARYOTES

1. The independent assortment of two genes located on different chromosomes.

Mendel's Law of Independent Assortment: during gamete formation, segregation of one gene pair is independent of other gene pairs because the traits he studied were determined by genes on different chromosomes. Consider two genes, each with two alleles A a and B b on separate (different) chromosomes.

50% parental : 50% recombinants

Gametes of non-homologous chromosomes assort independently at anaphase producing 4 different genotypes AB, ab, Ab and aB with a genotypic ratio 1:1:1:1. So, when genes are on different chromosomes, **50%** of the gametes produced by a doubly-heterozygous individual are **recombinant**, when compared to the gametes produced by its parents (50% are **parental)**.

2. If two genes occur on the same chromosome: they may not assort independently at anaphase of meiosis. These genes are said to be linked and demonstrate linkage in genetic crosses.

The two genes are on the same chromosome (NO CROSSING OVER):

100% parental gametes

If there were no **crossing over**, all the alleles on a single chromosome would segregate together and would end up in the same gamete. This means that **A and B are linked.**

The two genes are on the same chromosome (WITH CROSSING OVER):

50% parental : 50% recombinants

With crossing over, we get **recombination** of alleles on the same chromosomes. Since crossover occurs in the 4-strand stage of meiosis, and involves only two of the four chromatids, each crossover event results in **50% recombinant gametes, and 50% parental gametes**.

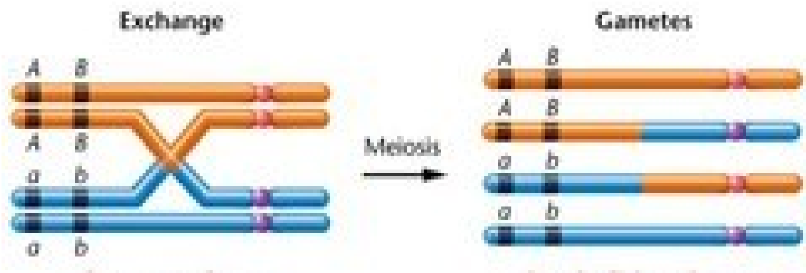
Genetic Mapping

Genetic or chromosome mapping: Graphic representations of the spatial (position) relationships between linked genes on a chromosome.

Genes with recombination frequencies less than 50% are present in the same chromosome (linked). Two genes that undergo independent assortment, indicated by a recombination frequency of 50 percent, are either on non-homologous chromosomes or are located far apart in a single chromosome. However crossing over does not occur between linked genes in every meiotic event, especially when the positions of the genes on the chromosome are very near one another.

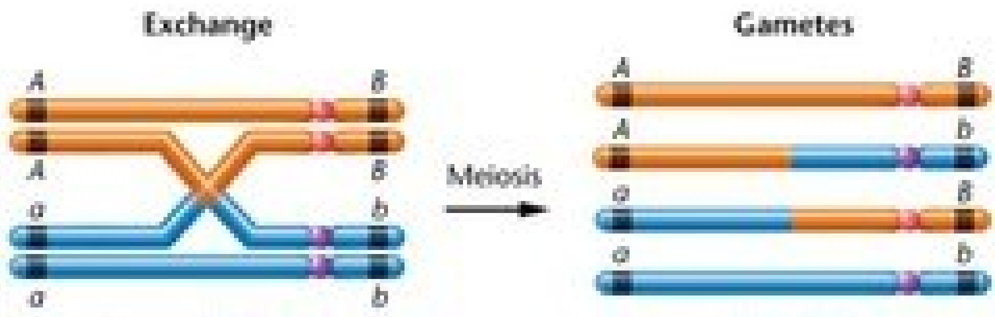
The frequency with which crossing over occurs between any two linked genes is proportional to the distance between the loci along the chromosome:

1. At very small distances, crossover is very rare = most gametes are parental.



Segments of the 2 non-sister chromatids are exchanged without changing the linkage between the alleles AB and ab. By crossing over, all the alleles on a single chromosome would segregate together and would end up in the same gamete. **A and B are linked forming 100% parental gametes.**

2. As the distance between two genes increases, crossover frequency increases=More recombinant gametes, fewer parental gametes.
3. When genetic loci are very far apart on the same chromosome, crossing over nearly always occurs = the frequency of recombinant gametes approaches 50%.



Segments of the 2 non-sister chromatids are exchanged, changing the linkage between the alleles AB and ab. By crossing over, 4 different genotypic gametes are formed AB, Ab, aB and ab forming 50% parental and 50% recombinant gametes.

How do we determine how much crossing-over occurs between linked genes (and therefore get an idea of how far apart two loci are)? The answer is critical, because this is the first step in constructing a genetic map.

Map units

When large numbers of mutations are available for a species, genes on the same chromosome will show evidence of linkage to one another (<50% recombination frequency). Genes will fall into **LINKAGE GROUPS.** The number of linkage groups will equal the haploid number of chromosomes. The linkage of genes on a chromosome can be represented on a genetic map (linkage map, chromosome map). A genetic map shows the linear order of the genes along a chromosome with distance **proportional to the frequency of recombination.** Unit of distance in linkage map is a map unit.

1 map unit (mu) or centimorgan (cM) is equal to 1% recombination.

So, two genes that recombine with a frequency of 1% are said to be 1 map unit or 1 centimorgan apart.

A map unit is also equivalent to the physical distance along a chromosome which will experience 1 crossover event in every 50 meiotic divisions

1 crossover in 50 meiotic divisions = 1 recombinant gametes in every 100 = 1 % recombination

How to calculate the recombination frequency?

Recombination Frequency (%) = (# of recombinant gametes/Total # of gametes) X 100

How to Solve Linkage Map Problems

I. All linkage map distances are calculated as the percentage recombination between two loci. This is reflected in the percentage of recombinant offspring.

A. If two genes are completely linked, all of the offspring will be parental (have the same linkage as the parents); there will be no recombinant offspring. Thus the linkage map distance between the two genes would be zero centimorgan (cM) or Linkage Map Units (LMU).

B. If the genes are on separate chromosomes (or are far enough apart that crossing over between them occurs in virtually all meiosis), half of the offspring will be recombinant, the other half parental. Thus the distance between them would calculate as 50 cM, and 50 cM reflects independence.

C. Linkage Map Units are not additive, and there is no physical distance which is equal to one LMU.

1. Locations of crossovers are not random; crossovers are repressed close to the centromere, and the presence of one crossover will repress a second one close by.
2. Some crossover events are always missed because it is possible that multiple crossovers have occurred between the two points you are mapping; thus you will underestimate the total amount of recombination. The distance calculated between two genes will always be somewhat dependent upon how many interior points you are monitoring. (ie, you will calculate a greater distance between two genes if you are doing a three point cross including a gene between the two in question than if you are doing a simply two point cross.

II. Straightforward calculation of the percent recombination between two genes is called a two point cross.

A. To perform a mating cross, mate a parent who is heterozygous for both genes to one who is homozygous recessive for both genes. Thus the recombination in the offspring will reflect crossing over of only one parent, reducing the complexity of the problem.

B. X linked genes can be mapped by mating a female heterozygous for both genes in question to any male, then scoring only the male offspring, which will directly reflect what happens to the female's X chromosomes.

C. Establish linkage by determining that the results of the mating do not indicate independence.

D. Calculate the map distance between the two genes by calculating the percentage of the offspring which have recombined the two genes.

III. Most useful linkage mapping is done with three point crosses, which involve mapping three genes relative to each other. The results of many three point crosses can be combined together to create an overall map of many genes.

A. Again, a useful mating for mapping would be between a parent heterozygous for all three genes and a homozygous recessive partner, or for X linked genes, between a triple heterozygous female and any male. Unless you have complete linkage, you will get eight phenotypic classes of offspring.

B. Confirm linkage by determining that the results of the cross do not meet expectations for independence.

C. Identify parental offspring and double crossover offspring. The most numerous offspring classes will be the parental classes. There should be two, and they should be reciprocals. The least numerous classes should be the double crossover classes (those that resulted from crossing over between both pairs of genes). Again, there should be two of these classes, and they should be reciprocals.

D. Determine gene order by determining which of the three genes is in the middle. This is done by comparing the parental and double crossover classes. Since the double crossovers crossed over between the first and second genes, then crossed back between the second and third genes, the only gene which is actually recombined is the middle one.

1. Select one parental class, then choose the double crossover class which is most like it. They should differ only in one gene. This is the middle gene.

2. Examine the other parental and the other double crossover; they should give you the same result.

E. Rewrite all of your offspring phenotypic classes, putting the genes in the correct order. It doesn't matter which end gene you decide is the "first" gene, but you must get the correct one in the middle.

F. You have four single crossover classes. Two of them resulted from crossing over between the first two genes; the other two from crossing over between the second and third genes. Figure out which of these are which, again by comparing them to the parental classes which are most like them. If a single crossover class differs from a parental class only in the first gene, then it represents a crossover between the first and second genes. If it differs only in the last gene, then it represents a crossover between the second and third genes. Again, you should have to pairs of reciprocal phenotypes.

G. Calculate the distance between the first two genes by calculating the percentage of your offspring that recombined those two genes. Remember that the double crossovers count as recombinants here.

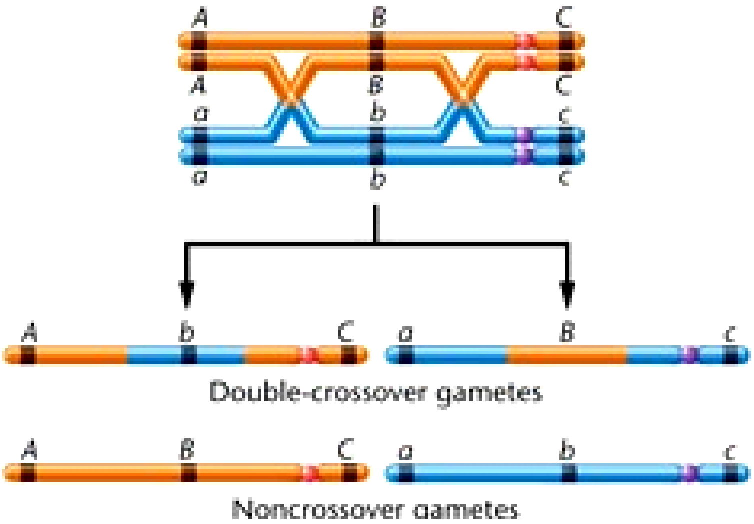
H. Calculate the distance between the second and third genes by calculating the percentage of your offspring that recombined those two genes. Again, the double crossovers count here.

I. The distance between the end genes can be calculated simply by adding the two intervening distances.

IV. Note that the distance you calculated between your end genes is different from the distance you would have calculated if you were simply doing a two point cross. You would have scored only the single crossovers as recombining the end genes; the double crossovers—each of which actually represents two crossovers between those end genes—would have been scored as parentals, since the end genes are still in the parental association.

Multiple Crossover events

Unless genes are very close together, more than one crossover event can occur in a single meiotic division. **Multiple crossovers can make mapping inaccurate if genes are far apart on the same chromosome.**



WORK SHEET

Problem 1.

Long stem (A) is dominant While short stem (a) is recessive

Rounded seed (B) is dominant While wrinkled seed (b) is recessive

Smooth stem (C) is dominant While rough stem (c) is recessive

A heterozygous trihybrid corn plant with Long stem, Rounded seed and Smooth stem was test crossed.

The Results of F1 was:

410 Long stem, Rounded seed and Smooth stem

410 short stem, wrinkled seed and rough stem

50 Long stem, wrinkled seed and rough stem

50 short stem, Rounded seed and Smooth stem

40 Long stem, wrinkled seed and Smooth stem

40 short stem, Rounded seed and rough stem

Construct a chromosome map showing the relative position for these genes.

Answer:

<u>Problem 2.</u>

Long stem (A) is dominant While short stem (a) is recessive

Rounded seed (B) is dominant While wrinkled seed (b) is recessive

Smooth stem (C) is dominant While rough stem (c) is recessive

A heterozygous trihybrid plant with Long stem, Rounded seed and Smooth stem was test crossed.

The Results of F1 was:

415 Long stem, Rounded seed and Smooth stem

415 short stem, wrinkled seed and rough stem

35 Long stem, wrinkled seed and Smooth stem

35 short stem, Rounded seed and rough stem

45 Long stem, wrinkled seed and rough stem

45 short stem, Rounded seed and Smooth stem

5 Long stem, Rounded seed and rough stem

5 short stem, rough seed and Smooth stem

Construct a chromosome map showing the relative position for these genes.

Answer:

Problem 3.

Long stem (A) is dominant While short stem (a) is recessive

Rounded seed (B) is dominant While wrinkled seed (b) is recessive

Smooth stem (C) is dominant While rough stem (c) is recessive

A heterozygous trihybrid corn plant with Long stem, Rounded seed and Smooth stem was test crossed.

Among 1000 Plant of F1the following new combinations resulted

50 Long stem, wrinkled seed and rough stem

50 short stem, Rounded seed and Smooth stem

40 Long stem, wrinkled seed and Smooth stem

40 short stem, Rounded seed and rough stem

Calculate the percentage of parental combinations.

Answer:

Problem 4.

Long stem (A) is dominant While short stem (a) is recessive

Rounded seed (B) is dominant While wrinkled seed (b) is recessive

Smooth stem (C) is dominant While rough stem (c) is recessive

A heterozygous trihybrid plant with Long stem, Rounded seed and Smooth stem was test crossed.

The Results of F1 was 1000 plant of which:

40 Long stem, wrinkled seed and Smooth stem

40 short stem, Rounded seed and rough stem

Calculate the distance between A and C knowing that the distance between A and B is 18 centimorgan and C lies between A and B.

Answer:

Problem 5.

Long stem (A) is dominant While short stem (a) is recessive

Rounded seed (B) is dominant While wrinkled seed (b) is recessive

Smooth stem (C) is dominant While rough stem (c) is recessive

A heterozygous trihybrid corn plant with Long stem, Rounded seed and Smooth stem was test crossed.

The Results of F1 was 1000 plant:

Y_1 Long stem, Rounded seed and Smooth stem

Y_2 short stem, wrinkled seed and rough stem

35 Long stem, wrinkled seed and Smooth stem

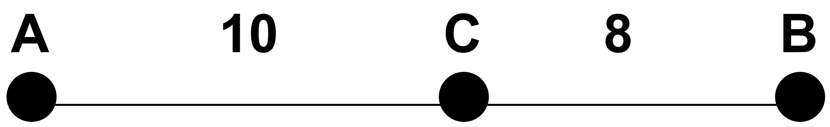
35 short stem, Rounded seed and rough stem

45 Long stem, wrinkled seed and rough stem

45 short stem, Rounded seed and Smooth stem

X_1 Long stem, Rounded seed and rough stem

X_2 short stem, wrinkled seed and Smooth stem



Using the previous diagram Find X_1+X_2 and Y_1+Y_2

Answer:

Problem 6.

Long stem (A) is dominant While short stem (a) is recessive

Smooth stem (C) is dominant While rough stem (c) is recessive

Using the previous diagram what are the predicted ratios for phenotypes result from selfcross of heterozygous dihybrid corn plant having long smooth stem?

Answer:

Problem 7.

Long stem (A) is dominant While short stem (a) is recessive

Rounded seed (B) is dominant While wrinkled seed (b) is recessive

Smooth stem (C) is dominant While rough stem (c) is recessive

A heterozygous trihybrid corn plant with Long stem, Rounded seed and Smooth stem was test crossed.

The Results of F1 was:

225 Long stem, Rounded seed and Smooth stem

225 short stem, Rounded seed and Smooth stem

225 Long stem, wrinkled seed and rough stem

225 short stem, wrinkled seed and rough stem

25 Long stem, wrinkled seed and Smooth stem

25 short stem, wrinkled seed and Smooth stem

25 Long stem, Rounded seed and rough stem

25 short stem, Rounded seed and rough stem

Which of these genes are linked? If so, what is the distance between them?

Answer:

Student name: **Code number:**

STUDENT'S ASSINMENT

Give it to your laboratory instructor

Choose the correct answer of the followings:

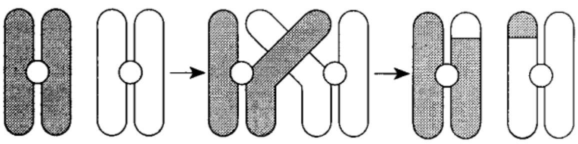
- Which process is illustrated in the diagram below?

  a. diffusion

  b. replication

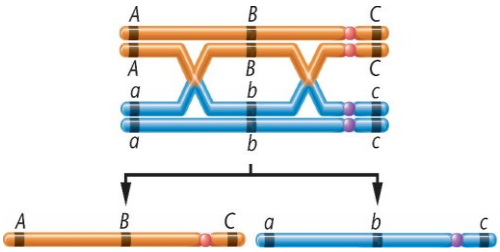
  c. mitosis

  d. crossing over



- If two genes on the same chromosome exhibit complete linkage, what is the expected F2 phenotypic ratio from a selfed heterozygote with the genotype AaBb?
  a) 1:2:1
  b) 1:1:1:1
  c) 1:1
  d) 9:3:3:1
  e) 3:1

- The gametes produced below result from ________ crossover.
  a) double
  b) single
  c) undetected
  d) no
  e) reciprocal



Problem 1.

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45 short stem, Rounded seed and Smooth stem

5 Long stem, Rounded seed and rough stem

5 short stem, wrinkled seed and Smooth stem

Did the result show evidence for double crossing over? Determine the genes bordering the chromosome segment in which double crossing over occurred and the length of this segment.

Answer:

Problem 2.

Long stem (A) is dominant, while short stem (a) is recessive.

Rounded seed (B) is dominant, while wrinkled seed (b) is recessive.

Smooth stem (C) is dominant, while rough stem (c) is recessive.

A heterozygous trihybrid corn plant with Long stem, Rounded seed and Smooth stem was test crossed.

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40 short stem, Rounded seed and rough stem

Construct a chromosome map showing the relative position for these genes.

Answer: