CHROMOSOME STRUCTURE, MEIOSIS, AND GENETICS

Pre-lab Preparatory Information

Know this material prior to attending your lab. You may be quizzed on this material.

Objectives: by the end of the lab, you will be able to…

…describe the detailed structure of a chromosome.

…explain the process of meiosis itself, identify the stages microscopically and explain its significance to genetics.

…use your knowledge of chromosomes and meiosis to analyze a human karyotype for abnormalities such as Kleinfelter's Syndrome.

…collect the quantitative data used in genetic analyses including monohybrid and dihybrid crosses.

…conduct and interpret the results of a statistical method used to determine whether observations conformed to predictions.

…explain how meiotic events lead to independent assortment.

…solve genetics problems including monohybrid and dihybrid crosses and X-linked inheritance.

A. Meiosis

As you have seen in the plant evolution labs, meiosis is the second important kind of nuclear division; recall that its etymology is "to lessen." Meiosis resembles mitosis in many ways, but the consequences of meiotic divisions are very different from those of mitotic divisions. While mitotic division may occur in almost any living cell of an organism, meiosis occurs only in special cells. In animals, meiosis is restricted to cells that form gametes (eggs and sperm).

Normal condition: Each species has a characteristic number of chromosomes per somatic cell. Fruit flies have 8; normal humans have 46. They exist as **homologous** pairs (partners) that are similar in size and shape and carry the same kinds of genes. Thus humans have 23 homologous pairs. The full complement of 46 chromosomes is referred to as the **diploid** number (referring to the fact that each kind of chromosome is represented twice). In plants and animals, when an egg is fertilized, the egg and sperm fuse to form a single cell called a **zygote** that develops into a new organism.

haploid egg + haploid sperm $\xrightarrow{\text{gamete fusion}}$ diploid zygote

In-Laboratory Exercises:

I. Chromosome Structure: A Review

Mitosis in Onion Root Tips

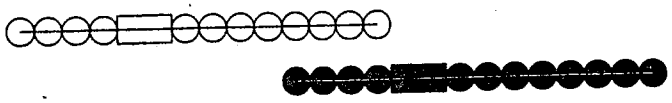
1. Study a prepared slide of onion root tips. Locate the four stages of mitosis.

2. Using a high power lens, carefully observe the chromosome structure during metaphase. Note that each chromosome is made of two chromatids. Also note that although each chromatid has a centromere, the location of this structure varies with different chromosomes.

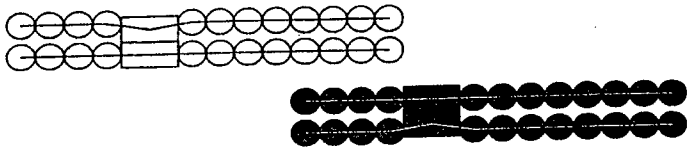
II. Meiosis

A. Artificial Chromosomes:

Begin as you did when you constructed artificial chromosomes to study mitosis, by making a **homologous pair** of chromosomes (one red, one yellow) that is unduplicated. Also draw a large round cell and nucleus with chalk on your lab benchtop.



Premeiotic S phase (during the premeiotic interphase). Construct ("synthesize") a sister chromatid for each chromosome.

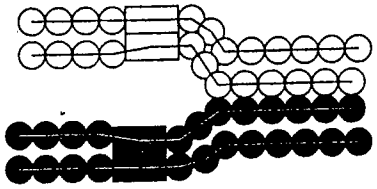


---------- **Meiosis I** (the 1st meiotic division) ----------

Prophase I - very long and complex-divided into distinctive substages.

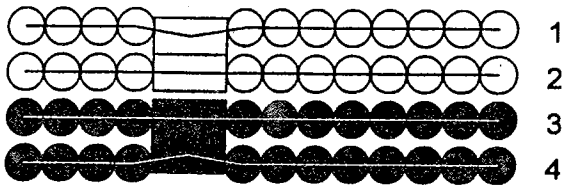
1. **Leptotene** ("thin band") - The chromosomes start to become visible as they condense.

2. **Zygotene** ("yoked band") - This is the first indication that something unusual is happening. The homologous chromosomes begin to pair (synapse) with one another along their lengths. You can demonstrate this process using your artificial chromosomes.



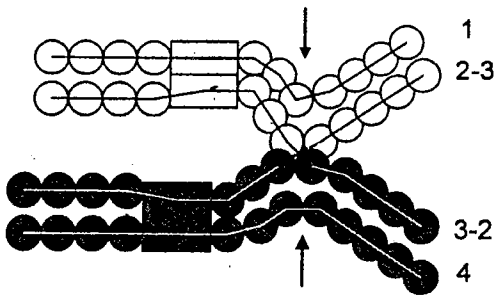
A pair of homologous chromosomes in the process of synapsis

3. **Pachytene** ("thick band") - the chromosomes are now fully synapsed along their lengths. Demonstrate this using your artificial chromosomes. The resulting structure is called a tetrad because it consists of four chromatids (2 chromosomes with two chromatids each).



pachytene tetrad

4. **Diplotene** ("double band") - The paired chromosomes show characteristic kind of "looped out" appearance. The homologous chromosomes appear to be "repelling" one another except at special regions of contact. You can demonstrate this with your artificial chromosomes.



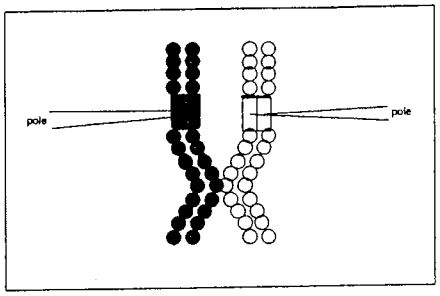
The three-dimensional nature of this "tetrad of chromatids" is more easily observed using clay. **Carefully examine the clay model on the demonstration table.**

What about the special region of contact? This is a place where two non-sister (homologous) chromatids (#2 and #3 in our example) have broken in corresponding locations and have become rejoined so that the chromosomes have now exchanged genetic material. This process is called crossing over and is manifested as the X shaped region of contact known as a chiasma (pl. chiasmata) shown at the arrows. Most of the available evidence is consistent with the view that crossing over occurs at pachytene, but we are unaware of it until the chiasmata become evident at diplotene.

5. **Diakinesis** - The chromosomes become shorter and the thicker and homologous chromosomes still appear to be "repelling" one another. However, the tetrads do not fall apart because they are held together at the regions of the chiasmata. At the end of diakinesis the nuclear envelope breaks down (so erase the one you made with chalk!)

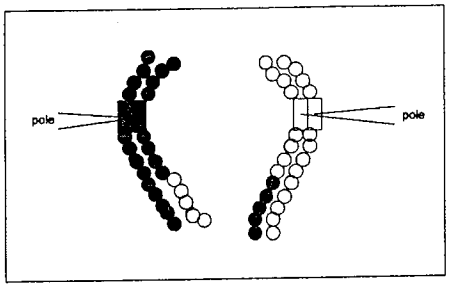
Metaphase I

The **tetrad** of chromatids lines up on the equatorial plane (metaphase plate). In contrast to the situation in mitotic metaphase, the sister centromeres are oriented to the same pole and homologous centromeres are oriented to opposite poles. You may demonstrate this with your artificial chromosomes.



Anaphase I

The homologous centromeres move to opposite poles and the chromatids trail behind forming a "double" V configuration because the sister centromeres adhere to one another.



Telophase I

The chromosomes reach the poles and in most organisms decondense. The nuclear envelopes are formed and cytokinesis often occurs to form 2 cells. In your artificial example how many chromosomes does each cell contain? How many chromatids?

Meiotic interphase may be long, brief, or simply non-existent, but the important thing to remember is that **no DNA synthesis occurs.**

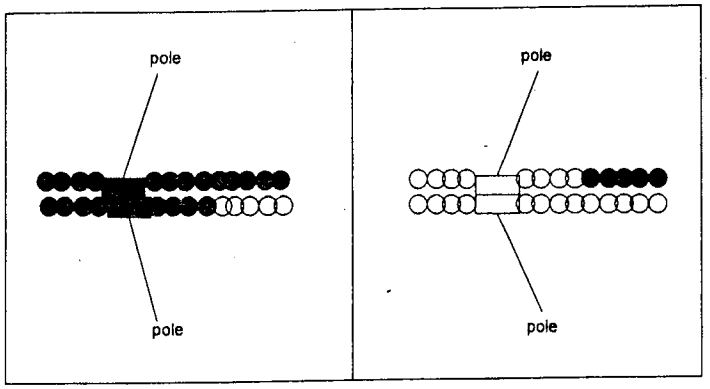
---------- **Meiosis II (the 2nd meiotic division)** ----------

Prophase II

The chromosomes again become visible and we see that their structure is very similar to the structure of mitotic chromosomes. At the end of prophase II nuclear envelope disappears and the spindle fibers attach to the centromeres.

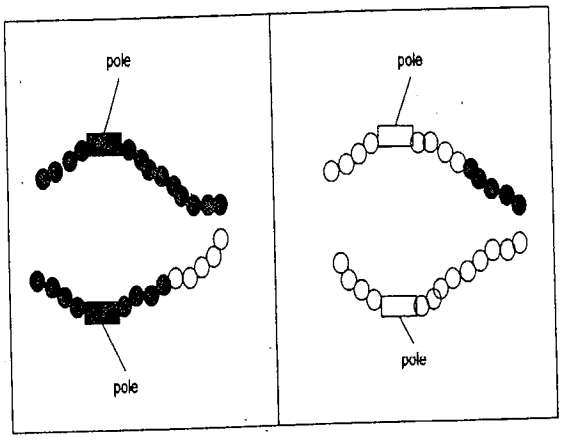
Metaphase II

The chromosomes line up on the metaphase plate. Note that, as mitotic metaphase, the sister centromeres are attached by spindle fibers to opposite poles.



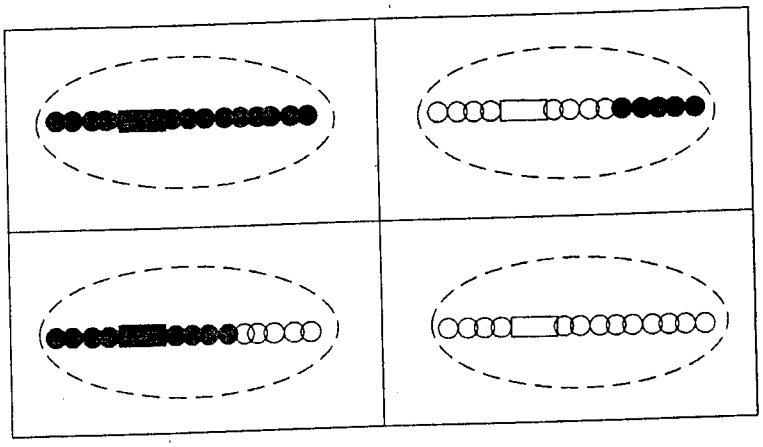
Anaphase II

The sister centromeres move to opposite poles and the attached chromatids are carried long, trailing out behind in a v-shape.



Telophase II

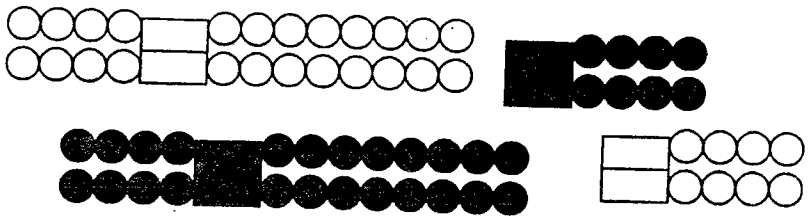
The chromosomes now reach the poles and decondense. In our example each nucleus contains a single unreplicated chromosome. Demonstrate this using your artificial chromosomes. Is each nucleus now haploid or diploid? Has the purpose of meiosis been accomplished? Has the ploidy been "lessened?"



[NOTE: For plants, this is now called a "tetrad of microspores" -- not to be confused with a "tetrad of chromatids" in Prophase I.]

2a. Get four more "centromeres" and enough pop-it beads to make a second pair of homologous chromosomes. To distinguish them from the first pair, make this pair much shorter and put the "centromere" at the end. Start at the end of premeiotic S phase. Remember, one chromosome must be made of all red pop-it beads (= genes) with two identical strands

(= sister chromatids). The other must be all yellow (= alternative alleles) but is the same size and shape as the red chromosomes.

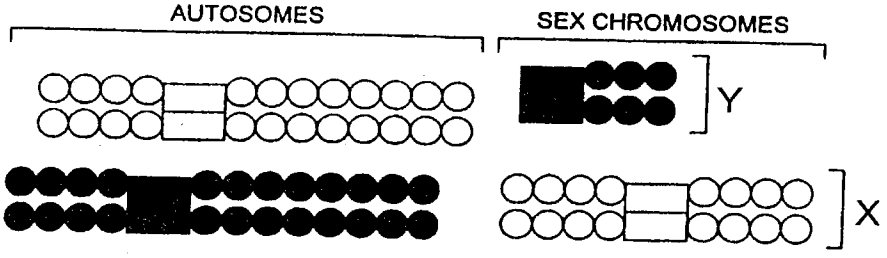


For this series, we shall skip the events in prophase I, assuming no crossing over. Line up the chromosomes as in metaphase I with both yellow chromosomes on one side and both red chromosomes on the other. DRAW what you see.

Continue through meiosis I and II, stopping at each phase to DRAW the distribution of chromosomes. At the end, note that two haploid cells have all red chromosomes; the other two, all yellow.

2b. Now return the chromosomes to their arrangement in metaphase I, but this time put one yellow and one red chromosome on one side and the other two on the other. Continue through meiosis I and II, drawing each stage. Note the distribution of chromosomes (and therefore, alleles) in each haploid cell formed at the end of meiosis.

3. Obtain some more red pop-it beads, enough to have a few on each side of the "centromere" of the shorter red chromosome. You now have two long homologous chromosomes that we'll now identify as autosomes. The shorter, yellow chromosome is the X chromosome; the dinky red chromosome, the Y chromosome.

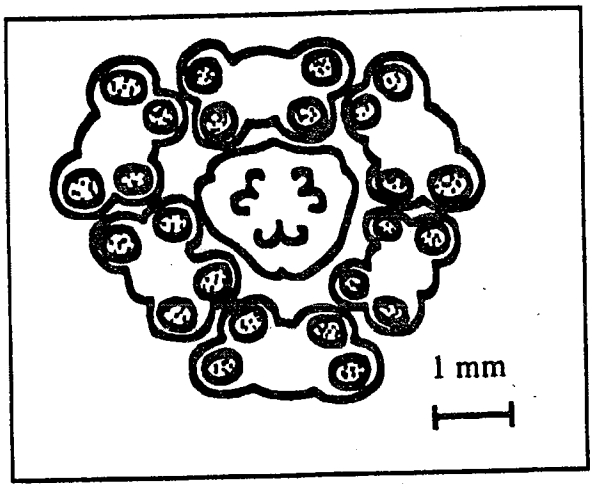


Move the chromosomes through meiosis one more time, noting how the sex chromosomes are segregated. In this instance, continue through the sexual cycle through fertilization to remind you that the genes segregated in meiosis subsequently come together at zygote formation.

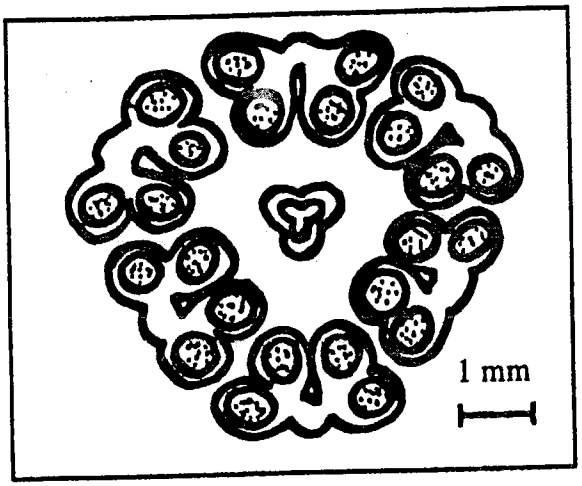
Are the X and Y chromosomes homologous? Why or why not?

B. **Prepared slides:** Lily (*Lilium*) anthers

Figure 10-1. Cross sections through lily flower at level of anthers.



A. Section through six anthers with ovary in center.



B. Section through six anthers with style in center.

Briefly review the structure of the flower (Lab 8). The stamen is composed of the anther and filament; there are six in the monocot lily. Each anther has two pair of microsporangia (pollen sacs). Each has many microsporocytes that go through meiosis to form four microspores. The microspore nucleus then divides by mitosis to form the pollen grain (microgametophyte).

Early in meiosis, the filament is rather short so that the anther is down by the ovary portion of the pistil (Fig. 10-1A). In the pistil you can see three pair of ovules. You might even see a megasporocyte, a large cell with a large nucleus and a bright red nucleolus.

As the cells in the anther go through meiosis the filament elongates so that by the end of meiosis the anther is beside the style (Fig. 10-1B). By the time the pollen is released, the anthers are near the top of the flower (Fig. 8-1). The length of the filament in lily can be used to predict whether the anther is in meiosis or mitosis.

Although the phases of meiosis can be observed most plants and animals, the anther is usually used because of the ease in which the materials can be obtained. Again, remember that you are looking at a two-dimensional slice of three-dimensional reality, so that the whole cell cannot be seen in each section. (Squashes would be preferable, as used for the photographs in your textbook, but they are not available commercially.)

Don't forget that you can test your ability to recognize the different phases of meiosis at the VIRTUAL LABORATORY FOR BIOLOGY 101.

Meiosis in the anther starts with the diploid **microsporocyte** ("microspore mother cell"). Each nucleus has a diploid number of duplicated chromosomes. (You may wish to refer again to Fig. 2.1A in Lab 2.) As illustrated in Fig. 10.2A, these cells are still attached in the microsporangium. In each of the following diagrams, traced from actual sections, the mottled green materials in the cytoplasm have been omitted for clarity. All of the diagrams are drawn to approximately the same magnification.

Obtain a slide labeled "early meiosis." Examine each of the microsporangia, two pairs in each anther. Depending upon the slide, you may see any of the following stages of meiosis.

If the cells are in **early prophase I** (Fig. 10.2B), the chromosomes will be long and slender, and the bright red nucleoli will still be evident (at least in sections through some of the cells). The nuclear region will still be clear, compared to the green cytoplasmic region around each nucleus.

If the cells are in **late prophase I** (Fig. 10.2C), the short, thick chromosomes are highly condensed. The nucleoli are missing, but the nuclear region may still be clear.

Once the cells are in **metaphase I** (Fig. 10.2D), the short, thick chromosomes will have lined up across the equatorial plane, or "metaphase plate." If you focus carefully with the condenser diaphragm closed enough to optimize contrast, you may see the spindle fibers. However, you will only get this image for cells where the section is at a right angle to the metaphase plate (cells to right in Fig. 10.2D). If the section is parallel to the metaphase plate, the cells will appear similar to those in prophase I (compare cells to left of Fig. 10.2D to those in Fig. 10.2C). Fortunately, the cells in each microsporangium are fairly synchronous, so if you see one cell that is in metaphase I, they are all in metaphase I. Therefore, you must look at all of the cells in a cavity to recognize the specific phase of meiosis!

If there is a slide labeled "late meiosis I", you may see the following.

Remember that the homologous chromosomes separate (disjoin) in **anaphase I**, with each resulting cell getting one complete set of chromosomes. This will be evident in some of the cells sectioned, as near the center in Fig. 10.2E. Most of the other cells will only have fragments -- or no chromosomes at all.

By **telophase I** (Fig. 10.2F), the chromosomes start to decondense in the nuclear regions and a new cell plate forms between them. This new cell wall (arrows) forms within the microsporocyte cell wall. Remember, that you are observing sections, so that the new wall will not be apparent in each cell. Each nucleus now has a haploid number of chromosomes, and each chromosome is still made of two chromatids. (Each chromosome is still duplicated; recall Fig. 2.1C from Lab 2).

Since there is little time in lily between meiosis I and meiosis II, the cells in telophase I resemble those in **prophase II**. Remember that there is no DNA replication between meiosis I and meiosis II.

Next examine a slide labeled "meiosis II." Once again, examine each of the microsporangia. Depending upon the slide and the microsporangium, you may see any of the following stages of meiosis.

By this time, the cells in each microsporangium are less synchronous, so you may see more than one phase in each region. During **metaphase II**, the chromosomes once again line up along the metaphase plate. In this case, the new plate is usually at a right angle to the cell wall formed in telophase I. If you look long enough and hard enough, you will see a section through at least one cell that shows this alignment (open arrow in Fig. 10.2G). In a section through another cell (lower left in Fig. 10.2G) you may see that the sister chromatids have begun to separate (disjoin) in **anaphase II.** However, the presence of the cross wall in some of the cells clearly indicates meiosis II.

Finally, during **telophase II**, the chromosomes once again decondense and a new cell plate forms at a right angle to the one formed in telophase I -- all enclosed in the original microsporocyte cell wall. This final tetrad of **microspores** signals the end of meiosis, with each of the four nuclei having one set of unduplicated chromosomes (as in Fig. 2.1D). Again, it is unlikely that a section will be through all four cells. But as you see three cells, the other cell was part of the original three-dimensional structure.

Recall that in lily, each of these microspores separates, and each nucleus divides by mitosis to form an individual mature pollen grain (Lab 8).

Review:

Anaphase I: homologous chromosomes separate (disjoin)

Anaphase II: sister chromatids separate (disjoin)

Segregation of alleles on nonhomologous chromosomes depends upon how the chromosomes line up in metaphase I.

Recombination of linked genes occurs when there is an exchange of segments between chromatids of homologous chromosomes in Prophase I; the sister chromatids (that are no longer identical) are separated in Anaphase II.

If you have difficulty finding the above phases of meiosis, a video tape on *Cell Structure and Function, Mitosis, and Meiosis,* produced by a Rutgers undergraduate biology major, is available in the MSLC. Simply fast-forward to the meiosis section and review the same slides you saw in lab. Then check out a slide set at the MSLC and try again. Or use the TV camera set up with one of the microscopes in the MSLC and go over the slides as a group!

III. Human Chromosomes: Karyotyping

You will EACH be given a copy of a hypothetical chromosome spread for your karyotype analysis. You are to construct the karyotype for this patient to look for possible chromosomal abnormalities. YOU are the cytogeneticist.

Some of the chromosomal abnormalities you might encounter include:

Abnormalities in the number of sex chromosomes:

XO - Turner Syndrome
XXX - Triple-X
XXY - Klinefelter Syndrome
XYY - XYY Karyotype

Abnormalities in the autosomes:

Trisomy 13 - Patau Syndrome
Trisomy 18 - Edwards Syndrome
Trisomy 21 - Down Syndrome
Trisomy 22 - (no common name)
14/21 translocation - normal carrier for Down syndrome
14/21 translocation - inherited Down syndrome

The characteristics of most of these diseases may be found in your textbook.

A normal karyotype is given on page 18; is it for a male or female? How would it differ for the other sex?

Procedure:

1. Use a pencil to draw a circle around each chromosome on the karyotype your TA gives you. In these examples, there are no overlapping chromosomes. How many chromosomes are in your unknown karyotype?

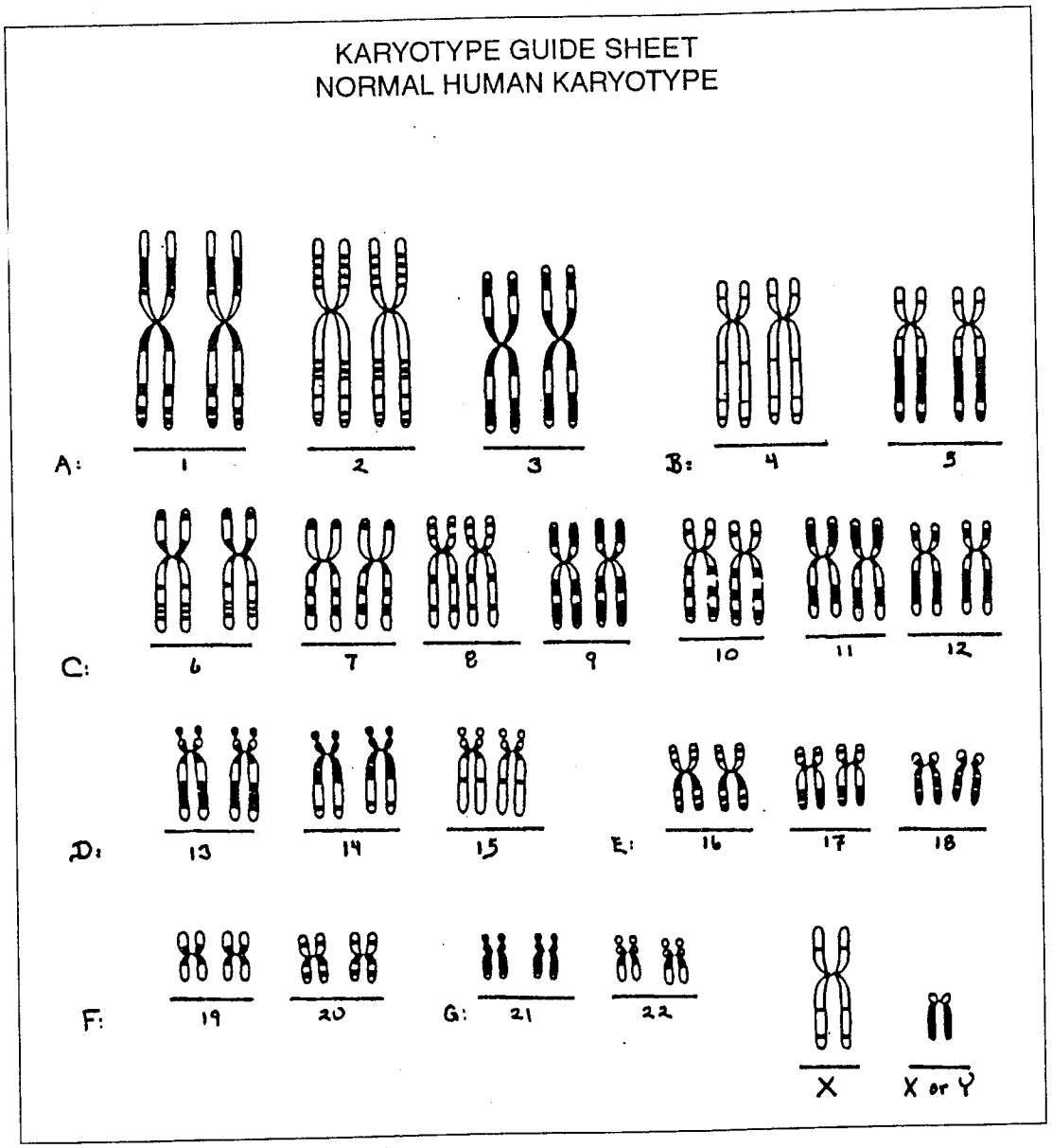
2. Now identify each chromosome using the Karyotype Guide Sheet on p. 18. Write the chromosome number or letter next to each chromosome on your unknown karyotype, and check off that particular chromosome on the Guide Sheet. If you find an abnormality, such as three chromosome 21s, note at the top of the sheet, but continue until you have identified all of the chromosomes. Also note at the top of the sheet whether the patient was male or female.

3. Cut out the chromosomes and arrange them on the Karyotype Guide Sheet. **Do not throw away paper scraps until you have accounted for all of the chromosomes.** If you will need to complete the task at home, only cut out the chromosomes that you can immediately identify.

4. Write a brief report explaining whether the patient has a chromosomal abnormality.

If your diagnosis suggests an abnormality, explain (a) how you came to that conclusion and (b) what you anticipate to be the clinical symptoms (do some research). (c) Include a diagram of meiosis (using only 2 or 3 pairs of chromosomes) to show how such a condition could arise. If the karyotype is normal, describe normal meiosis.

Don't forget to examine the demonstration slide of **real human chromosomes.** Are they as large as you had expected? Would working with the real human chromosomes make the karyotyping a bit more difficult?



From: Caroline Purser. 1987. Karyotype success rate increases with stylized chromosomes. *American Biology Teacher* 49(6): 360-363.

IV. Genetic Crosses

A. Monohybid cross

Obtain one ear of corn for the monohybrid cross. This ear represents the F_2 progeny of a pair of heterozygous plants. What do you predict will be the ratio of purple to yellow kernels?

Count at least 200 purple and yellow kernels and enter the data below.

phenotypes

purple kernels = 105
yellow kernels = 95

200 Total

Be sure to enter your data as actual numbers and not as percentages.

What results did you expect from this cross on theoretical grounds given the information provided (one pair of alleles, A (purple) dominant, a (yellow) recessive)? The easiest way to make such predictions is by use of the Punnett square method.

Monohybrid Cross Punnett Square

genotypes of parents Aa X Aa

Possible gametes 1/2 A 1/2 A

1/2 a 1/2 a

F_2 generation (the kernels we are classifying)

	A	a
A	AA	Aa
a	Aa	aa

Expected genotypes: 1 AA (purple)

2 Aa (purple)

1 aa (yellow)

Expected phenotypes 3 purple
1 yellow

How well do your data conform to these theoretical expectations? It would be rare for the data to fit precisely. We expect some deviation, but how much is acceptable? To make determine if the difference between what you expected (predicted from our understanding of Mendelian genetics) and what you observed is acceptable, you will do a Chi-square test. Recall from the pre-lab material that in a Chi-square test, we first compare the observed number with the theoretical expected number (which we have calculated) for each class of progeny and then calculate the difference (or deviation).

$$\chi^2 = \sum \frac{(o - e)^2}{e}$$

where
χ^2 = Chi square
o = observed number
e = expected number

The Chi-square test: We will apply the Chi-square test to the data you collected for the monohybrid cross. Use the table on the next page to organize your calculations.

Write your observed values and chi square value on the blackboard. Was the observed always the same as the theoretical?

When you have calculated the value of Chi-square value you evaluate it by referring to the table on the next page. The value (c-1) refers to the number of classes in which a deviation can occur, minus one. This value is called the degrees of freedom. The following table shows the maximum permissible χ^2 values allowable if the deviations are due to chance alone. For the monohybrid cross there are 2 classes of phenotypes (purple and yellow) so c-1=1; the degrees of freedom = 1.

	Purple	Yellow	Total
Observed numbers (o)	175	55	230
Expected proportions	**3/4**	**1/4**	**1**
Expected numbers (e)	173	58	230
(o – e)	2	-3	
$(o-e)^2$	4	9	
$\frac{(o-e)^2}{e}$	$\frac{4}{173}$	$\frac{9}{58}$	
$\Sigma \frac{(o-e)^2}{e}$	.023238		

Interpreting the Chi-square value and your results:

Using the Chi-square value you calculated above, locate where it falls in the Chi-Square Table below. Recall that your Degree of Freedom (c-1) = 1, hence you look in the first row of the table. If the chi-square falls within the left-hand box, we will conclude that the difference between the observed and the expected is nonsignificant - due purely to chance. If it falls within the right-hand side, the difference is significant - not due to chance.

If your chi-square value is 3.841 or larger, such a deviation would be expected to occur by chance alone less than 5% of the time. If it is 6.635 or larger, it would be expected to occur by chance less than 1% of the time. Hence you would have reason to doubt your hypothesis regarding the inheritance of these characters. What did you find? Was Mendel correct?

CHI-SQUARE TABLE

	O = E							O ≠ E		
	Probability							Probability		
C-1	.99	.95	.80	.50	.30	.20	.10	.05	.02	.01
1	.0016	.0039	.064	.455	1.074	1.642	2.706	3.841	5.412	6.635
2	.0201	.103	.446	1.386	2.408	3.219	4.605	5.991	7.824	9.210
3	.115	.352	1.005	2.336	3.665	4.642	6.251	7.815	9.837	11.341
4	.297	.711	1.649	3.357	4.878	5.989	7.779	9.488	11.668	13.277
5	.554	1.145	2.343	4.351	6.064	7.289	9.236	10.070	13.388	15.086

B. Test cross

Now obtain an ear of corn corresponding to a test cross between a plant with yellow kernels and one with purple kernels. On a separate piece of paper work out the Punnett squares when the parent with purple kernels is homozygous and when it is heterozygous.

Without bothering to count the kernels, what were the genotypes of the parents in this cross? Now count the number of yellow and purple kernels and record your data. Calculate the Chi square value for your data. Did your quantitative analysis confirm your prediction of the parent genotypes?

Again, place your observed values and Chi square on the blackboard to see whether your conclusion is reproducible.

	Purple	Yellow	Total
Observed numbers (o)	100	100	200
Expected proportions	½	½	1
Expected numbers (e)	100	100	
(o - e)	0	0	
$(o - e)^2$	0	0	
$\frac{(o - e)^2}{e}$	0	0	
$\sum \frac{(o - e)^2}{e}$	0		

What is the probability of obtaining this great (or greater) deviation from expectation? Do you accept your original hypothesis?

we got exactly 100 corn all each. so we ~~were~~ accurately predicted the number of female [illegible]

C. Dihybrid cross

Obtain an ear of corn that corresponds to a dihybrid cross. The parents of these kernels were both heterozygous for color and for seed texture. Classify the kernels and record the data. On a separate sheet of paper work out the Punnett square. Then use that information to determine your expected numbers for each class and calculate χ^2 value for your data.

	Purple, Smooth	Purple, Wrinkled	Yellow, Smooth	Yellow, Wrinkled	Total
Observed number (o)	127	29	31	13	200
Expected proportions	$\frac{9}{16}$	$\frac{3}{16}$	$\frac{3}{16}$	$\frac{1}{16}$	1
Expected number (e)	112.5	37.5	37.5	12.5	200
(o - e)	14.5	-8.5	-6.5	.5	
$(o - e)^2$	210.25	72.25	42.25	.25	
$\frac{(o - e)^2}{e}$	1.868	1.926	1.126	.02	
$\sum \frac{(o - e)^2}{e}$	4.94				

What is the value of c-1 (degrees of freedom) for the dihybrid cross? What is the probability of obtaining this great (or greater) deviation from expectation? Do you accept your original hypothesis?

D. Demonstration of Independent Assortment Using Artificial Chromosomes

Obtain a set of pop-it beads (artificial chromosomes). Use these to review how independent assortment arises as a consequence of meiotic events. Additional pop-it beads in various colors will be available to represent alleles at two or more loci.

V. Genetics Problems

A. Set 1

Use the following information to answer Questions 1 through 9.

Short hair in rabbits is governed by a dominant gene L and long hair by its recessive allele l. Black hair results from the action of the dominant gene B and brown hair is the result of the action of its recessive allele b. These loci are unlinked. A female from a pure-breeding line of short haired, brown rabbits is mated to a male from a pure-breeding line for long-haired black rabbits.

Key: L = short hair
l = long hair

B = black hair
b = brown hair

1. What is the genotype of the female rabbit?

LLbb

2. List all of the possible gametes she is capable of producing.

Lb

3. What is the genotype of the male rabbit?

llBB

4. List all of the possible gametes he is capable of producing.

lB

5. What is the genotype of the F_1 rabbits?

bL , Bl

6. What is the phenotype of the F_1 rabbits?

BL, Bl, bL, bl Black hair, Short hair

7. List all of the possible gametes that an F_1 rabbit is capable of producing.

BbLl ~~B~~

8. Use the Punnett square method to show all of the genotypes which would be expected among the F_2 progeny. 4 types

BbLl
BBLl
Bb LL

9. What is the ratio of phenotypes which would be expected among the F_2 progeny? BBll

9:3:3:1

B. Set 2

Practice Problems: **Autosomal inheritance**

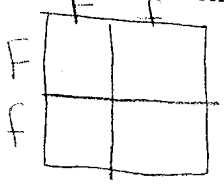
Using the following information in answering the questions below. Assume that each pair of alleles represents a different locus and that all of these loci are unlinked. (Also assume complete penetrance.)

A	=	free earlobes
a	=	attached earlobes
W	=	"widow's" peak
w	=	straight hairline
F	=	freckles present
f	=	no freckles
P	=	polydactyly (extra digits)
p	=	normal five digits
T	=	ability to taste a chemical called PTC ("taster")
t	=	non-taster

1. If a woman and a man who are both heterozygous for freckles produce a child, what is the probability that it will not have freckles?

Ff Ff

a. 0
b. 1
c. 1/4
d. 1/2
e. 3/4
f. None of the above



2. When both the husband and wife are polydactylous but each had one normal parent, what is the probability that they will have a polydactylous child?

a. 0
b. 1
c. 1/4
d. 1/2
e. 3/4
f. None of the above

3. If a husband and wife are both heterozygous for the same dominant trait, what is the probability that they will have a child with a genotype identical to their own (for this locus)?

a. 0
b. 1
c. 1/4
d. 1/2
e. 3/4
f. None of the above