

# Meiosis and Tetrad Analysis Lab

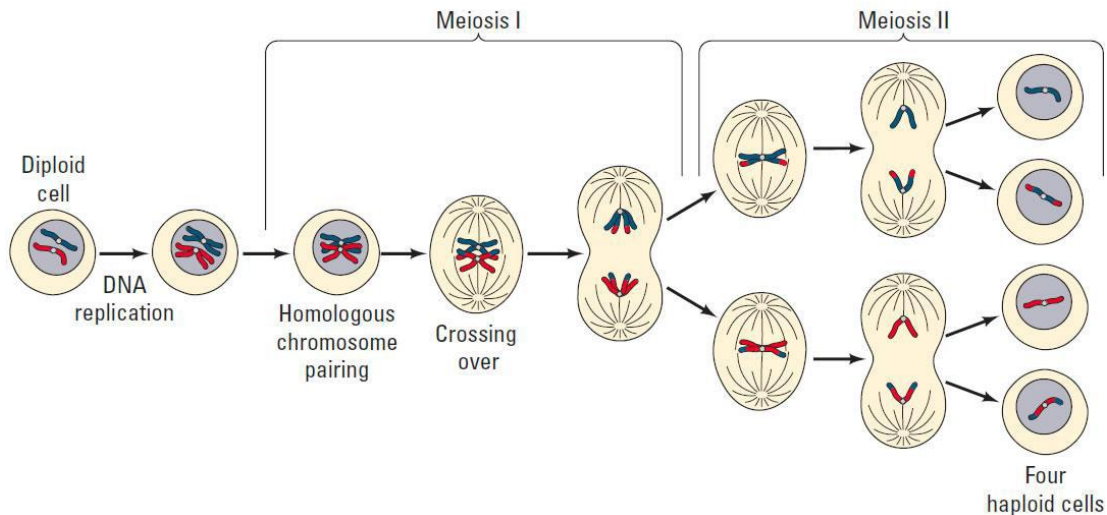
## Objectives:

- Explain how meiosis and crossing over result in the different arrangements of ascospores within asci.
- Learn how to calculate the map distance between a gene and the centromere of the same chromosome.

## Introduction:

All new cells come from previously existing cells. The process of cell division, which involves both division of the nucleus and division of the cytoplasm, forms new cells. There are two types of nuclear division: mitosis and meiosis. Mitosis typically results in new somatic (body) cells. Formation of an adult organism from a fertilized egg, asexual reproduction, regeneration, and maintenance or repair of body parts are accomplished through mitotic cell division. Meiosis results in the formation of either gametes (in animals) or spores (in plants). These cells have half the chromosome number of the parent cell.

**Meiosis** involves two successive nuclear divisions that produce four haploid cells. Meiosis I is the reduction division. It is this first division that reduces the chromosome number from diploid to haploid and separates the homologous pairs. Meiosis II, the second division, separates the sister chromatids. The result is four haploid gametes (see **Figure 1**).

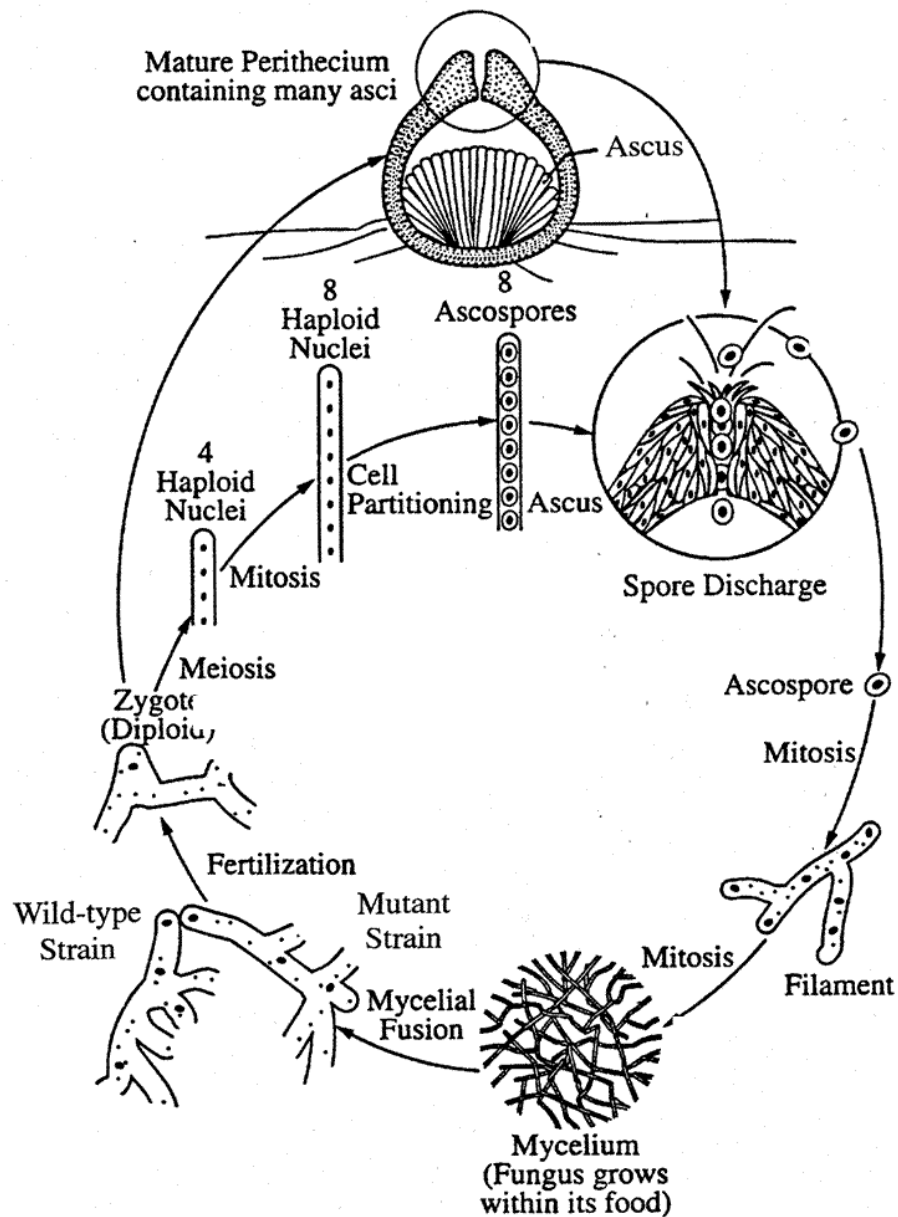


**Figure 1: Meiotic Cell Division Emphasizing Chromosome Movement**

Each diploid cell undergoing meiosis can produce  $2^n$  different chromosomal combinations, where  $n$  is the haploid number. In humans, the number is  $2^{23}$ , which is more than eight million combinations. Actually, the potential variation is even greater because, during meiosis I, each pair of chromosomes (homologous chromosomes) comes together in a process known as **synapsis**. Chromatids of homologous chromosomes may exchange parts in a process called **crossing over**. The relative distance between two genes on a given chromosome can be estimated by calculating the percentage of crossing over that takes place between them.

*Sordaria fimicola* is an ascomycete fungus that can be used to demonstrate the results of crossing over during meiosis. *Sordaria* is a **haploid** organism for most of its life cycle. It becomes **diploid** only when the fusion of the mycelia (filament-like groups of cells) of two different strains results in the fusion of the two different types of haploid nuclei to form a diploid nucleus. The diploid nucleus must then undergo meiosis to resume its haploid state.

Meiosis, followed by one mitotic division, in *Sordaria* results in the formation of eight **haploid ascospores** contained within a sac called an **ascus** (plural, asci). Many asci are contained within a fruiting body called a **perithecium** (ascocarp). When ascospores are mature the ascus ruptures, releasing the ascospores. Each ascospore can develop into a new haploid fungus. The life cycle of *Sordaria fimicola* is shown in **Figure 2** below.



**Figure 2: Life Cycle of *Sordaria fimicola*.**

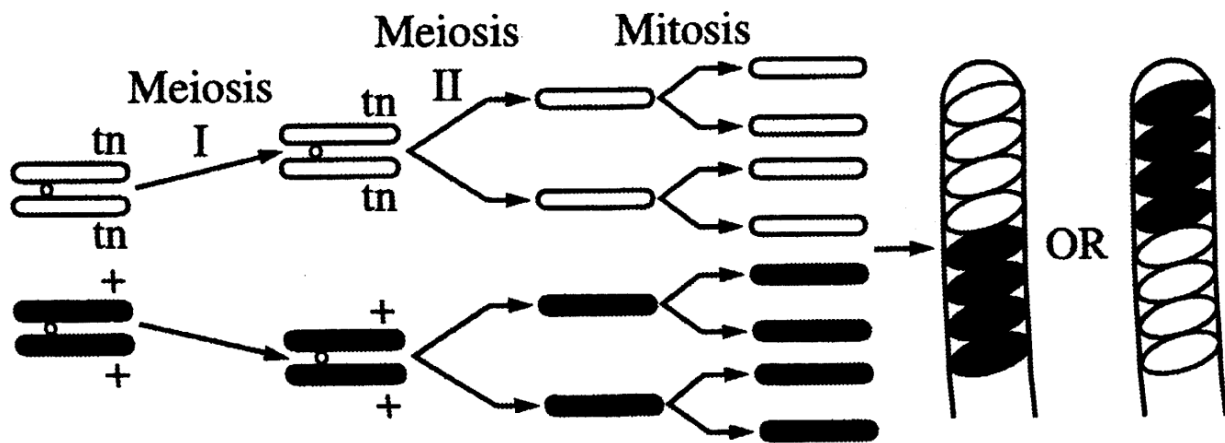
The spore color of the normal (wild type) *Sordaria*, is black. This phenotype is due to the production of the pigment melanin and its deposition in the cell walls. Several different genes are involved in the control of the melanin biosynthetic pathway and each gene has two possible allelic forms. The gene for spore color has two forms: a wild type allele (+) which shows a black color, and a mutant allele (tn) which shows a tan color.

To observe crossing over in *Sordaria*, one must make hybrids between wild type and mutant strains of *Sordaria*. The arrangement of the spores directly reflects whether or not crossing over has occurred. In **Figure 3** no crossing over has occurred. **Figure 4** shows the results of crossing over between the centromere of the chromosome and the gene for ascospore color.

**To Cross Over or NOT To Cross Over... that is the question.**

The following two examples take a look at a tan X wild cross. Two homologous chromosomes line up at metaphase I of meiosis. The two chromatids of one chromosome each carry the gene for tan spore color (tn) and the two chromatids of the other chromosome carry the gene for wild type spore color (+).

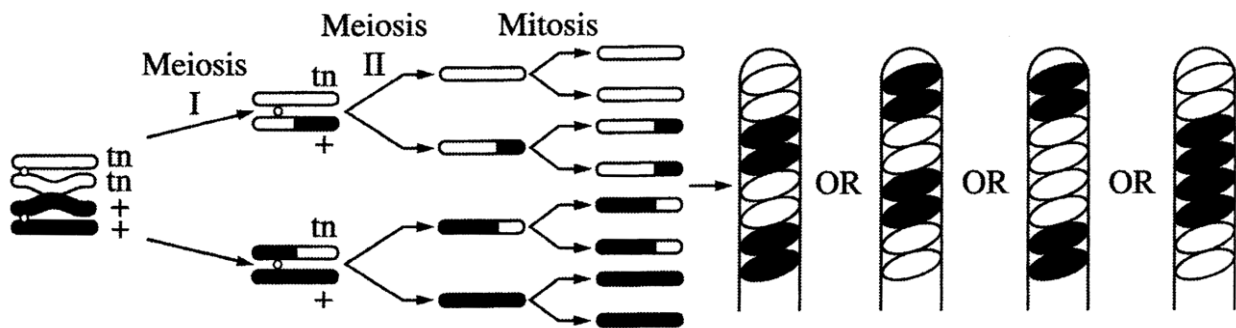
The first meiotic division results in two cells, each containing just one type of spore color gene (either a tan or wild type). Therefore, segregation of these alleles has occurred at the first meiotic division. Each cell is haploid at the end of meiosis I. The second meiotic division results in four haploid cells, each with the haploid number of chromosomes. A **mitotic division** following meiosis simply duplicates these cells, resulting in 8 spores – without any crossing over event. They are arranged in the 4:4 pattern shown below. Due to the independent assortment of the chromosomes in metaphase, the mutant allele chromosome could switch places with the wild type chromosome giving the other arrangement of spore colors shown in the picture.



**Figure 3: Meiosis with NO Crossing Over**

In the following example, crossing over HAS occurred in the region between the gene for spore color and the centromere. The homologous chromosomes separate during meiosis I. This time, because of the genetic shuffling, the first meiotic division results in two genotypically different chromosome arrangements – each containing both different alleles (1 tan, 1 wild type); therefore, the genes for spore color have not yet segregated, although the cells are haploid. The second meiotic division results in the segregation of the two types of genes for spore color – resulting in two phenotypically different cells.

A **mitotic** division following meiosis results in 8 spores arranged in the 2:2:2:2 or 2:4:2 pattern. Any one of these spore arrangements would indicate that crossing over has occurred between the gene for spore coat color and the centromere. Again, independent assortment of the chromosomes during metaphase I and II will result in all the possible combinations of spore colors.



**Figure 4: Meiosis with Crossing Over**

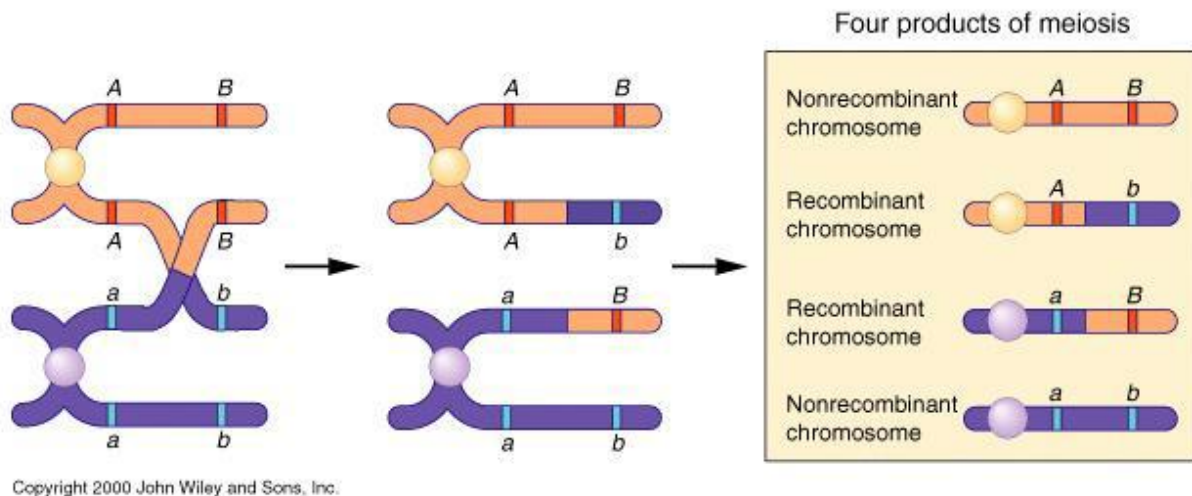
You should understand that there can be multiple crossing over events, you might sometimes see the 8 ascospores in a 2:1:1:1:1:2 or other 'weird' ratios. These would normally count as a double-crossover event, but realize, you also can have a double-crossover result in a 4:4 if it crossovers twice in between the centromere and the gene locus. So we will only be counting the ratios shown in Figure 3 and 4 in the lab.

Published gene map locations for the two genes that we are studying are *approximately 27 map units for the tan spore gene*. More on mapping genes in the final introduction section.

### Mapping Genes on Chromosomes

The exchange of genetic material between homologous chromosomes which occurs during crossing over creates a major exception to Mendel's principle of segregation. Recall that the segregation of alleles from the two parents occurs during anaphase I of meiosis, that is, during the first division of meiosis. If crossing over occurs, however, the alleles rearranged by the crossover are not segregated until anaphase II of meiosis, which is during the second division of meiosis. Thus, it is said that crossing over leads to second division segregation of the alleles involved in the crossover. Gene mapping became possible when it was realized that the frequency of second division segregation was related to the physical distance separating the genes involved.

If we assume that crossing over can occur at any point along a chromosome, it is logical that the probability of a crossover occurring between a gene locus and the centromere will be proportional to the locus-centromere distance. In **Figure 5**, we would expect a higher rate of crossover events with Gene B than Gene A.



**Figure 5: Differential Crossing Over due to Gene Loci Distance from Centromere**

Therefore, we can use the frequency (proportion) of crossover-produced ascospores as a measure of the relative distance separating the gene locus and the centromere. Here, the recombinant frequency would be equal to the number of recombinant asci per 100 total asci (both recombinant and non-recombinant).

$$\text{recombinant frequency} = \frac{\text{recombinant asci}}{\text{total asci}} \times 100$$

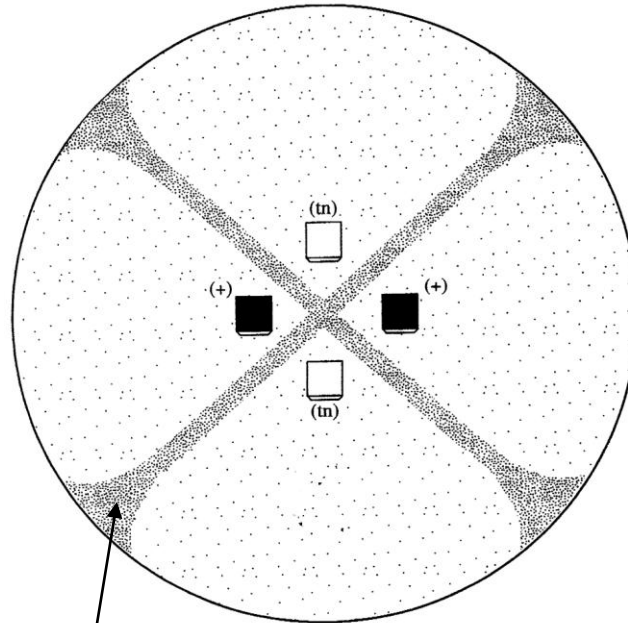
Geneticists define a crossover map unit as the distance on a chromosome that produces one recombinant post-meiotic product per 100 post-meiotic products. The distance is given in the units of centi-Morgans (cM). Given that map units express the percent recombinant asci resulting from crossovers and each single crossover produces 4 recombinant spores and 4 non-recombinant spores (so only 4 out of the 8 are 'different'), the map unit distance for our *Sordaria* cross is always one half the frequency of crossing over for the gene. See the textbook for more information on gene mapping.

$$\text{map units} = \frac{\text{recombinant frequency}}{2}$$

Using tetrad analysis, geneticists have been able to obtain genetic maps of chromosomes of many organisms. These maps indicate the sequence of genes on chromosomes and the relative location of these genes. However, because a genetic map is based on crossover frequencies, the relative distances between genes DO NOT correspond to real, physical distances. That is, although the sequence of genes is correct, some genes may be closer together and others farther apart than genetic maps indicate. This is because some regions of chromosomes have a greater, or lesser, tendency to form crossovers than other regions. For example, the centromere seems to inhibit crossing over and genes located close to it do not crossover as much as they should based solely on their physical location.

Procedure:

In the example below, two strains of *Sordaria* (wild type and a tan mutant variety) have been inoculated on a nutrient plate. Where the mycelia of the two strains meet, **Figure 6**, fruiting bodies called perithecia develop. Meiosis occurs within the perithecia during the formation of the asci.



**Figure 6: Sordaria Cross Plate**

1. In a group of 4 students, you will set up and work on the wild/tan cross.
2. Use a scalpel to gently scrape the surface of the nutrient medium where the two strains intersect to collect perithecia. At the intersection of the two strains is the region to harvest the perithecia.
3. Place the perithecia in a drop of water on a slide. Cover with a coverslip and return to your work area. Using the eraser end of a pencil (or a toothpick), press down the coverslip gently so that the perithecia rupture but the ascospores remain in the asci – this is tricky to do. You want to rupture the fruiting body, but not each spore case.
4. View your slide using the 10X objective and locate a group of hybrid asci (those containing both mutant and wild ascospores). Try to go to 40X if possible.
5. Count at least 50 hybrid asci for each mutant cross and enter your data in Table 1.
6. Determine the distance between the gene for spore color and the centromere. Calculate the recombinant frequency by dividing the number of crossover asci (2:2:2:2 or 2:4:2) by the total number of asci X 100. To calculate the map distance, divide the percentage of crossover asci by 2. This is done since only half of the spores in each ascus are the result of crossing over.

**Table 1: Sordaria fimicola Tetrad Analysis**

Sordaria Phenotype Crossed	Number of 4:4 Asci (Non-Crossover Events)	Number of Asci Showing Crossover	Total Asci	% Asci Showing Crossover (Recombinant Frequency)	Calculated Gene to Centromere Distance (cM)
Wild X Tan					

7. Use the chi-square distribution test (Tables 2) to statistically analyze the data using the book value determined by Olive, 1956 for the mutant cross.
  - a. Enter the number of non-crossover and crossover asci from your mutant cross as observed (o).
  - b. Calculate the percentage of crossover asci expected from Olive’s “book value”. (HINT – it is NOT the same as the map distance...) This also will give you the percentage of asci that you WOULD NOT expect to have a crossover.
  - c. Multiply the percentages by the total number of asci count from your mutant cross; this will give you the expected numbers (e) for this cross based on Olive’s map distance.
  - d. Finish up the chi-square analysis using your calculations and Table 4 and then answer the questions on the last page.

**Table 2: Calculation of the Chi-Square Value for the Tan X Wild Cross.**

	o	e	(o – e)	(o – e) <sup>2</sup>	(o – e) <sup>2</sup> / e
Crossover Asci					
Non-Crossover Asci					
	$\chi^2$ total =				
	P value =				
	Accept or Reject =				

**Table 3: The Chi-square Distribution Table.**

degrees of freedom	probability value (p-value)									
	ACCEPT NULL HYPOTHESIS								REJECT	
	0.99	0.95	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01
1	0.001	0.004	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64
2	0.02	0.10	0.45	0.71	1.30	2.41	3.22	4.60	5.99	9.21
3	0.12	0.35	1.00	1.42	2.37	3.67	4.64	6.25	7.82	11.34
4	0.30	0.71	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28

### Lab Analysis Questions

1. Did the data you collected support Olive's map distance that was initially calculated more than 50 years ago? Use your statistical analysis to completely and correctly answer this.
2. Why did you divide the percentage of asci showing crossover (recombinant) by 2?
3. Think of some environmental factors that could affect the amount of crossing over that occurs in *Sordaria*. How would you set up an experiment to test this? Pose a scientific question, hypothesis, explanation of an experimental setup including the variables, and the possible results.
  - a. Example, how does humidity affect the crossover frequency in *Sordaria*?

### Review Questions

4. When is the DNA replicated during meiosis?
5. Are homologous pairs of chromosomes exact copies of each other?
6. What is crossing over? Explain the parts of the crossing over event.
7. What is meant by independent assortment?
8. What happens if a homologous pair of chromosomes fails to separate, and how might this contribute to genetic disorders such as Down syndrome and Edwards syndrome?
9. How does the distance between two genes or a gene and a centromere affect crossover frequencies?
10. Do you think the cell cycle described for mitosis could be applied to meiosis as well? Explain your answer.



# Meiosis and Tetrad Analysis Lab Data Sheet

**Table 1: Sordaria fimicola Tetrad Analysis**

Sordaria Phenotype Crossed	Number of 4:4 Asci (Non-Crossover Events)	Number of Asci Showing Crossover	Total Asci	% Asci Showing Crossover (Recombinant Frequency)	Calculated Gene to Centromere Distance (cM)
Wild X Tan					

**Table 2: Calculation of the Chi-Square Value for the Tan X Wild Cross.**

	o	e	(o - e)	(o - e) <sup>2</sup>	(o - e) <sup>2</sup> / e
Crossover Asci					
Non-Crossover Asci					
	$\chi^2$ total =				
	P value = Accept or Reject =				

**Table 3: The Chi-square Distribution Table.**

degrees of freedom	probability value (p-value)									
	ACCEPT NULL HYPOTHESIS								REJECT	
	0.99	0.95	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01
1	0.001	0.004	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64
2	0.02	0.10	0.45	0.71	1.30	2.41	3.22	4.60	5.99	9.21
3	0.12	0.35	1.00	1.42	2.37	3.67	4.64	6.25	7.82	11.34
4	0.30	0.71	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28