Lecture 2:

History and Review (I)

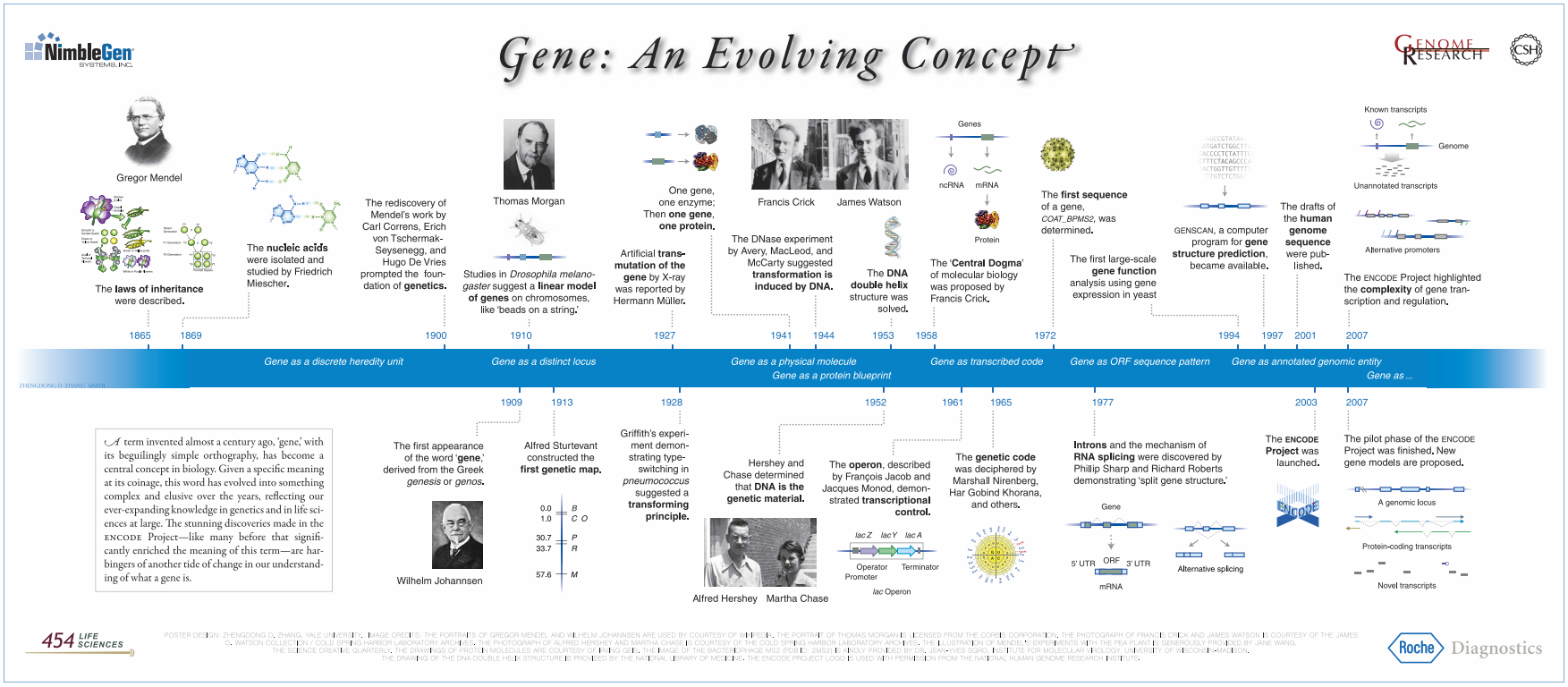
Course 485

Introduction to Genomics

AIMS

- Review basic molecular biology/genetics concepts.
- Highlight significance of findings and its relation to the study of genomics.

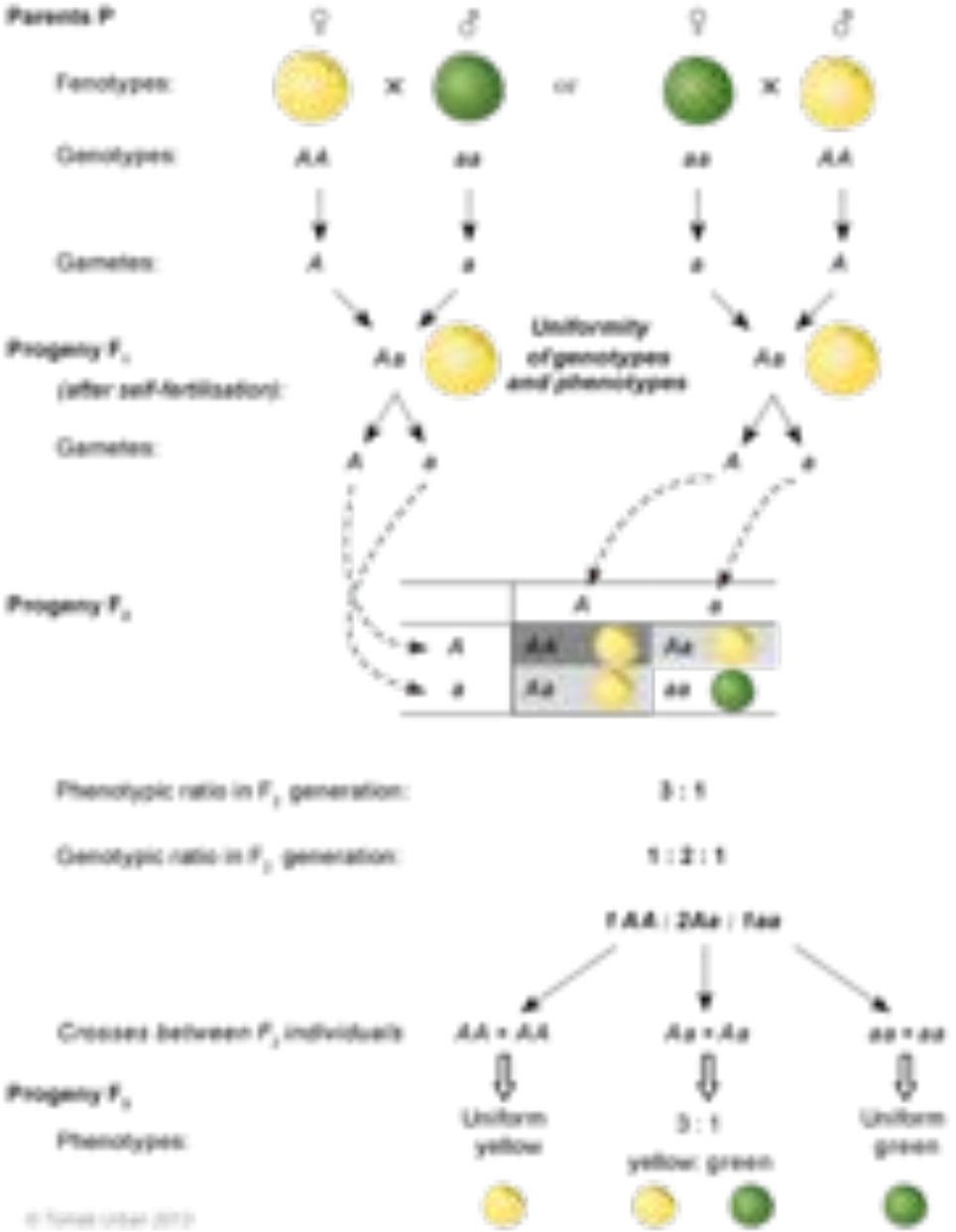
Chronological Review of concepts and finding in genetics and molecular biology



1865 - Mendel and his peas

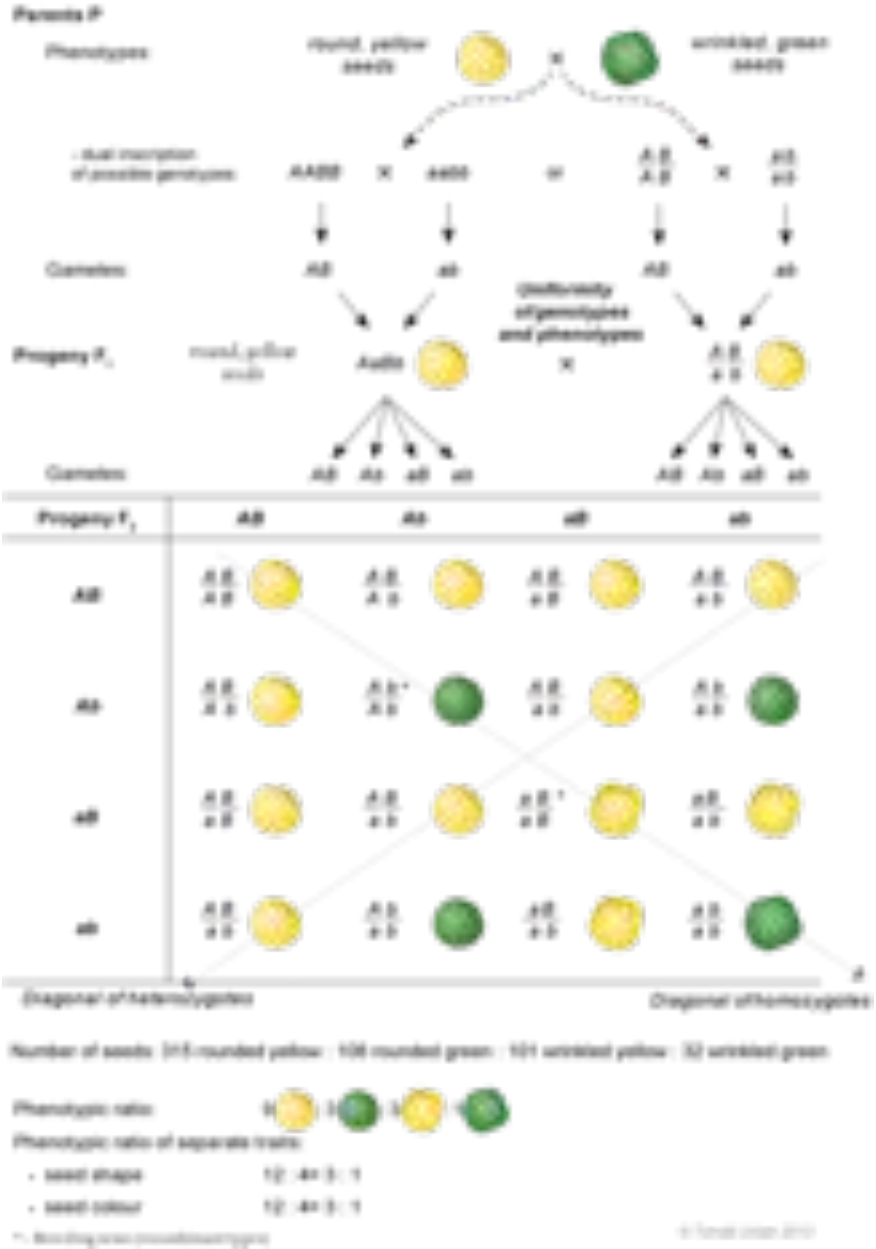
Gregor Mendel (Johann) studied heredity by the systematic breeding experiments of garden pea (*Pisum sativum*)

Monohybrid experiment



1st law:
Segregation of Mendel's factors (alleles)

Dihybrid experiment



2st law: Independent assortment of factors (alleles)

Do alleles of any two traits independently assort?

Lucky Mendel

Why didn't Gregor Mendel find linkage?

IT is quite often said that Mendel was very fortunate not to run into the complication of linkage during his experiments. He used seven genes and the pea has only seven chromosomes. Some have said that had he taken just one more, he would have had problems. This, however, is a gross oversimplification. The actual situation, most probably, is shown in Table 1. This shows that Mendel worked with three genes in chromosome 4, two genes in chromosome 1, and one gene in each of chromosome 5 and 7. It seems at first glance that, out of the 21 dihybrid combinations Mendel theoretically could have studied, no less than four (that is, *a–i*, *v–fa*, *v–le*, *fa–le*) ought to have resulted in linkages. As found, however, in hundreds of crosses and shown by the genetic map of the pea[1], *a* and *i* in chromosome 1 are so distantly located on the chromosome that no linkage is normally detected. The same is true for *v* or *le* on the one hand, and *fa* on the other, in chromosome 4. This leaves *v–le*, which ought to have shown linkage.

Mendel, however, seems not to have published this particular combination and thus, presumably, never made the appropriate cross to obtain both genes segregating simultaneously. It is therefore not so astonishing that Mendel did not run into the complication of linkage, although he did not avoid it by choosing one gene from each chromosome.

STIG BLIXT

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Received March 5; accepted June 4, 1975.

[1] Blixt, S., in *Handbook of Genetics*, 2 (edit. by King, R. C.), (Plenum, New York, 1974).

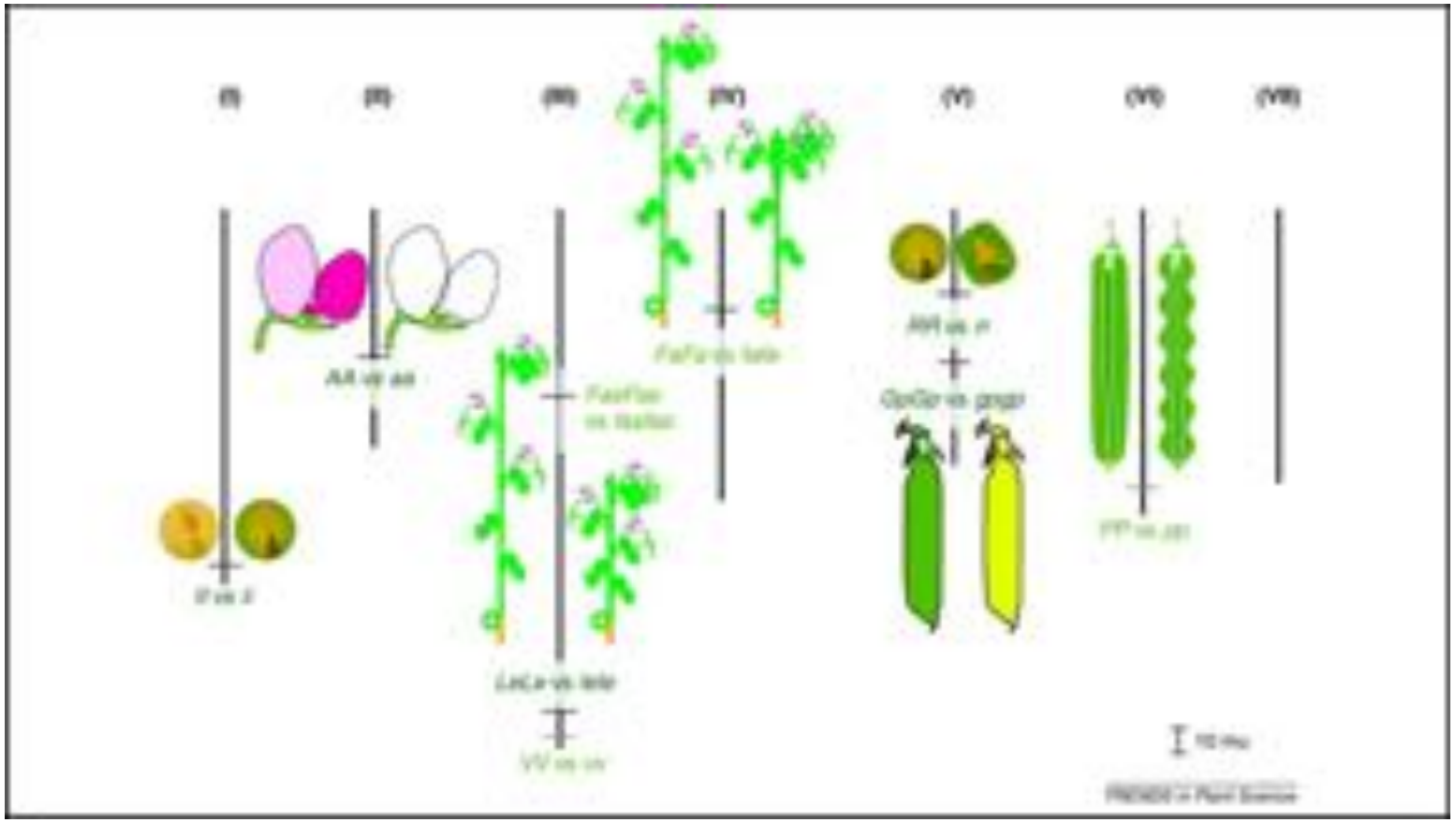
Table 1 Relationship between modern genetic terminology and character pairs used by Mendel

Character pair used by Mendel	Alleles in modern terminology	Located in chromosome
Seed colour, yellow–green	*I–i*	1
Seed coat and flowers, coloured–white	*A–a*	1
Mature pods, smooth expanded–wrinkled indented	*V–v*	4
Inflorescences, from leaf axils–umbellate in top of plant	*Fa–fa*	4
Plant height, >1m–around 0.5 m	*Le–le*	4
Unripe pods, green–yellow	*Gp–gp*	5
Mature seeds, smooth–wrinkled	*R–r*	7

Perfect independent assortment is due chromosomal location of genes responsible for the traits.

Blixt, S. Why didn't Gregor Mendel find linkage? Nature 256, 206 (1975)

Lucky Mendel



The genes of Mendel's traits are located on different chromosomes or far apart on one chromosome.

Ellis et. al. Mendel, 150 years on (2011) Trends in Plant Science, 16(11) 590-596

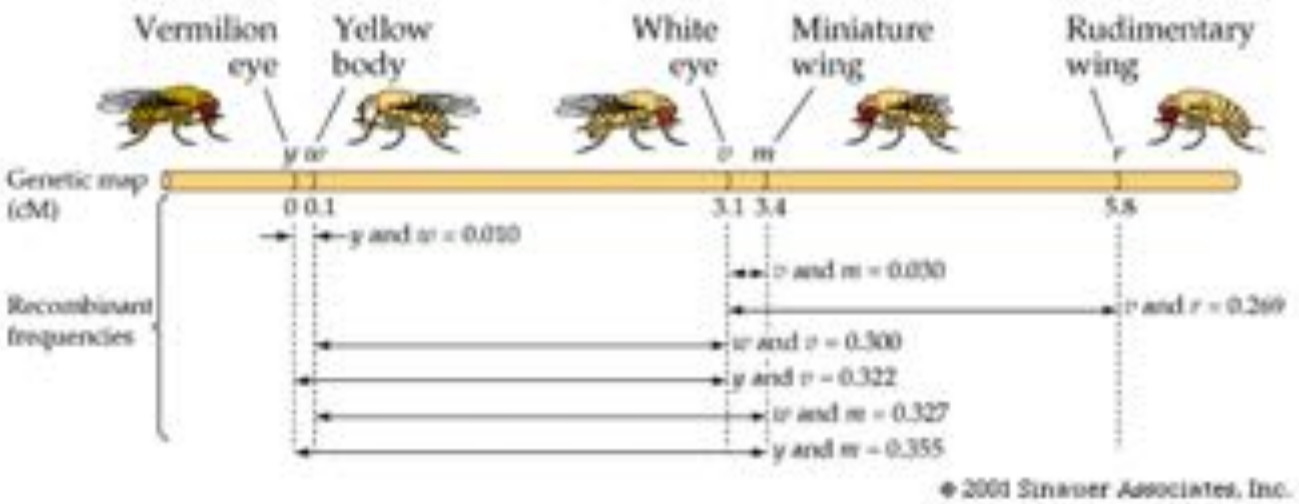
What if alleles do not independently assort?

Linkage maps

If not independent assortment then what?

Genes on the same chromosome and distance between genes can be determined through the numbers of resulting combinations.

We will revisit this later!



What is the chemical identity of Mendel's factors?

Nuclein: the chemical of the nucleus

Fredrick Miescher bloody bandages (1869)

XLV.

Ueber die chemische Zusammensetzung der Eiterzellen [1].

Von Dr. F. Miescher aus Basel.

Miescher, Friedrich (1871) "On the chemical composition of pus cells", Medicinisch-chemische Untersuchungen, 4 : 441–460.

Miescher's gray/white precipitate

Fig. 5. Glass vial containing nuclein isolated from salmon sperm by Friedrich Miescher while working at the University of Basel. The faded label reads "Nuclein aus Lachssperma, F. Miescher" (*Nuclein from salmon sperm, F. Miescher*). Possession of the Interfakultäres Institut für Biochemie (Interfacultary Institute for Biochemistry), University of Tübingen, Germany; photography by Alfons Renz, University of Tübingen, Germany.

Nuclein: the chemical of the nucleus

- The molecule is different than other molecules (contain C, O, N, H, and P – not known to be in proteins).
- Since the molecule came from nucleus, he called it **Nuclein (today called DNA)**.

daneben aber sehr reich an Phosphor. Die alte Tradition von den phosphorhaltigen Eiweissstoffen hat also doch einen reellen Hintergrund.

I. gr. 0,1915 lösliches Nuclein gaben 1811 Pt. = 13,47 N. Die Kerne waren nach der Isolation nicht mit Alkohol extrahirt. Die folgenden Versuche sind an ganzen, mit Alkohol heiss erschöpften Kernen gemacht.

Miescher, Friedrich (1871) "On the chemical composition of pus cells", Medicinisch-chemische Untersuchungen, 4 : 441–460.

Where do Mendel's factors reside?

Chromosome Theory

If Mendel factors segregate during meiosis, then something in the cell must do the same.

A physical entity with specific chemical properties inside the cell behaves similar to Mendel's factors.

Chromatin

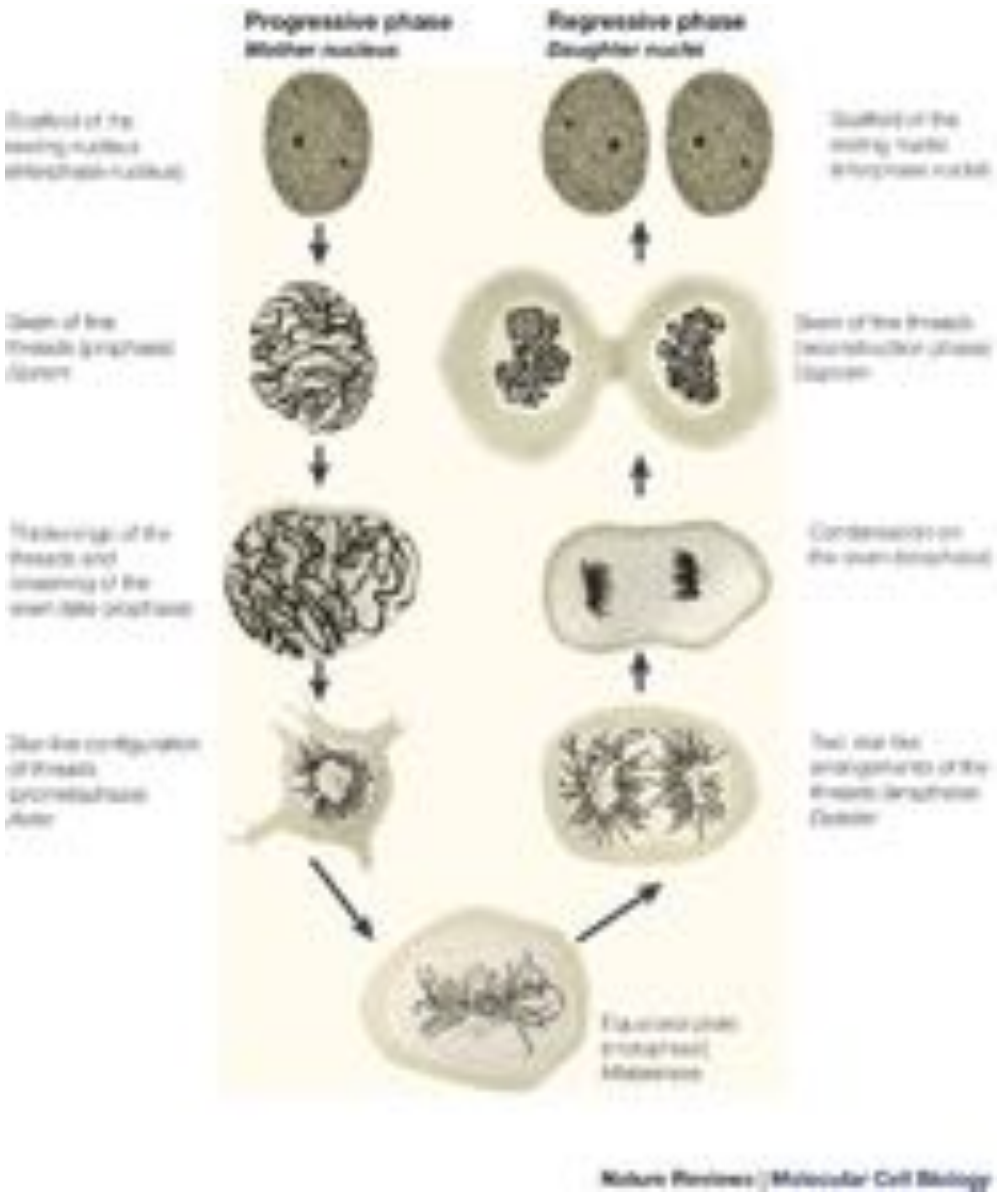
Walther Flemming band structure in dividing cells (1879)

- Used cells of salamanders and staining techniques to study cell division (he called it mitosis).
- The intensely stained parts of the nucleus he called **chromatin** (chroma is Greek for color).

Chromatin

The nucleus content separates during cell division.

What is this substance?



Chromosomes

Hermann Fol and Oscar Hertwig
(1870-1880)



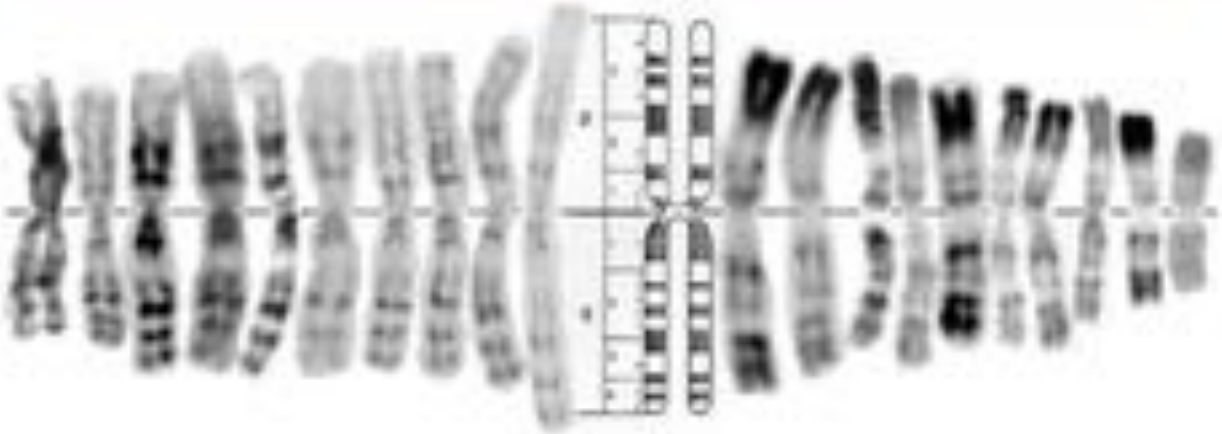
- Observed fertilization and fusion of the eggs and sperms nuclei.
- Chromatin is called **chromosomes**.

advancements in cell biology, microscopy, and staining techniques allowed visualizing the genetic material.

This also contributed to the development of “cytogenetic maps”

Cytogenetics

banal. Planets are named after gods, chemical elements after myths, heroes, and great cities. Chromosomes were named with all the creativity of shoe sizes. Chromosome one is the longest,



Factors on chromosomes

Thomas Morgan

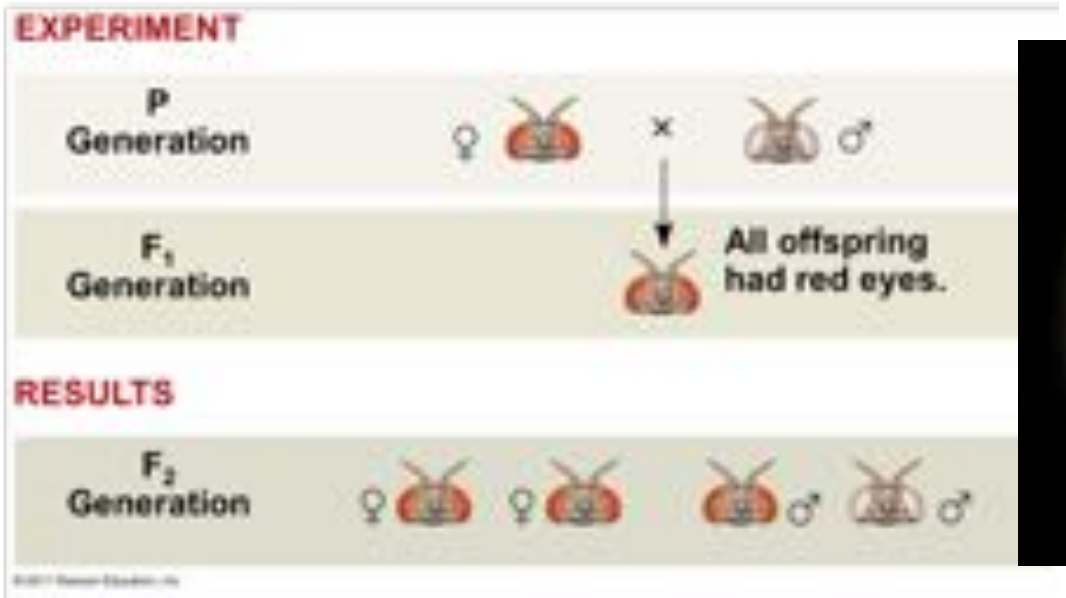
Studies in *Drosophila melanogaster* suggest a **linear model of genes** on chromosomes, like 'beads on a string.'

1910

Morgan and the fly room found evidence to associate factors (genes) to specific chromosome.

How?

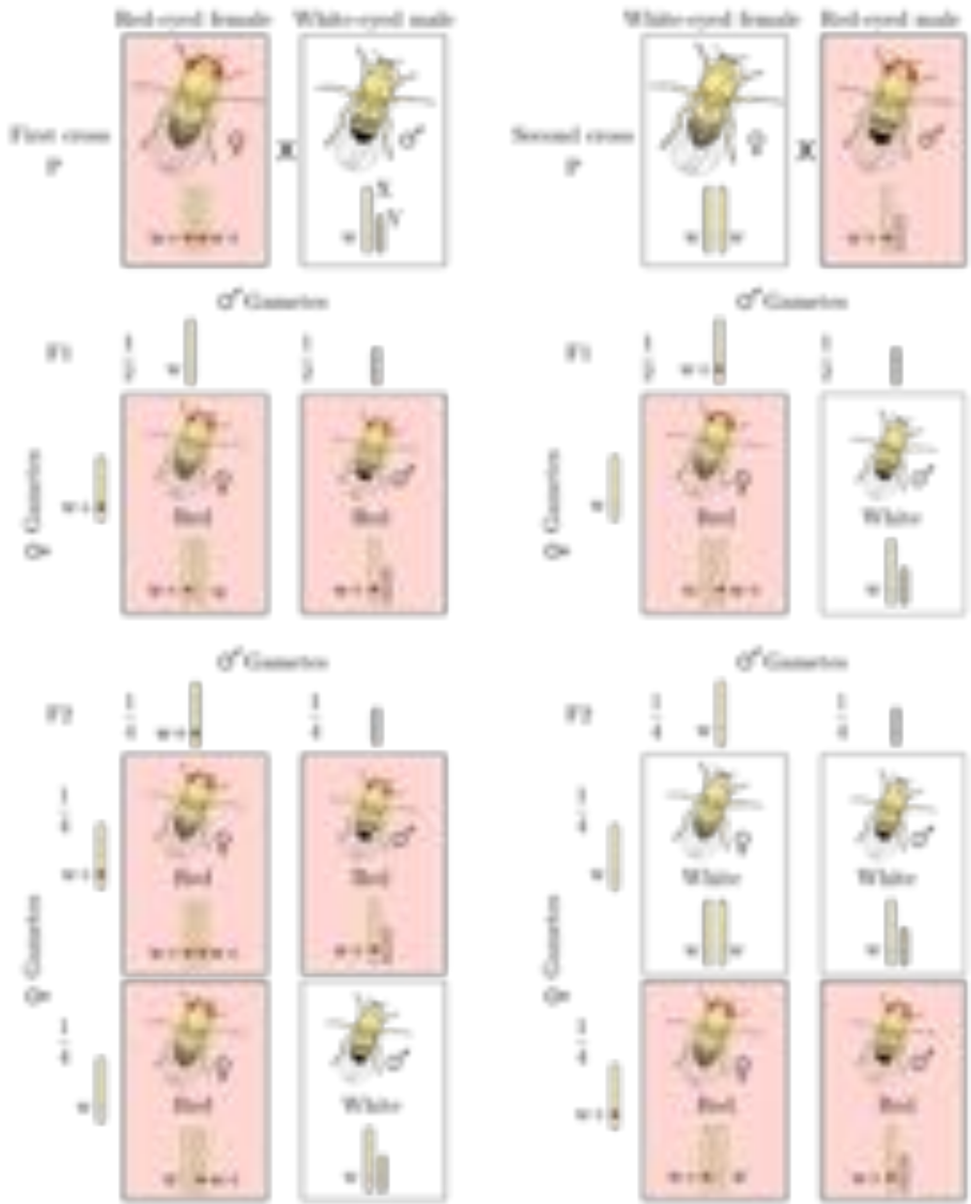
Genes on chromosomes



Using a mutant (white eyed fly) and series of crosses, Morgen found white-eye trait sex-linked (associated white eye factor (gene) on X chromosome).

Details?

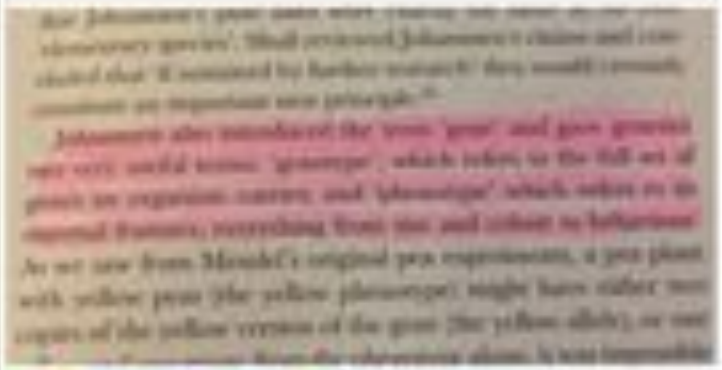
Genes on chromosomes



The Gene

It appears as most simple to use the last syllable 'gen' taken from Darwin's well-known word pangene.... Thus, we will say for 'das pangene' and 'die pangene' simply 'Das Gen' and 'Die Gene.

(Wilhelm Ludvig Johannsen)



First genetic map

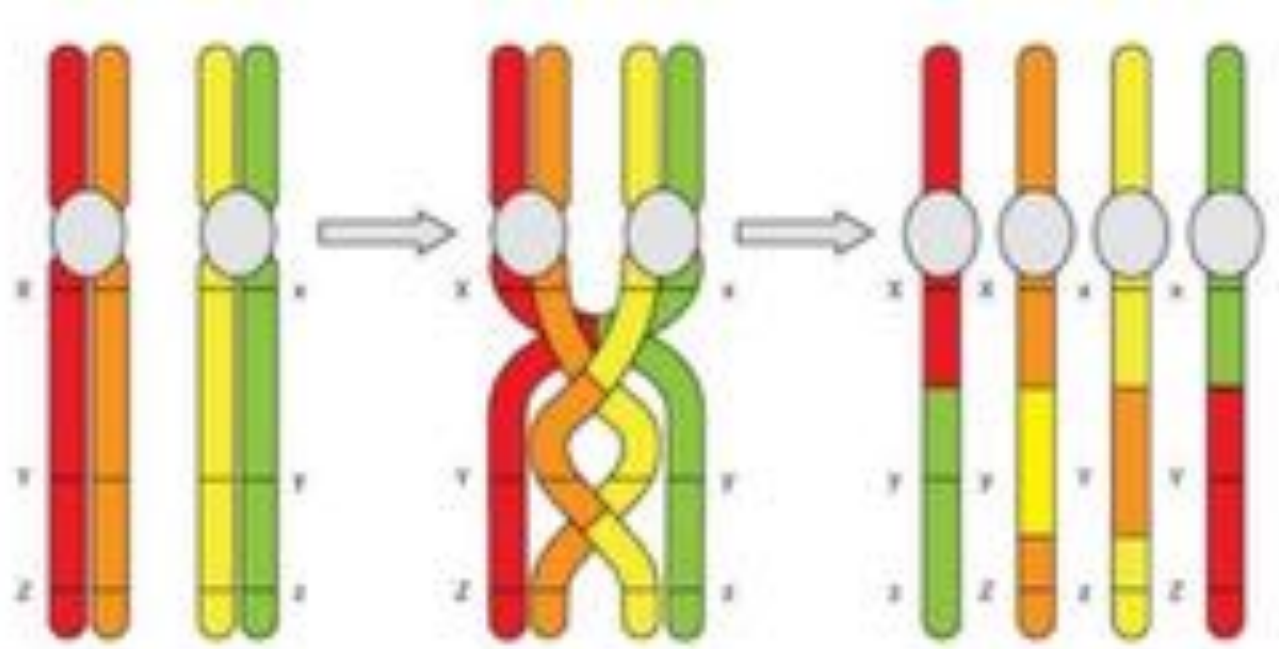
First genetic map constructed by Alfred Sturtevant (1913) - Morgan's student

First genetic map

When does assortment of chromosomes happen?

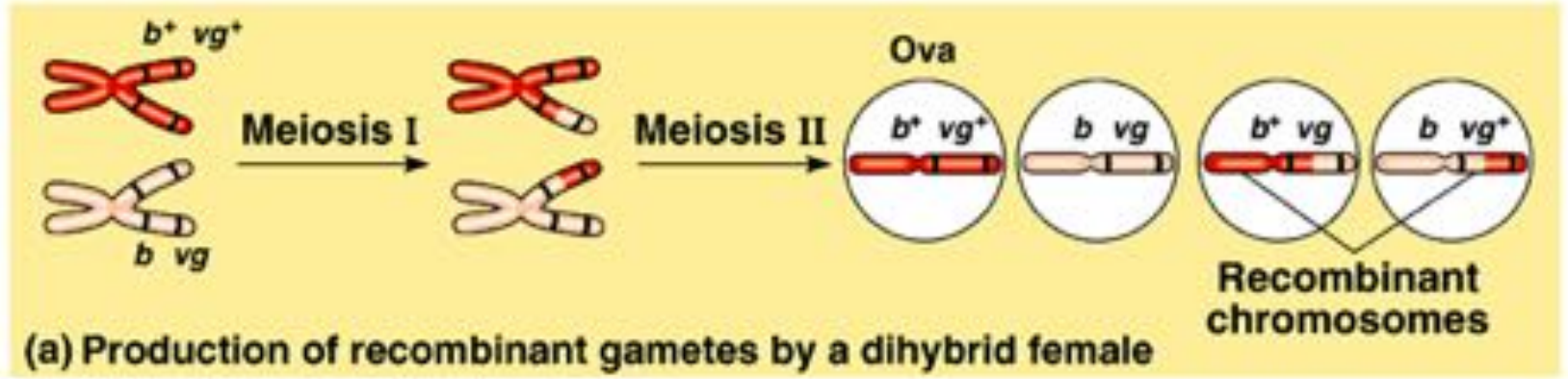
Do you know what cross-over is?

Cross-over



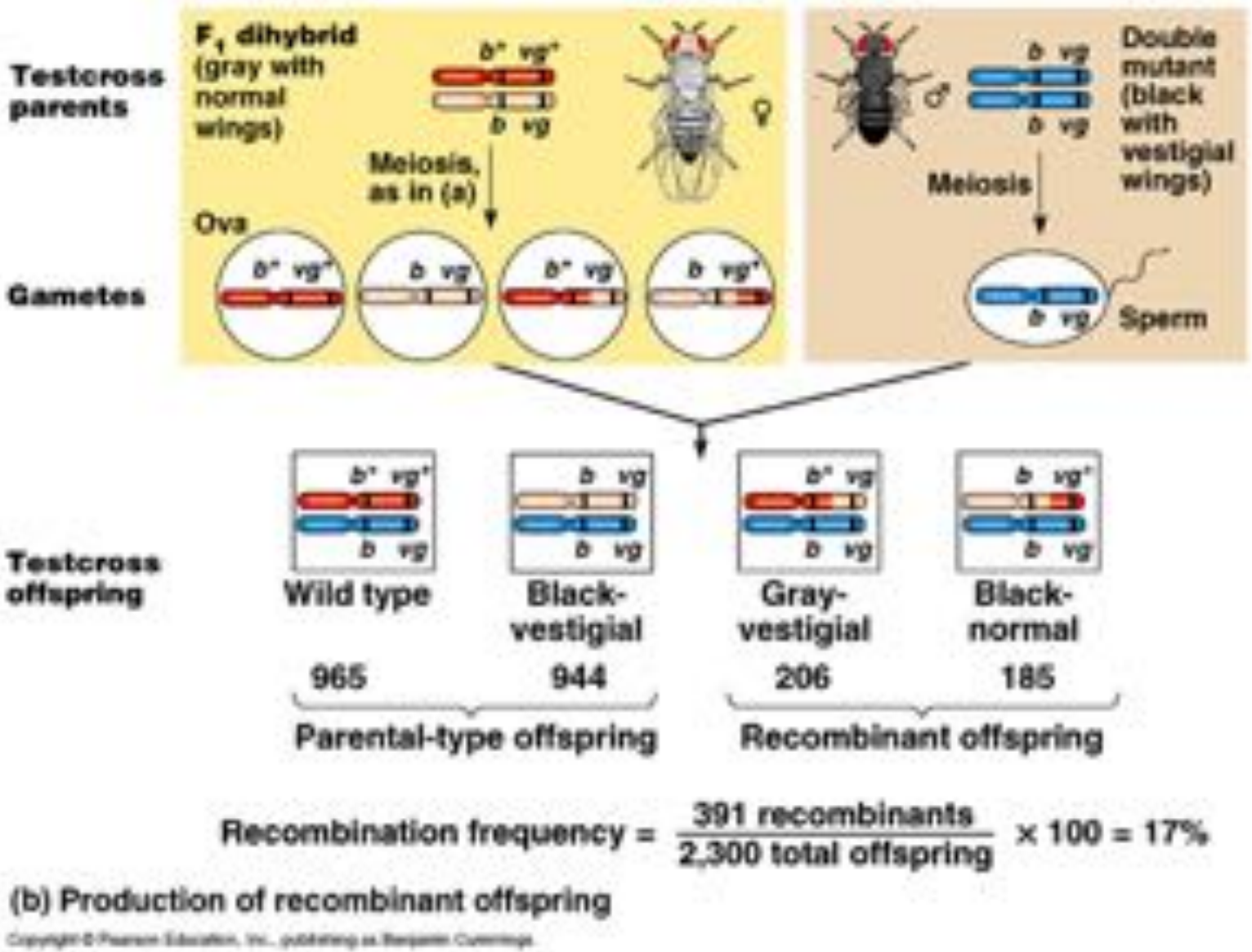
leaving the chromosome ineffective. Inversions can also hurt a chromosome's chances of crossing over – a huge loss. Crossing over (when twin chromosomes cross arms and exchange segments) allows chromosomes to swap genes and acquire better versions, or versions that work better together and make the chromosome more fit.

First genetic map



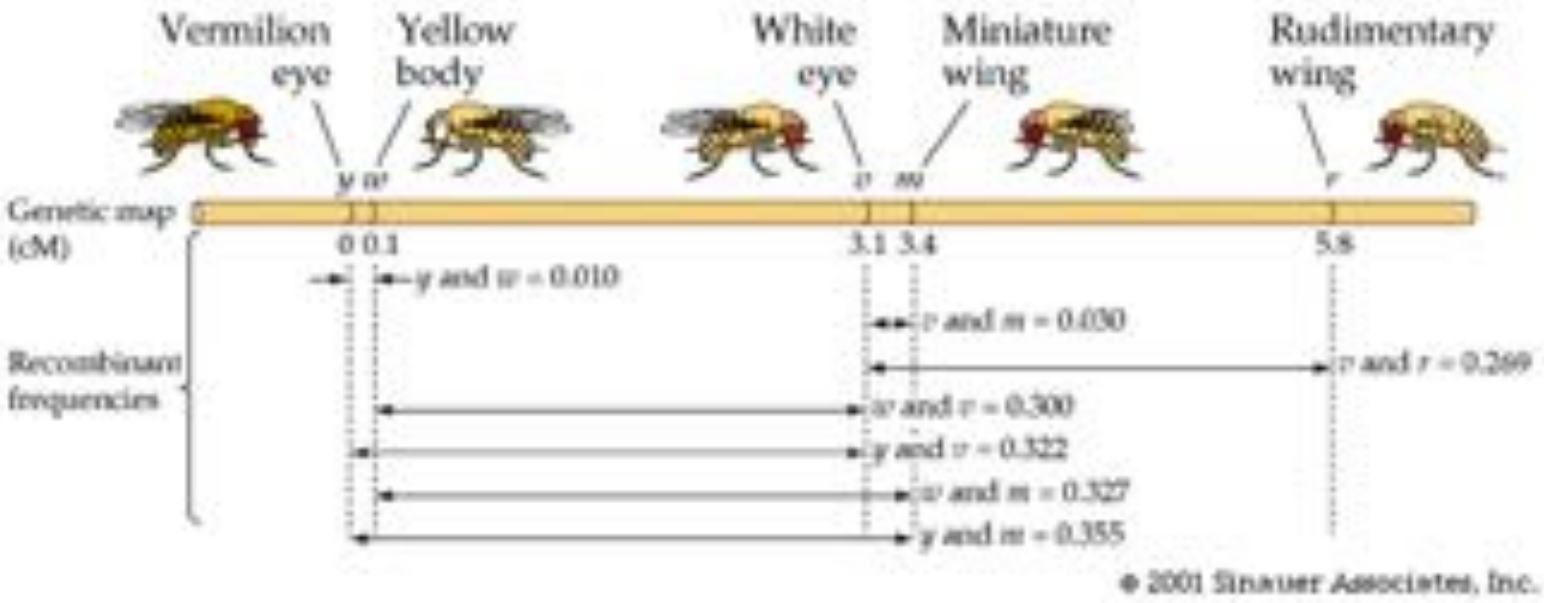
Dihybrid and cross over: what are the resulting gametes?

First genetic map



(b) Production of recombinant offspring

First genetic map



Based on the frequencies of recombinants, genetic distances between genes can be estimated.

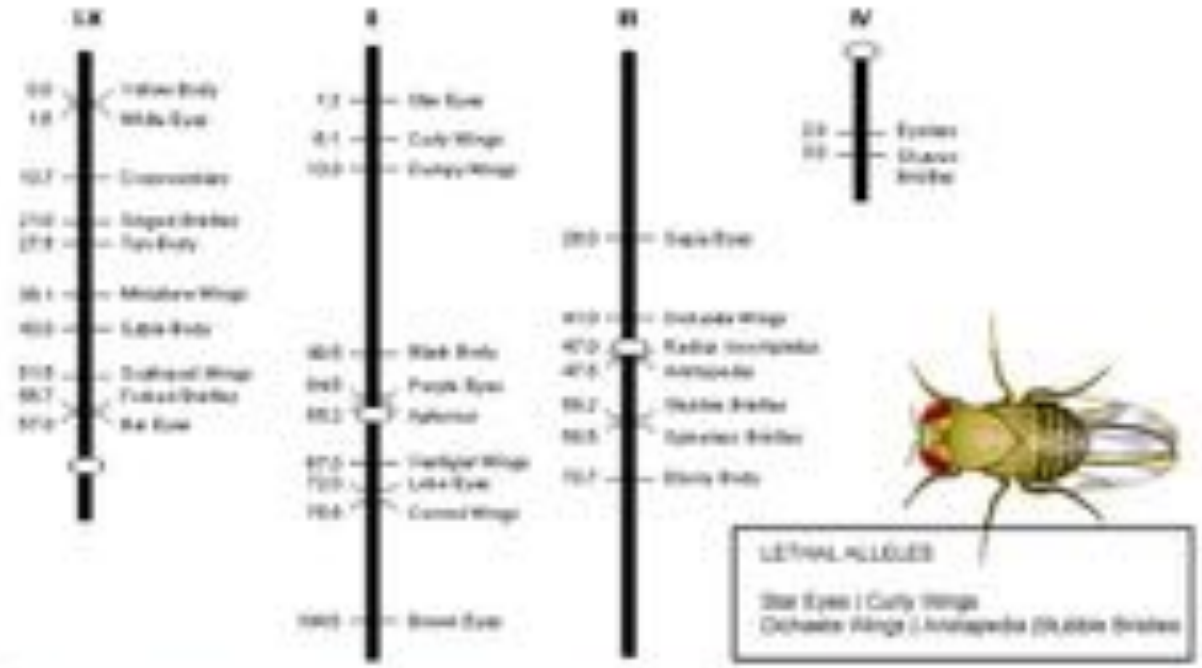
Fly Room - Significance

Genes on chromosomes

years, the fly boys began to [illegible]
the model that made Morgan's team so historically important. It
said that all traits were controlled by genes, and that these genes
resided on chromosomes in fixed spots, strung along like pearls
on a necklace. Because creatures inherit one copy of each chro-

Fly Room - Significance

Each chromosome - many genes

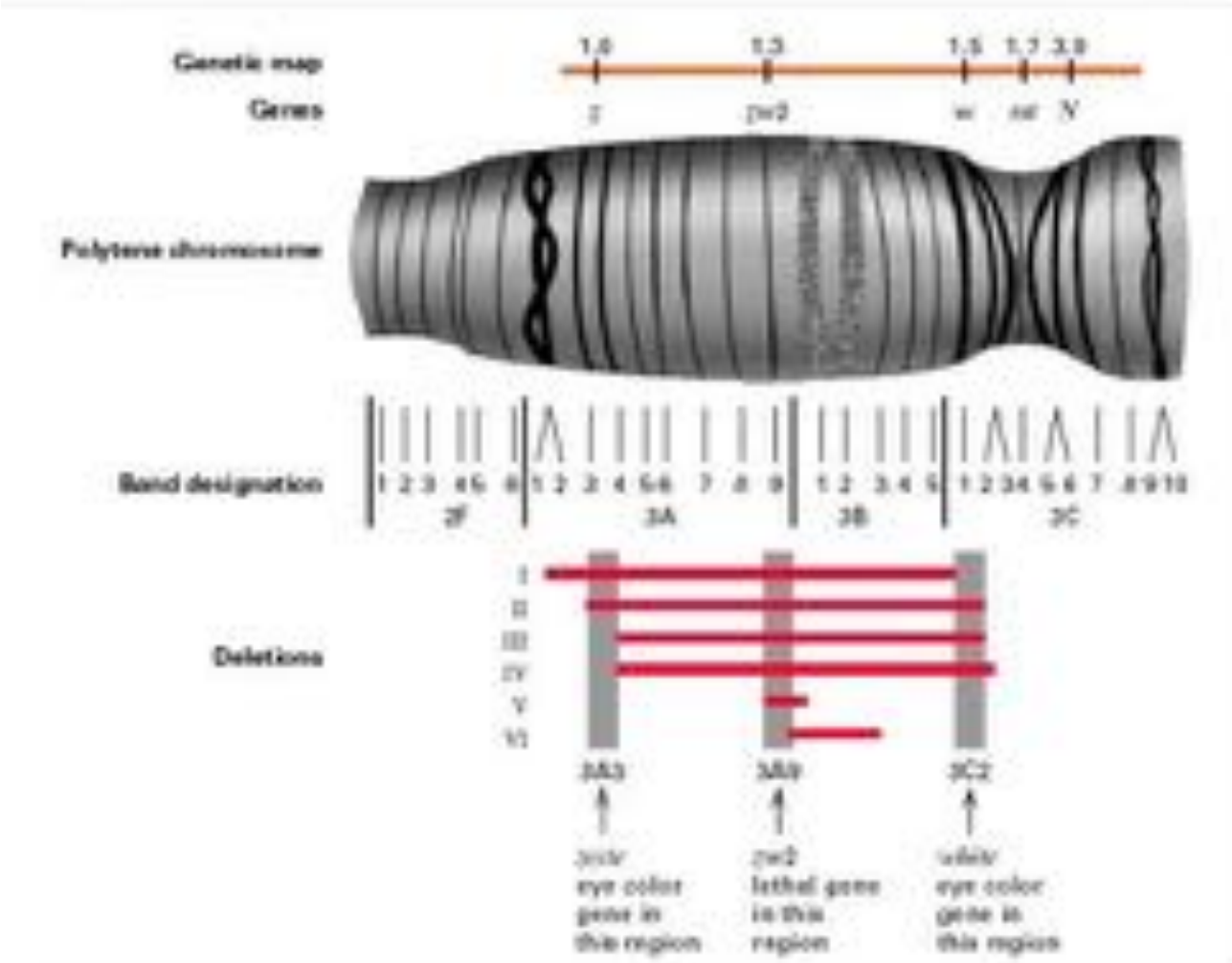


and protect everyone in genetics.

But historians can assign credit for some things. All the fly boys helped determine which clusters of traits got inherited together. More important, they discovered that four distinct clusters existed in flies—exactly the number of chromosome pairs. This was a huge boost for chromosome theory because it showed that every chromosome harbored multiple genes.

Fly Room - Significance

Linkage maps + cytogenetic maps



The chemical identity of the gene

What is the gene made of?

Griffith's transformation experiment

VOLUME XXVII JANUARY, 1928 No. 2

THE SIGNIFICANCE OF PNEUMOCOCCAL TYPES.

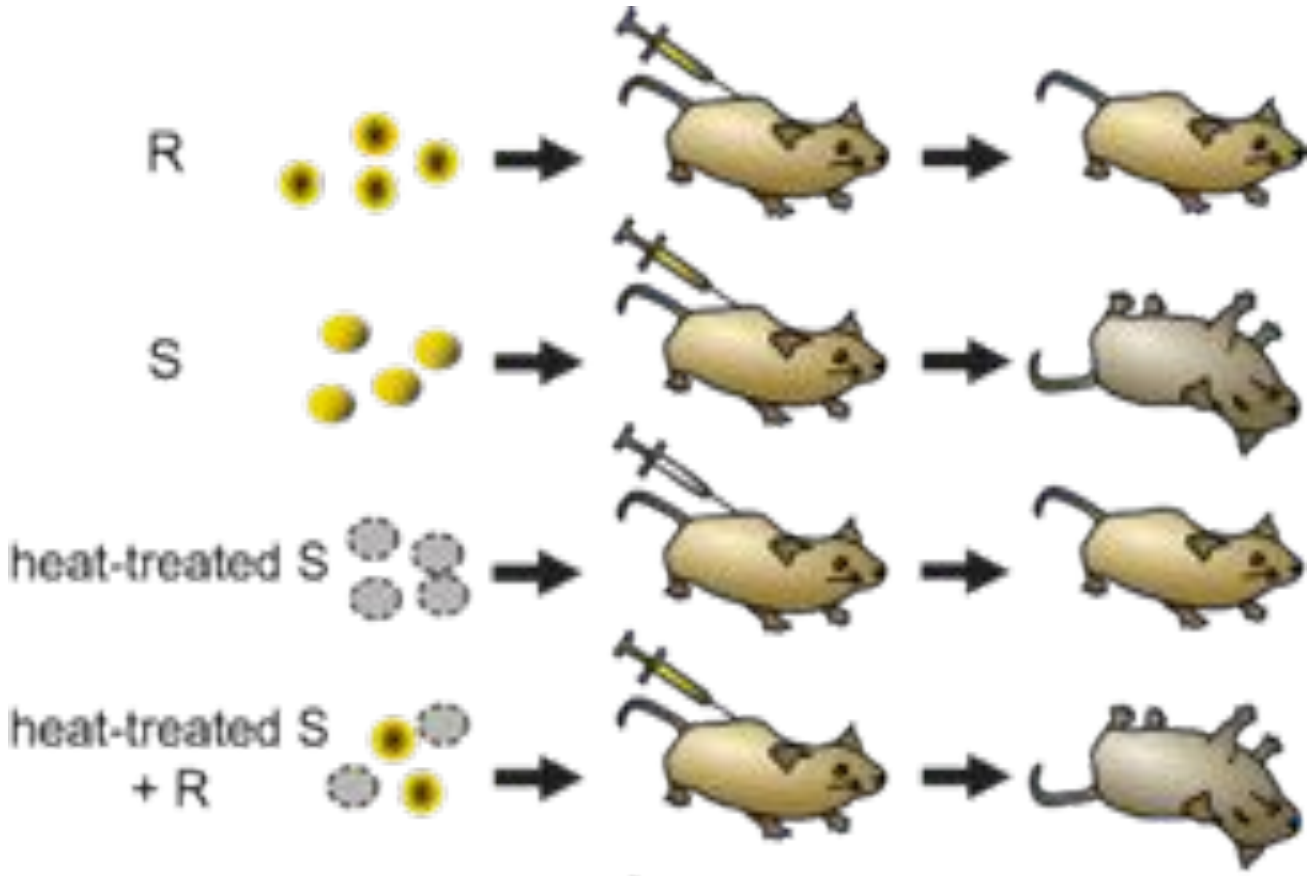
BY FRED. GRIFFITH, M.B.
(*A Medical Officer of the Ministry of Health.*)
(*From the Ministry's Pathological Laboratory.*)

CONTENTS.

Griffith, F. (1928). The Significance of Pneumococcal Types. The Journal of Hygiene, 27(2), 113–159.

Griffith's transformation experiment

- Griffith's conclusion: R changes into S by acquiring a molecule.
- He called the molecule (**transforming principle**).



Avery's transformation experiment

STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III

BY OSWALD T. AVERY, M.D., COLIN M. MacLEOD, M.D., AND MACLYN McCARTY,* M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

PLATE 1

(Received for publication, November 1, 1943)

Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters. Among microörganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of Pneumococcus. This phenomenon was first described by Griffith (1) who succeeded in transforming an attenuated and non-encapsulated (R) variant derived from one specific type into fully encapsulated and virulent (S) cells of a heterologous specific type. A typical instance will suffice to illustrate the techniques originally used and serve to indicate the wide variety of transformations that are possible within the limits of this bacterial species.

Griffith found that mice injected subcutaneously with a small amount of a living R culture derived from Pneumococcus Type II together with a large inoculum of heat-killed Type III (S) cells frequently succumbed to infection, and that the heart's blood of these animals yielded Type III pneumococci in pure culture. The fact that the R strain was avirulent and incapable by itself of causing fatal bacteremia and the additional fact that the heated suspension of Type III cells contained no viable organisms brought convincing evidence that the R forms growing under these conditions had newly acquired the capsular structure and biological specificity of Type III pneumococci.

The original observations of Griffith were later confirmed by Neufeld and Levinthal (2), and by Baerthens (3) abroad, and by Dawson (4) in this laboratory. Subsequently Dawson and Sia (5) succeeded in inducing transformation *in vitro*. This they accomplished by growing R cells in a fluid medium containing anti-R serum and heat-killed encapsulated S cells. They showed that in the test tube as in the animal body transformation can be selectively induced, depending on the type specificity of the S cells used in the reaction system. Later, Alloway (6) was able to cause

* Work done in part as Fellow in the Medical Sciences of the National Research Council.

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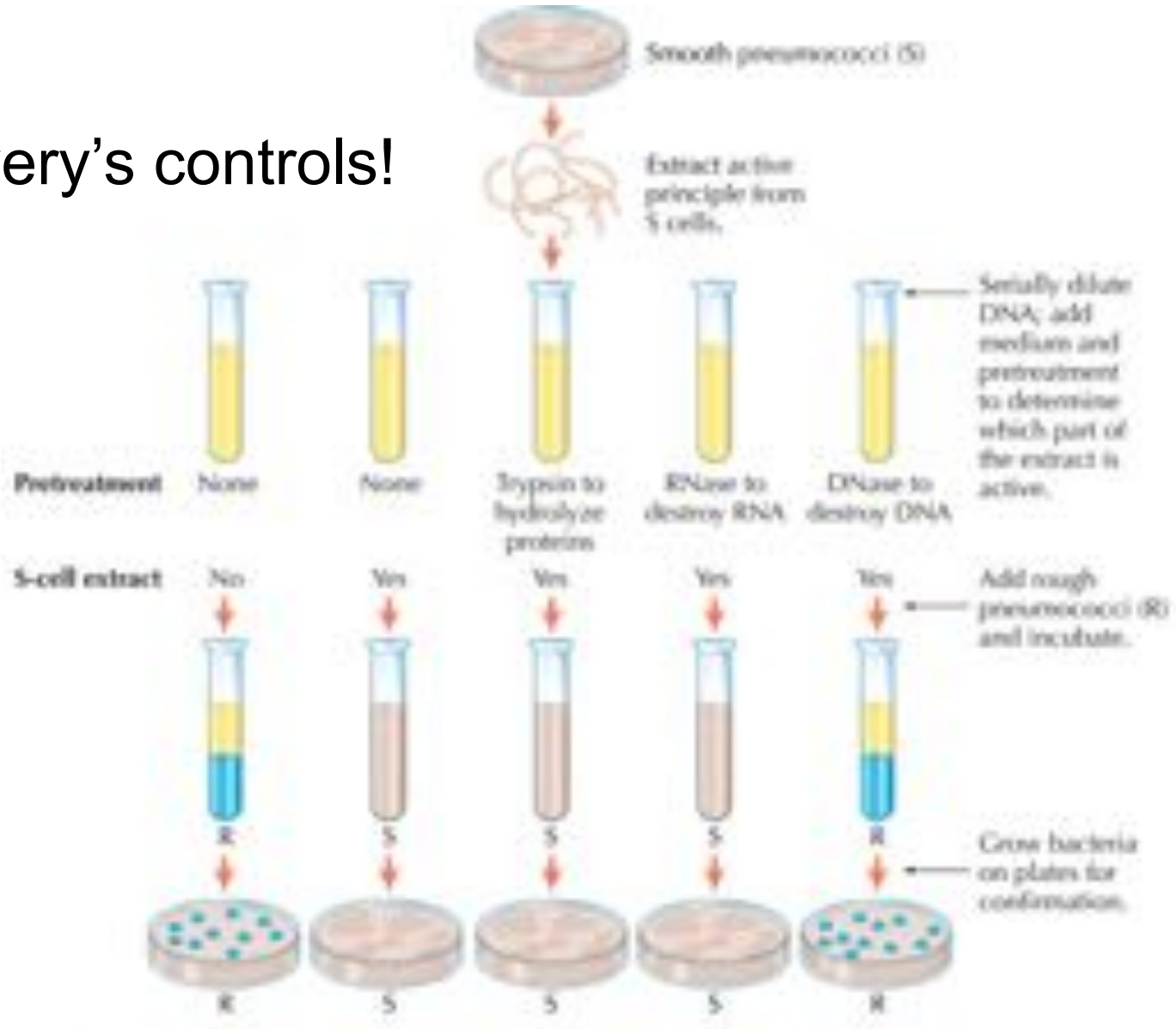
Avery, O. T., MacLeod, C. M., & McCarty, M. (1944). STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES : INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III. The Journal of Experimental Medicine, 79(2), 137–158.

Avery's transformation experiment

Avery's controls!



Hershey and Chase experiment

INDEPENDENT FUNCTIONS OF VIRAL PROTEIN AND NUCLEIC ACID IN GROWTH OF BACTERIOPHAGE*

By A. D. HERSHEY AND MARTHA CHASE

(From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island)

(Received for publication, April 9, 1952)

The work of Doermann (1948), Doermann and Dissoway (1949), and Anderson and Doermann (1952) has shown that bacteriophages T2, T3, and T4 multiply in the bacterial cell in a non-infective form. The same is true of the phage carried by certain lysogenic bacteria (Lwoff and Gutmann, 1950). Little else is known about the vegetative phase of these viruses. The experiments reported in this paper show that one of the first steps in the growth of T2 is the release from its protein coat of the nucleic acid of the virus particle, after which the bulk of the sulfur-containing protein has no further function.

Materials and Methods.—Phage T2 means in this paper the variety called T2H (Hershey, 1946); T2h means one of the host range mutants of T2; UV-phage means phage irradiated with ultraviolet light from a germicidal lamp (General Electric Co.) to a fractional survival of 10^{-4}.

Sensitive bacteria means a strain (H) of *Escherichia coli* sensitive to T2 and its h mutant; resistant bacteria B/2 means a strain resistant to T2 but sensitive to its h mutant; resistant bacteria B/2h means a strain resistant to both. These bacteria do not adsorb the phages to which they are resistant.

"Salt-poor" broth contains per liter 10 gm. bacto-peptone, 1 gm. glucose, and 1 gm. NaCl. "Broth" contains, in addition, 3 gm. bacto-beef extract and 4 gm. NaCl.

Glycerol-lactate medium contains per liter 70 mM sodium lactate, 4 gm. glycerol, 5 gm. NaCl, 2 gm. KCl, 1 gm. NH_4Cl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.01 gm. gelatin, 10 mg. P (as orthophosphate), and 10 mg. S (as $MgSO_4$), at pH 7.0.

Adsorption medium contains per liter 4 gm. NaCl, 5 gm. K_2SO_4, 1.5 gm. KH_2PO_4, 3.0 gm. Na_2HPO_4, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.01 gm. gelatin, at pH 7.0.

Veronal buffer contains per liter 1 gm. sodium diethylbarbiturate, 3 mM $MgSO_4$, and 1 gm. gelatin, at pH 8.0.

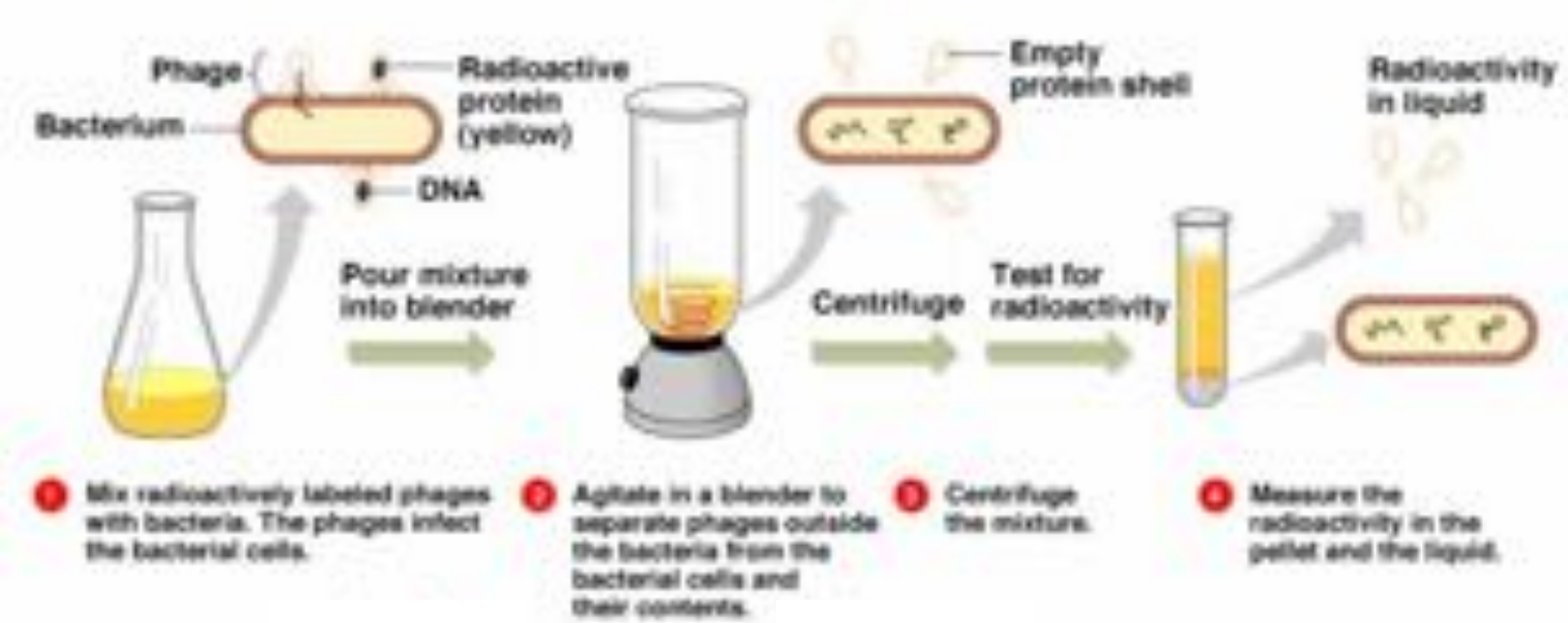
The HCN referred to in this paper consists of molar sodium cyanide solution neutralized when needed with phosphoric acid.

* This investigation was supported in part by a research grant from the National Microbiological Institute of the National Institutes of Health, Public Health Service. Radioactive isotopes were supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, United States Atomic Energy Commission.

Hershey A, Chase M (1952). "Independent functions of viral protein and nucleic acid in growth of bacteriophage" . J Gen Physiol 36 (1): 39–56.

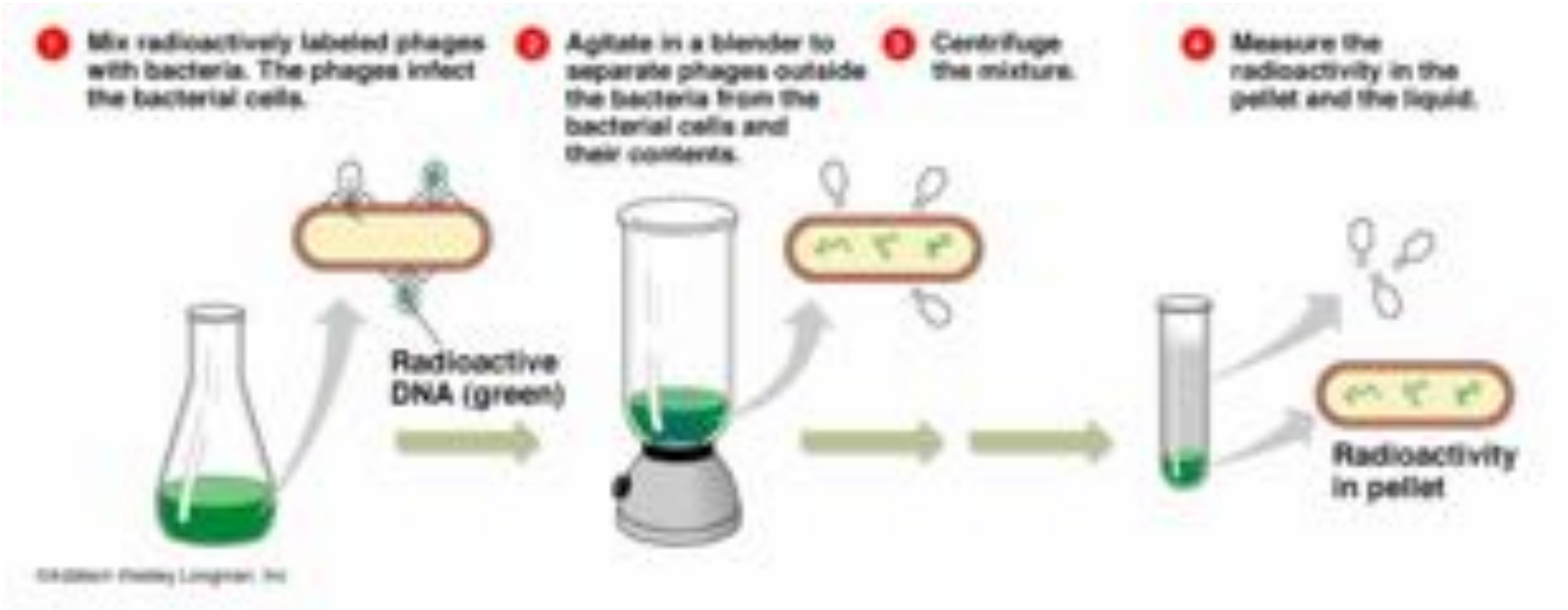
Hershey and Chase experiment 1

- Labeled proteins with radioactive S^{35}.
- Labeled proteins do not enter the bacteria cells.
- Proteins are not the genetic material.



Hershey and Chase experiment

- Labeled DNA with radioactive P^{32}.
- Labeled DNA enters the bacteria cells.
- DNA is the genetic material.



Expectations

- You knowledge about basic concepts strengthens.
- You know some of the key findings and its relation to the study of genomes.

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