Applied molecular biology

- Book: Glick, Pasternak, Molecular Biotechnology, *Principles and application of recombinant DNA*

- Biotechnology
- Molecular genetics

What Is Biotechnology?

- Using scientific methods with organisms to produce new products or new forms of organisms
- Any technique that uses living organisms or substances from those organisms to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses

Beer is an ancient foodstuff

Ancient beer was not just drink, but food

Thick drink with high caloric value as well as alcohol

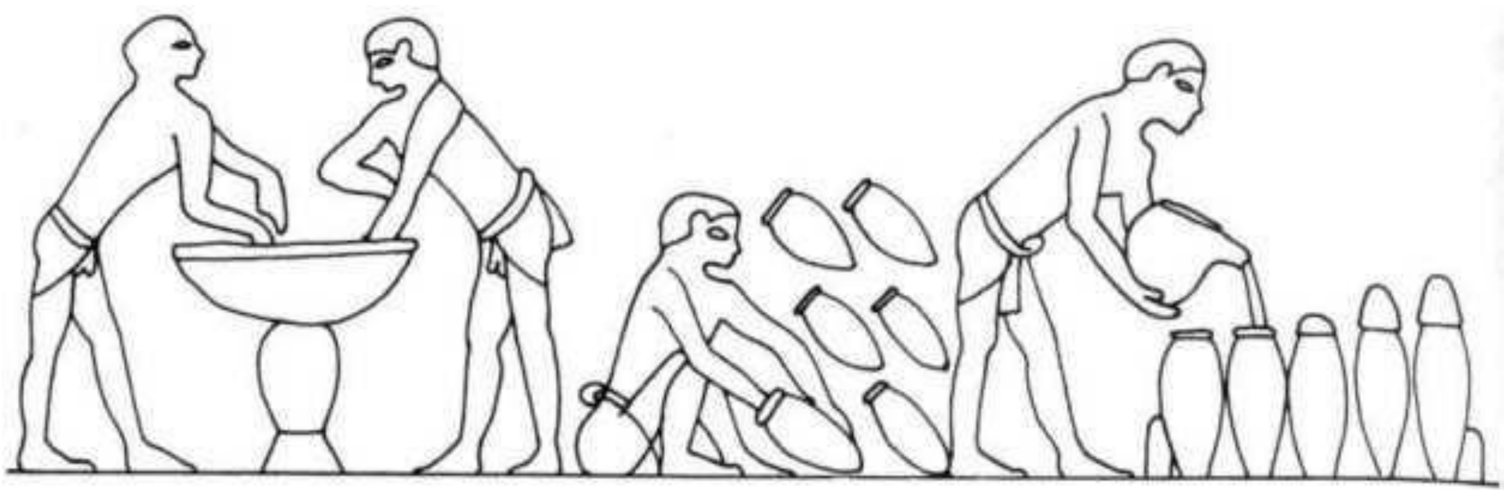


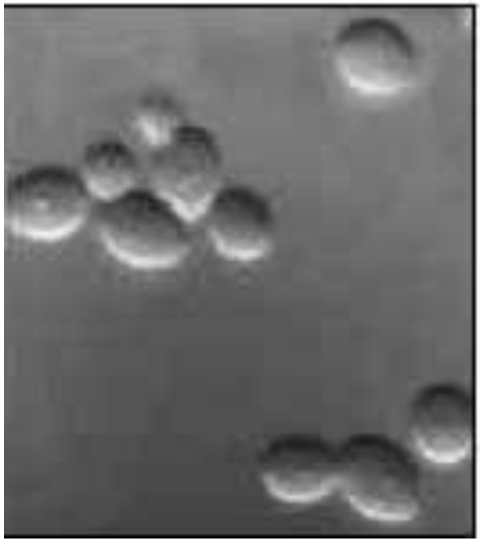
FIG. 106: *Beermaking in Egypt: a scene from a tomb of about 2500 B.C.*

Technology in the Ancient World
Engineering Library T16 .h64 1992

Both beer and bread were developed around the same time in the middle east

Early bread was flat, but when wild yeast contaminated the dough, a fluffier, sweeter bread was created

Beer arose out of the liquid soaked bread



Yeast cells

Cheese & yogurt also came about due to microbial contamination

Classical Biotechnology

- Refinement of fermentation techniques during 18th and 19th C.
- During 20th C. fermentation expanded to the production of:

  Glycerol

  Acetone

  Butanol

  Lactic acid

  Citric acid

Biopharmaceuticals

Herbal plants have been used since ancient times

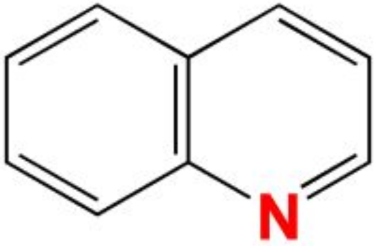
Even today, 25% of our common medicines contain at least some compounds obtained from plants

Why do plants create these compounds?

- Protection from herbivory and predation
- Allelopathy - plants secrete toxins from their roots that prevent the germination of other plants in their root zone

Alkaloids: Over 5,000 alkaloids have been identified in numerous plant families, most in the angiosperms

- Contain nitrogen
- Alkaline
- Bitter
- Physiological effect on animals, often on nervous system
- Names of most alkaloids end in "...ine"



http://www.life.umd.edu/classroom/bsci124/lec29.html
http://www.uky.edu/~dhild/biochem/26/quinoline.gif

Common Medicinal Alkaloids & their Sources:

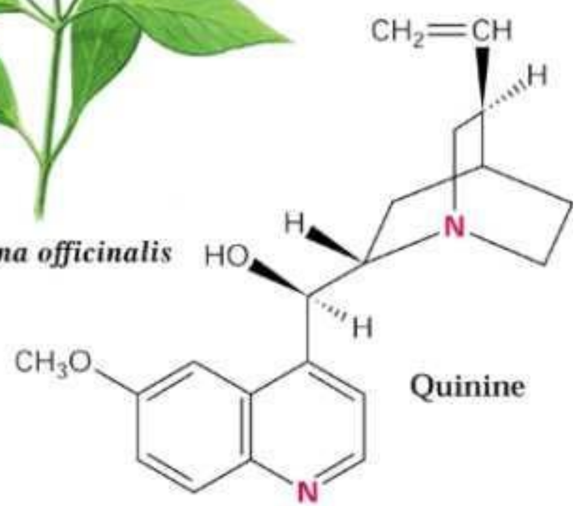
Morphine	Poppies
Caffeine	Coffee/Tea
Nicotine	Tabacco
Emetine	Ipecac
Atropine	Belladonna
Quinine	Cinchona Tree

During 19th C. quinine was critical to British colonial expansion

Extracted from the bark of the cinchona plant

Not enough could be extracted, another source was needed

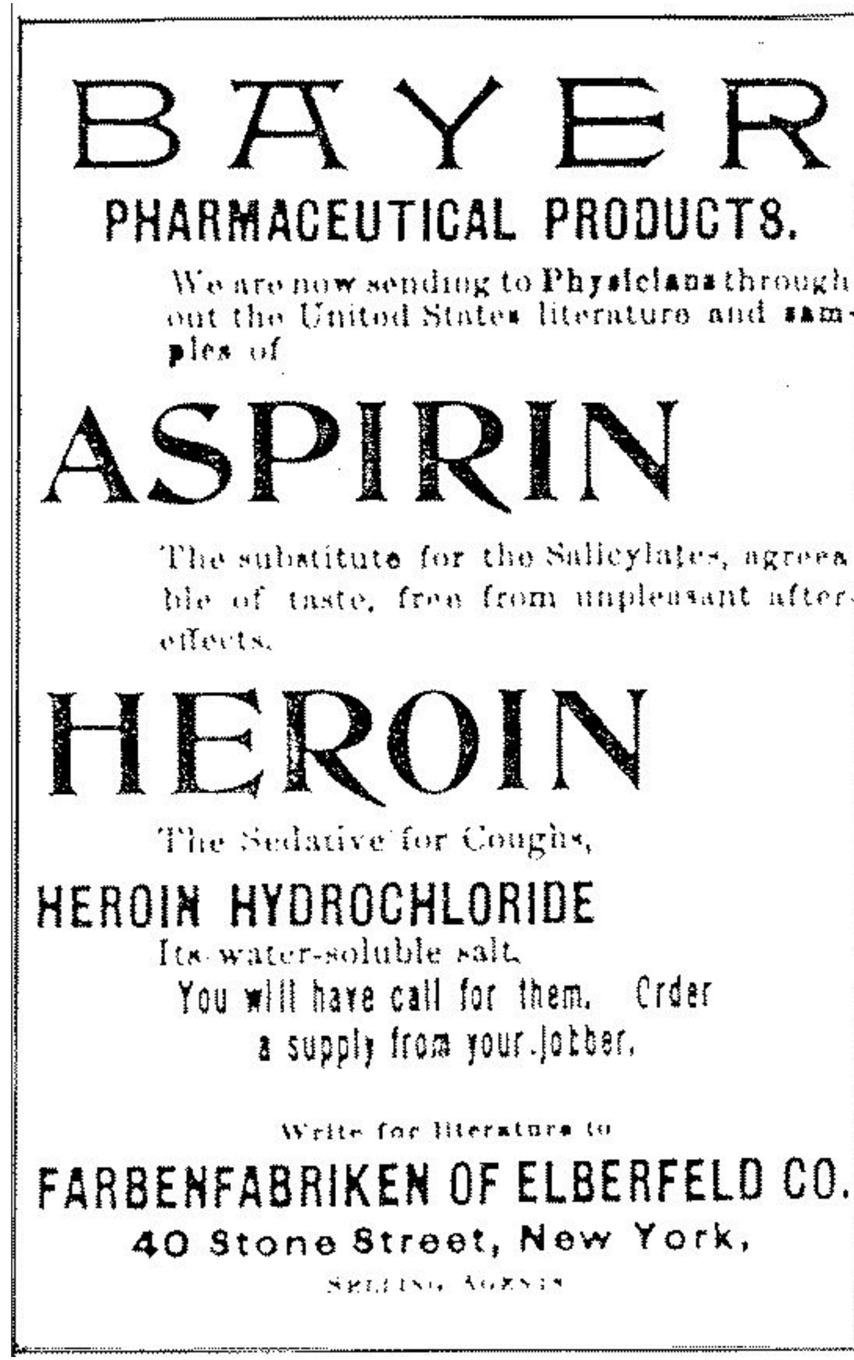
Cinchona officinalis



Quinine

http://www2.unil.ch/lpc/images/docu04/0_doc/quinine.gif

- Bayer discovered way to synthesize acetylsalicylic acid
- Known under its trade name, Aspirin

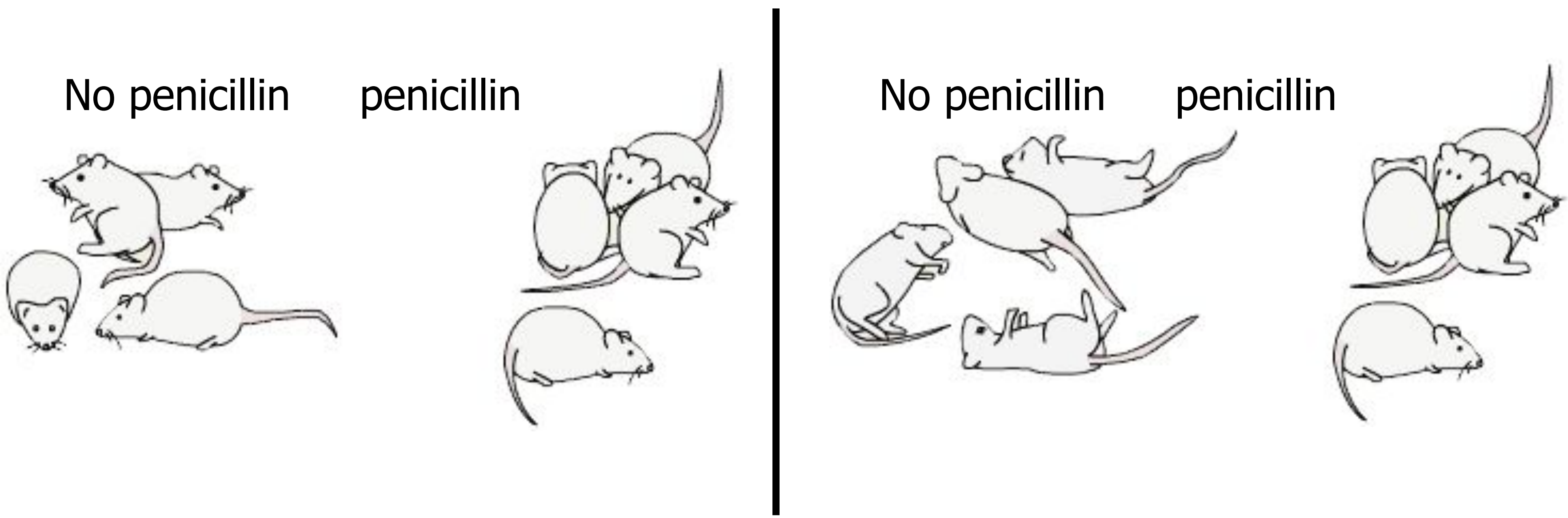


Penicillin

- In 1928 Alexander Fleming noticed something odd about a petri dish contaminated with mold
- The mold seemed to kill the bacteria
- Fleming was unable to isolate the bactericidal action

Penicillium mold

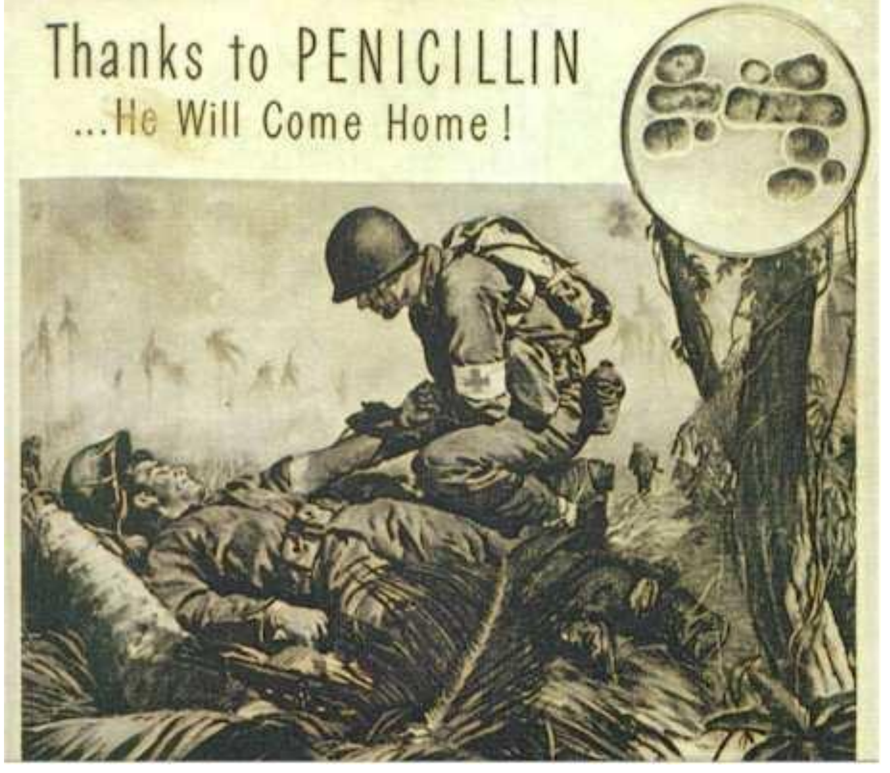
- In 1940 Norman Heatley finally showed that penicillin could stop infection
- Mice were infected with streptococcus bacteria
- Half were given penicillin.
- Those receiving penicillin survived, those that didn't died.

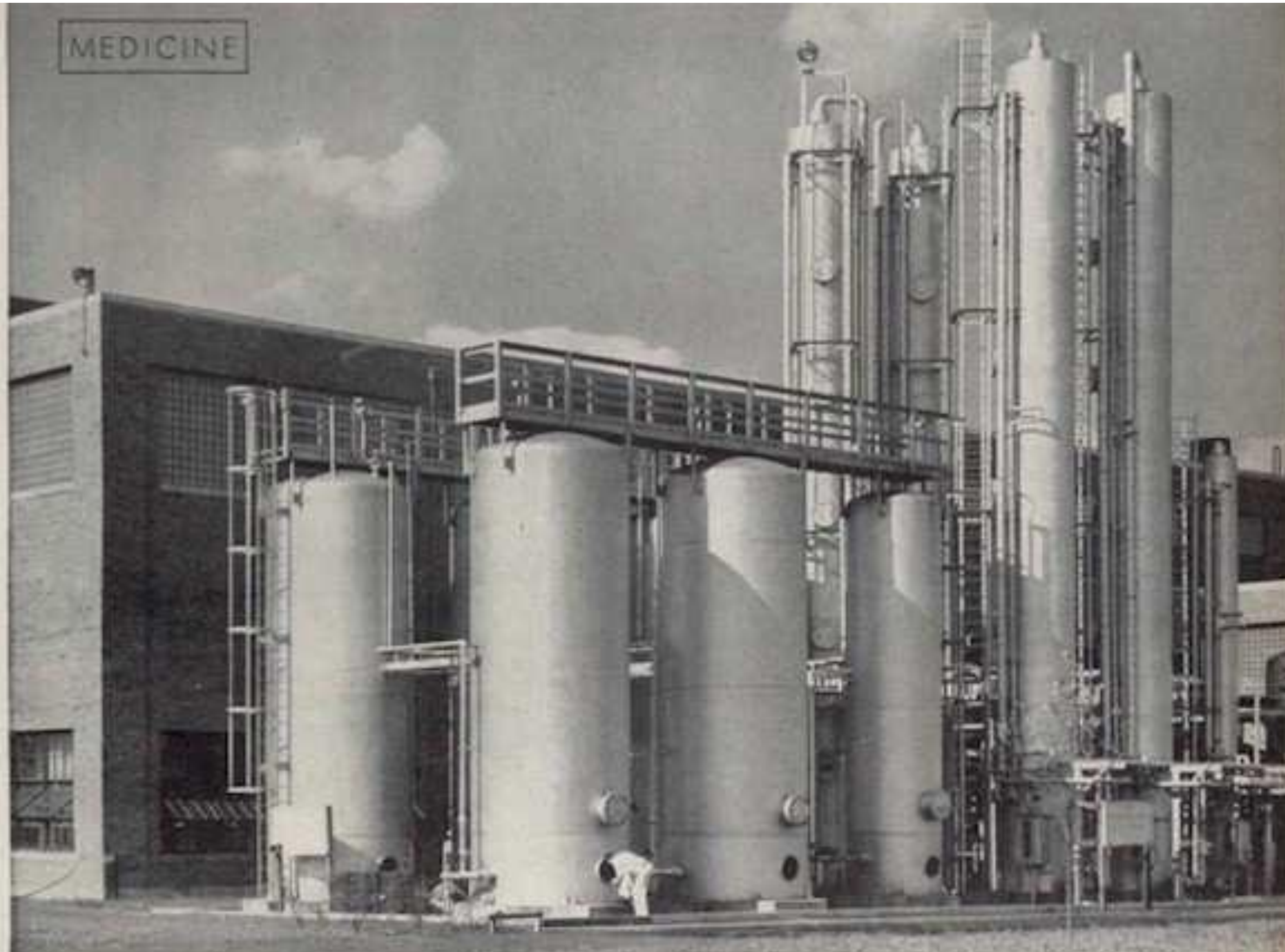


24 hrs. later

- 1st human patient was a policeman with staphylococcal & streptococcal infections which had already taken part of his face and an eye
- He began to recover, but later died
- 2nd patient was a 15 yr. old boy septic from a hip operation
- Two days after receiving penicillin his temperature dropped back to normal after being at 100° for 2 wks

- By D-Day the U.S. was making millions of doses.
- Unfortunately, neither Heatley nor his boss patented the discovery. This was done by U.S. firms.
- Thus for 25 yrs. England had to pay royalties on its own discovery.





HUGE TANKS AND STILLS ARE PART OF THE COMMERCIAL SOLVENTS' PENICILLIN PLANT IN TERRE HAUTE, IND. THIS IS ONE OF [illegible] U. S. PLANTS MASS-PRODUCING THE DRUG

PENICILLIN

MASS PRODUCTION OF DRUG REPLACES SLOW LABORATORY METHODS TO MEET MILITARY NEEDS AND PROVIDE A LIMITED CIVILIAN SUPPLY

Genetics - historical perspective

- Practical genetics 7,000 yeas ago
- corn breeding - Central America
- rice breeding - China
- horse pedigree - Babylon

Genetics - science - Mendel

A domesticated animal is one which has been bred in captivity

Thru artifical selection they are modified from their ancestors for use by humans

Before

After

http://www.doganswers.com/method.htm

http://www.billybear4kids.com/animal/whose-toes/toes-61a-wolf.html

Wolf/Dog domestication lead to:

- Alteration in body size
- Reduction in skull & tooth size
- Shortening of the jaw bones
- Affection for humans
- Variation in coat color
- Tendency towards barking

By 6000BC dog skeletons are found along side human remains

Modern sheep have been bred not to lose their wool

http://nemp.otago.ac.nz/read_speak/2004/read_comprehension/shrek.htm

Most domesticated species arose in SW Asia or China

- Of the ~150 species of terrestrial non-carnivores >100 lbs, only 14 have been domesticated
- 13 are of Eurasian origin, one from mesoamerica
- None derive from Australia or sub-Saharan Africa

Desirable Characteristics for Domestication of an Animal Species

- Value to humans as food, draft, fiber, or hunting
- Large herbivores offer energy use advantages
- Rapidly reach their desired size
- Must be able to breed in captivity
- Good disposition & social structure

Plant Domestication

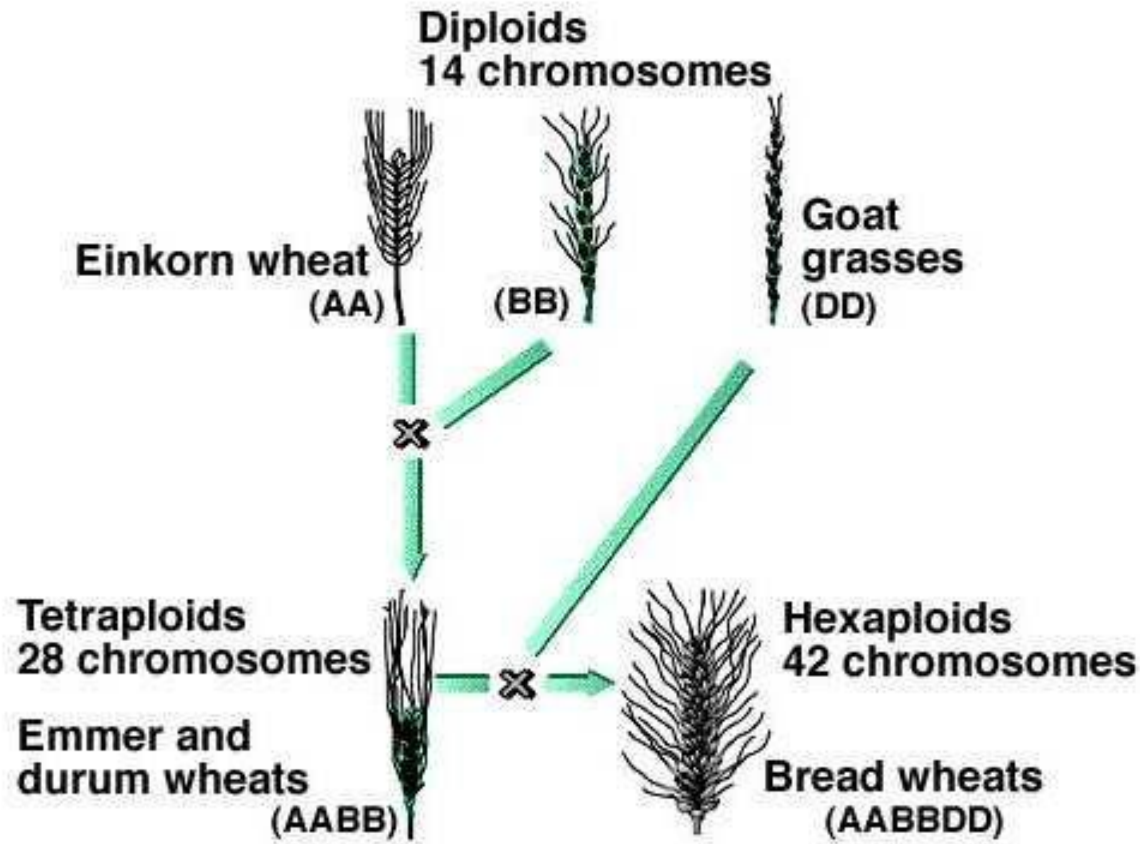
The switch from hunter-gatherer to farmer took place between 10 000 & 5 000 years ago

- Both Eurasia & the Americas developed large numbers of domesticated crops
- The development of agriculture required changes in wild plants such that they were amendable to cultivation
- Many of these changes were either brought about by humans or were capitalized by them

Example

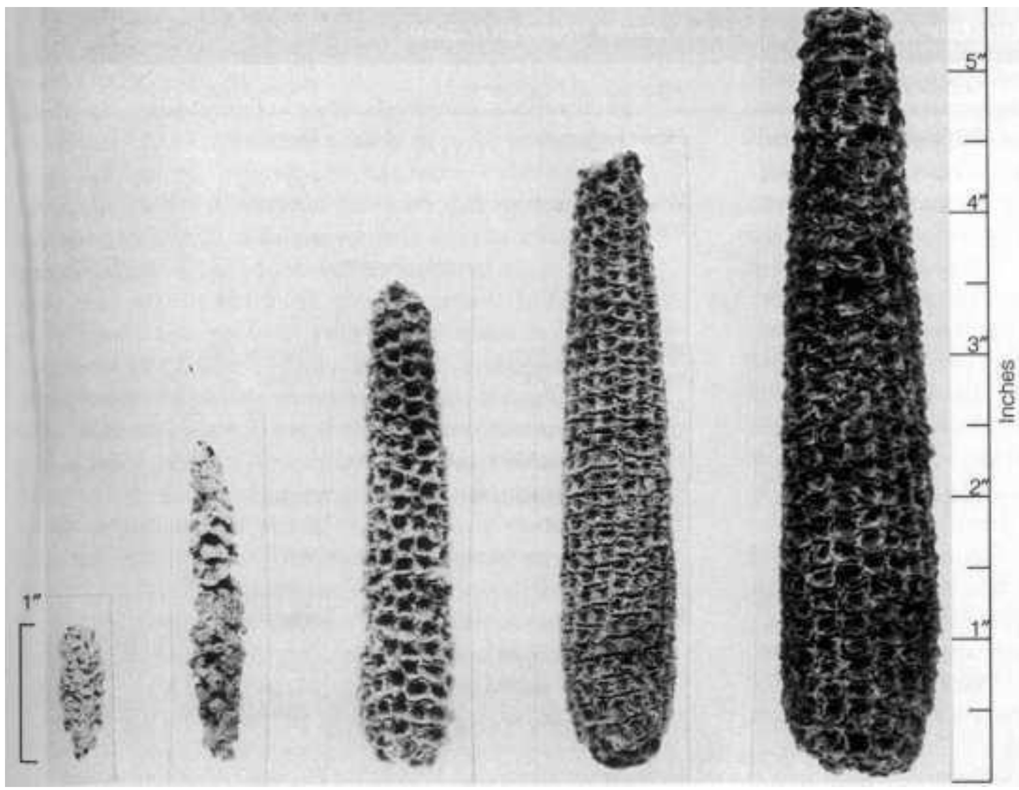
- Wheat is a grass spread seeds called grains
- Mutants developed that did not lose seed
- This made it easier for humans to collect

Hybridization played a role in the evolution of modern grains



The evolution of modern corn took several thousand years

Selection for larger ears by mesoamericans created modern corn by the time Europeans had reached the Americas



Changes in corn size from 5000 BCE to 1500CE

Mutation responsible for this change has been identified

It is not a change in a gene itself, rather it is a decrease in the expression of the gene *tb1*

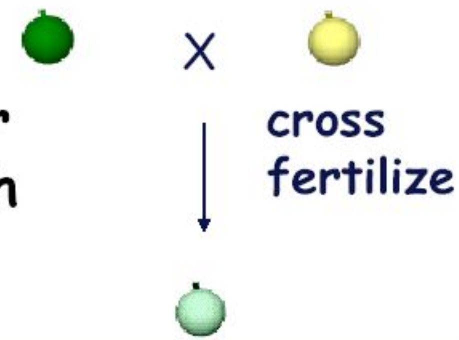
Mendelian Analysis

Gregor Mendel, Father of Genetics

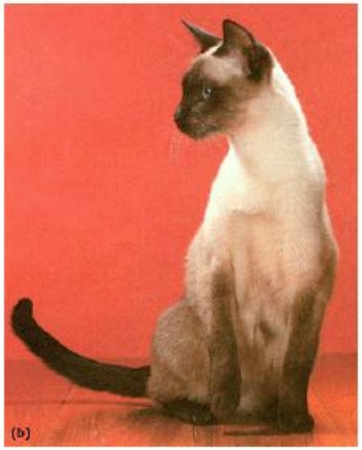
1822-1884

Prevalent Theories

- Blending inheritance:
  - Substances blended together to yield unique individual with traits from both parents



- Darwin:
  - Particles, called gemmules, were collected from all parts of body and became concentrated in germ cells



Mendel was skeptical of these ideas, and was particularly intrigued by some early observations by Kolreuter, 1840.

Crossed purple flowered plants with white flowered plants, the progeny were all purple, but then in the next generation, white flowered plants reappeared.

How can it be that traits can be lost in the hybrid,and then reappear in the next generation.

.

Who was Mendel?

What did he do differently?

Mendel was trained in several disciplines.

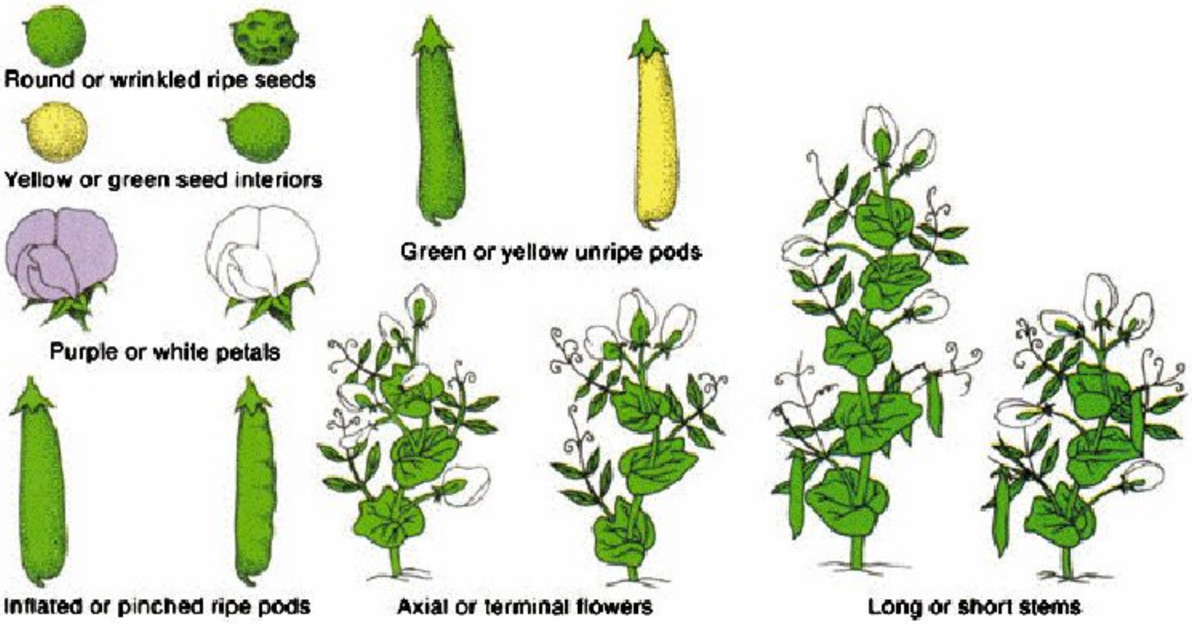
- Physics (with Christian Doppler)
- Mathematics
- Botany

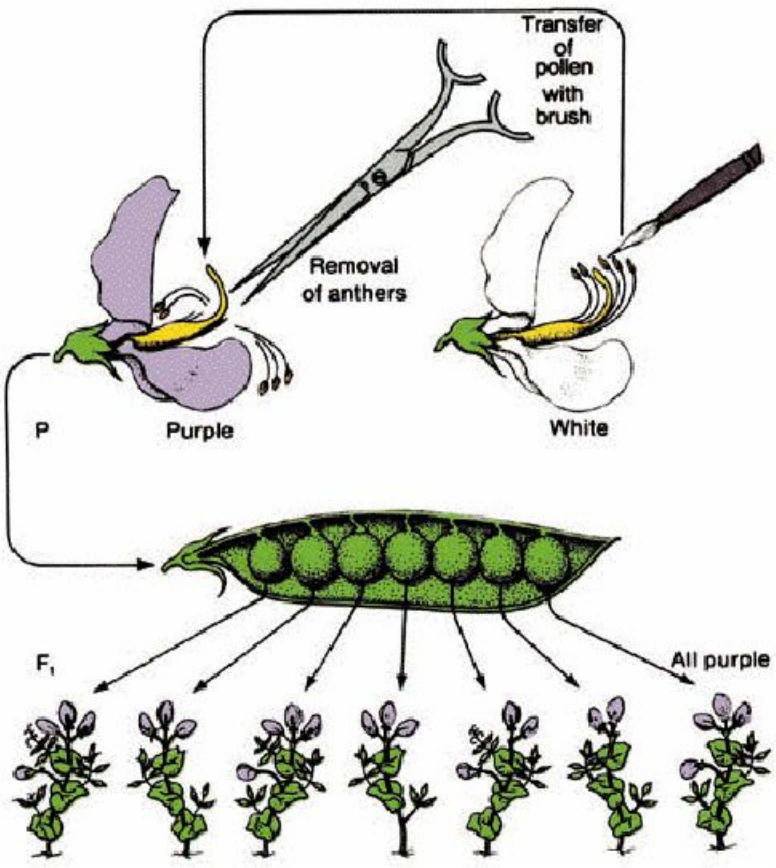
Mendel brought to Biology methods that were standard in Physics

- Limited the number of variables
- Quantitated results
- Came up with models that could be tested

Mendel did the following:

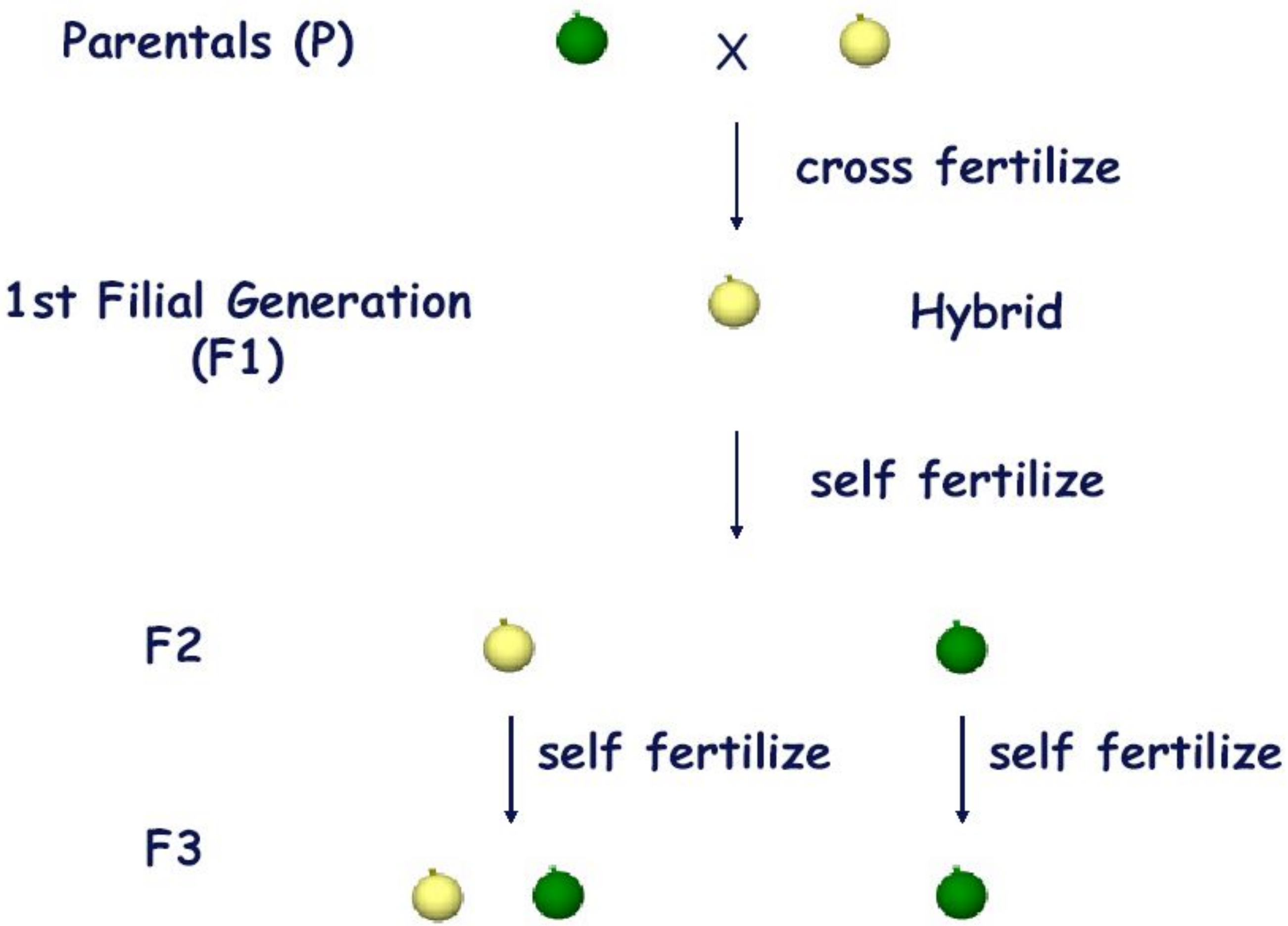
1. Isolated pure true-breeding lines of peas for seven different characteristics (plants that breed the same characteristics after selfing for at least two generations).





Cross fertilize:
Transfer pollen from one plant to the ovule of the second plant

Self fertilize:
Allow pollen of the plant to fertilize it's own ovules

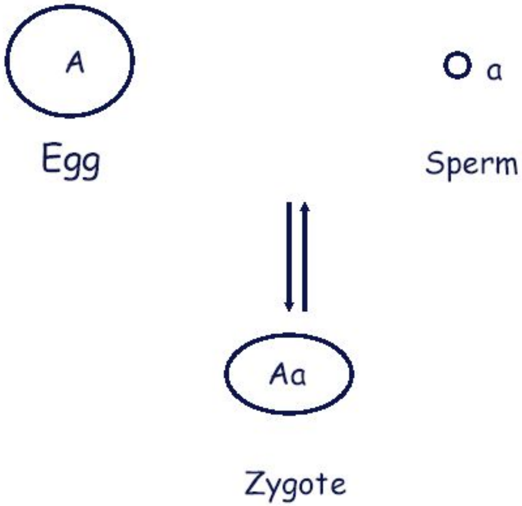


Parentals (P)
X
cross fertilize
1st Filial Generation
(F1)
Hybrid
self fertilize
F2
self fertilize
self fertilize
F3

Parents	F1 (hybrid)	self	F2	
yellow	yellow		yellow	green
X			6022	2001
green			3:1	

How could one explain the 3:1 ratios observed in monohybrid crosses?

Mendel had a strong background in probabilities and quickly developed a model



Single determinant in egg and sperm that come together in the zygote.

The simplest

Mendel's Theory

1-Hereditary determinants are of a particulate nature

2-Each adult pea has 2 determinants (which we now call alleles) for each character

3-The gametes only have 1 determinant for each character

4-Each determinant segregates equally into gametes

5-Union of 2 gametes occurs randomly with regard to genetic determinants

Schematically:

Y - dominant allele*, yellow
y - recessive allele, green

Each adult has two determinants:
If both are the same, homozygous
If they are different, heterozygous

* different forms of same gene

Using this nomenclature, lets go through the cross again.

P

Each adult has two determinants

genotype	YY	x	yy	(homozygotes, Pure breeding)
phenotype	yellow		green	

↓ ↓ Each determinant segregates equally

gametes	Y	y

↓ Random union of gametes

F1 Yy (heterozygote)

F2 results

Equal segregation of determinants

F1 male gametes (pollen)

Punnett Square

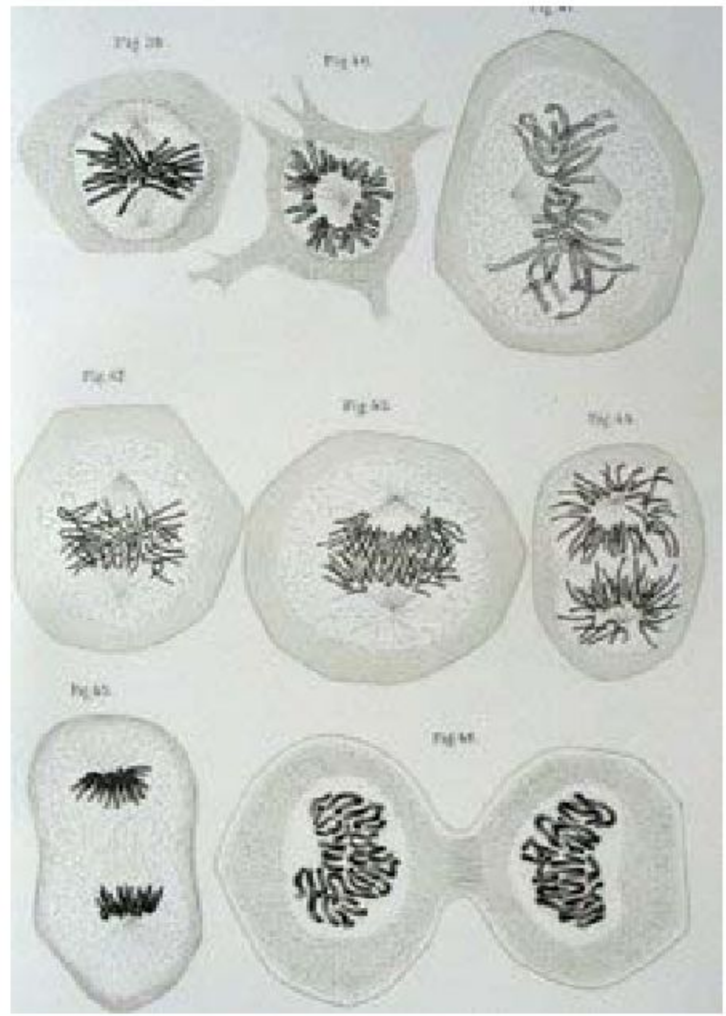
F1 female gametes (egg)	1/2 Y	1/2 y
1/2 Y	YY	Yy
1/2 y	Yy	yy

1/4 YY + 1/2Yy + 1/4 yy

Genotypic ratio. 1 : 2 : 1

1882 - Walter Fleming

- Stained cells with dyes and discovered rod-shapped bodies
- Chromosomes – colored bodies

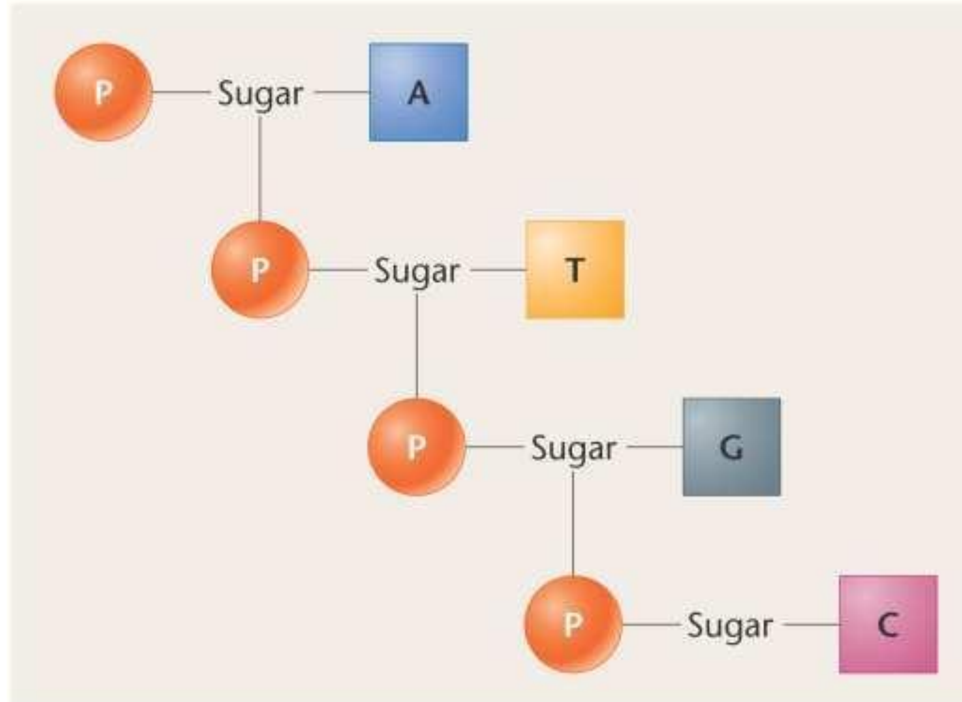


Later Concepts

- 1900 - Not until 34 years after its publication did Mendel's work receive additional attention, with publications in 1900 by three Botanists: Hugo de Vries, Carl Correns, and Erich von Tsernak;
- 1902 - Walter Sutton first integrated the concepts of chromosomes with Mendel's laws, in studies of grasshopper reproduction and cell division and concluded that Mendel's heritable factors must be on the chromosomes.
- 1907 – T.H. Morgan began his work with fruit flies, ultimately mapping gene locations.

First Structure

- By 1910 actual components known (nucleotides)
  - Phoebus Levene proposed a tetranucleotide structure for DNA



- Tetranucleotide repeat of ATCG
- Own data showed nucleotides not in 1:1:1:1 ratio
  - Differences "probably experimental error…"

So…

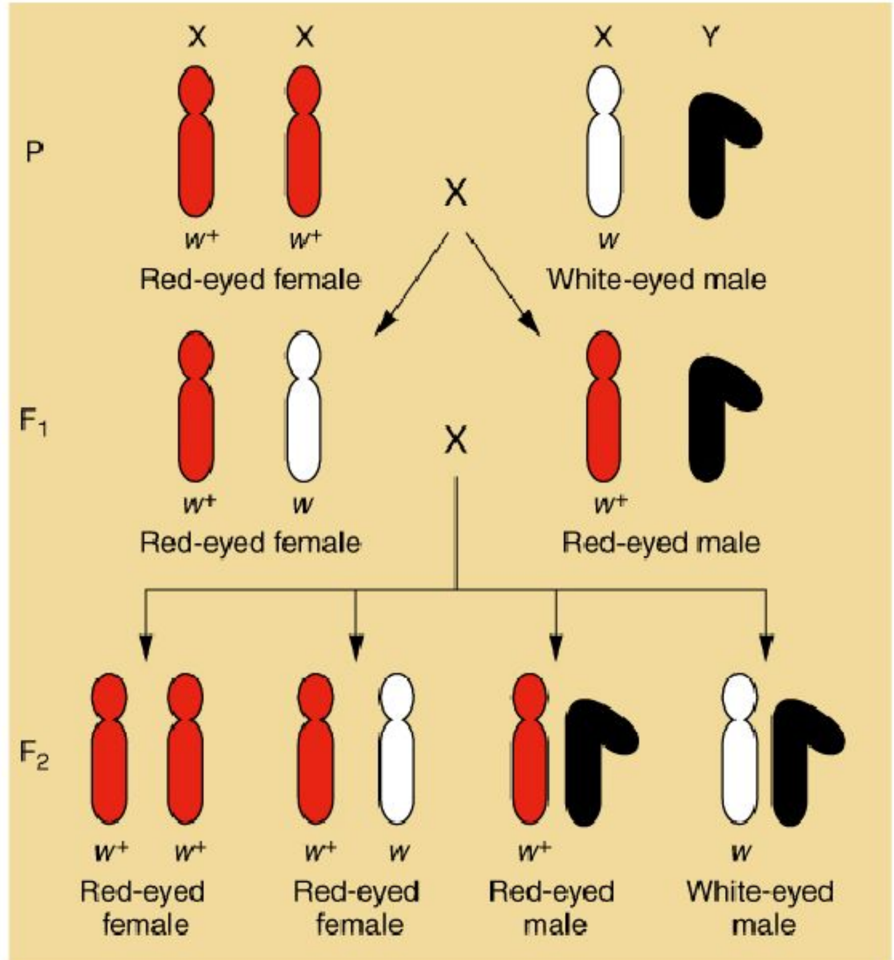
- If DNA was a single covalently bonded tetranucleotide structure then it couldn’t easily encode information
- Proteins, on the other hand, had 20 different amino acids and could have lots of variation
- Most geneticists focused on “transmission genetics” and passively accepted proteins as being the likely genetic material

T. H. Morgan's Fruit Flies 1907-1930s

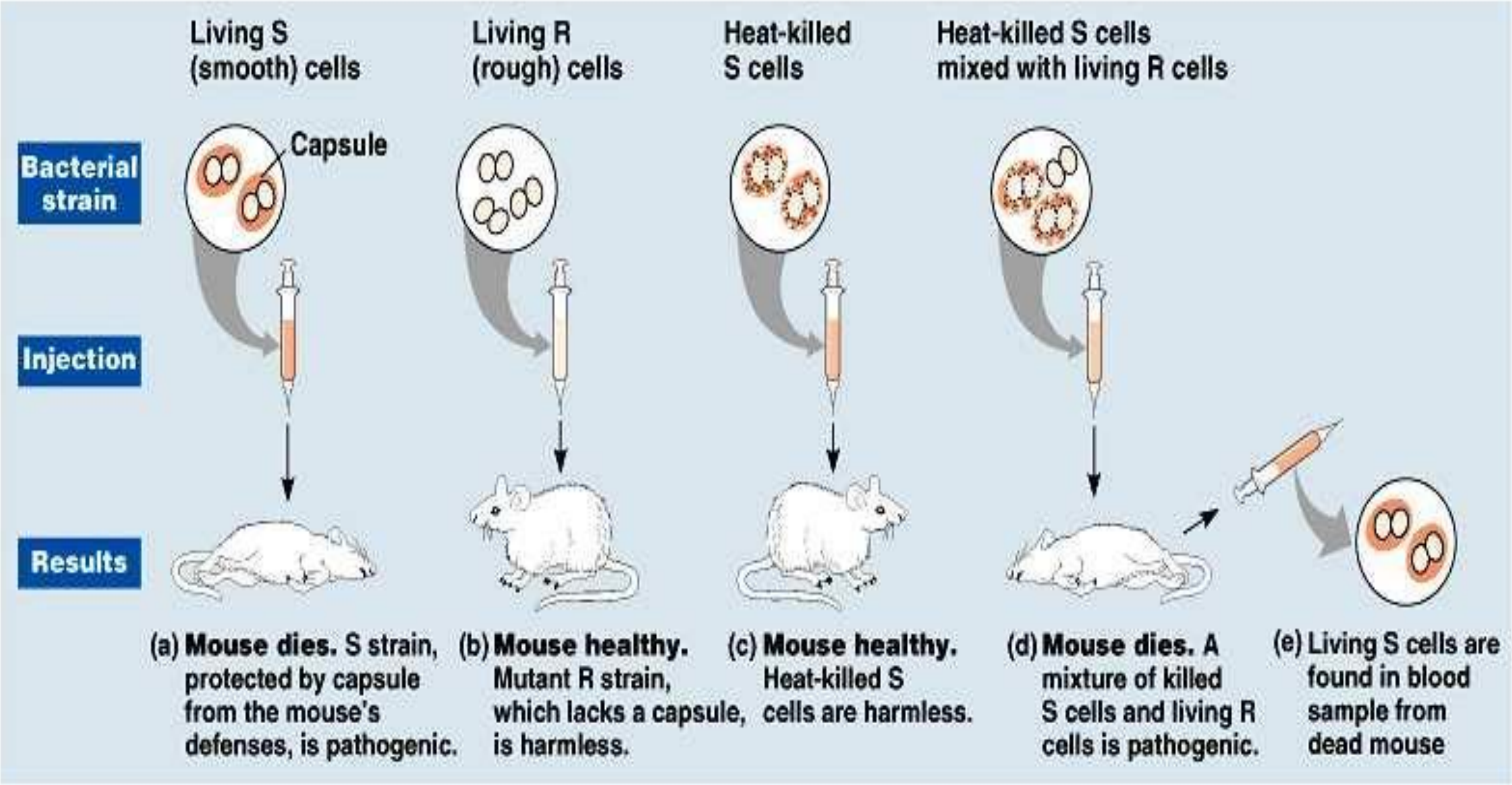
Fig. 6.3 Morgan's hypothesis diagramed

w is on X chr.

There is no *w+* allele on Y



Frederick Griffith, 1928
Transformation of Bacteria



Transforming factor ?

Avery, McCarty and MacLeod

- After 10 yrs of effort published work using Griffith's approach to assay for the genetic material
  - Used
    - Cell-free extract of S cells
    - From 75 liters of cell culture obtained 10-25 mg of "active factor
    - Proteases, RNases, DNases, etc.

- Transforming factor is DNA

Erwin Chargaff

- 1949-1953
- Digested many DNAs and subjected products to chromatographic separation
- Results
  - A = T, C = G
  - A + G = C + T (purine = pyrimidine)
  - A + T does not equal C + G
    - Members of a species similar but different species vary in AT/CG ratio

Table 10.3 DNA Base Composition Data

(a) Chargaff's data*

Organism's/Source	Molar proportions[a]			
	1	2	3	4
	A	T	G	C
Ox thymus	26	25	21	16
Ox spleen	25	24	20	15
Yeast	24	25	14	13
Avian tubercle bacilli	12	11	28	26
Human sperm	29	31	18	18

(c) G + C content in several organisms

Organism	%G + C
Phage T2	36.0
Drosophila	45.0
Maize	49.1
Euglena	53.5
Neurospora	53.7

(b) Base compositions of DNAs from various sources

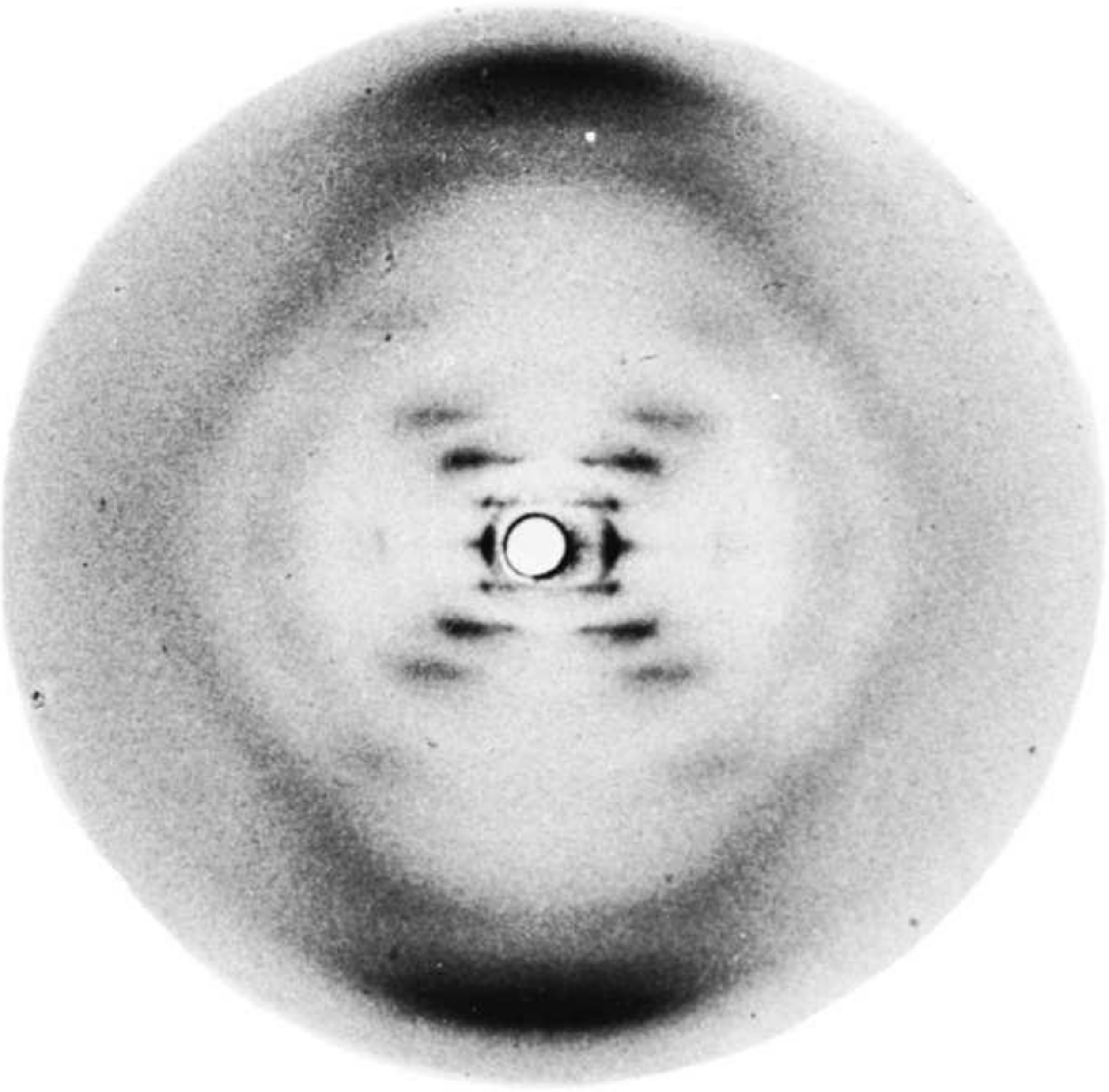
Source	Base composition				Base ratio		A + T/G + C ratio	
	1	2	3	4	5	6	7	8
	A	T	G	C	A/T	G/C	(A + G)/(C + T)	(A + T)/(C + G)
Human	30.9	29.4	19.9	19.8	1.05	1.00	1.04	1.52
Sea urchin	32.8	32.1	17.7	17.3	1.02	1.02	1.02	1.58
E. coli	24.7	23.6	26.0	25.7	1.04	1.01	1.03	0.93
Sarcina lutea	13.4	12.4	37.1	37.1	1.08	1.00	1.04	0.35
T7 bacteriophage	26.0	26.0	24.0	24.0	1.00	1.00	1.00	1.08

* *Source*: From Chargaff, 1950.

[a] Moles of nitrogenous constituent per mole of P. (Often, the recovery was less than 100 percent.)

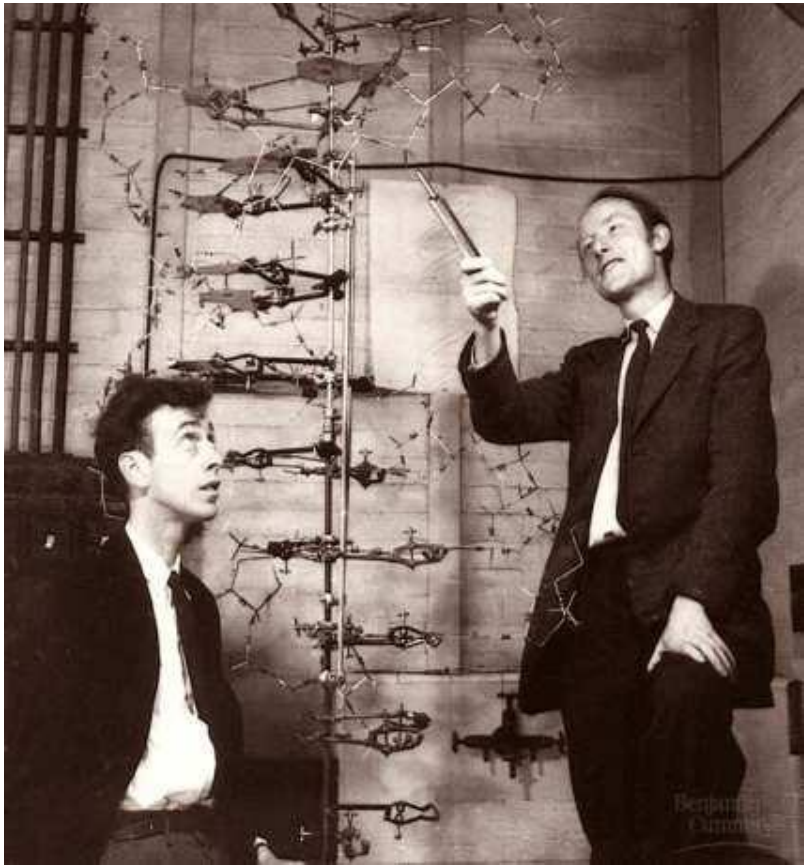
X-ray Crystallography of DNA

- Franklin and Wilkins



Watson and Crick

- 1953 propose double helix model
  - Right-handed double helix

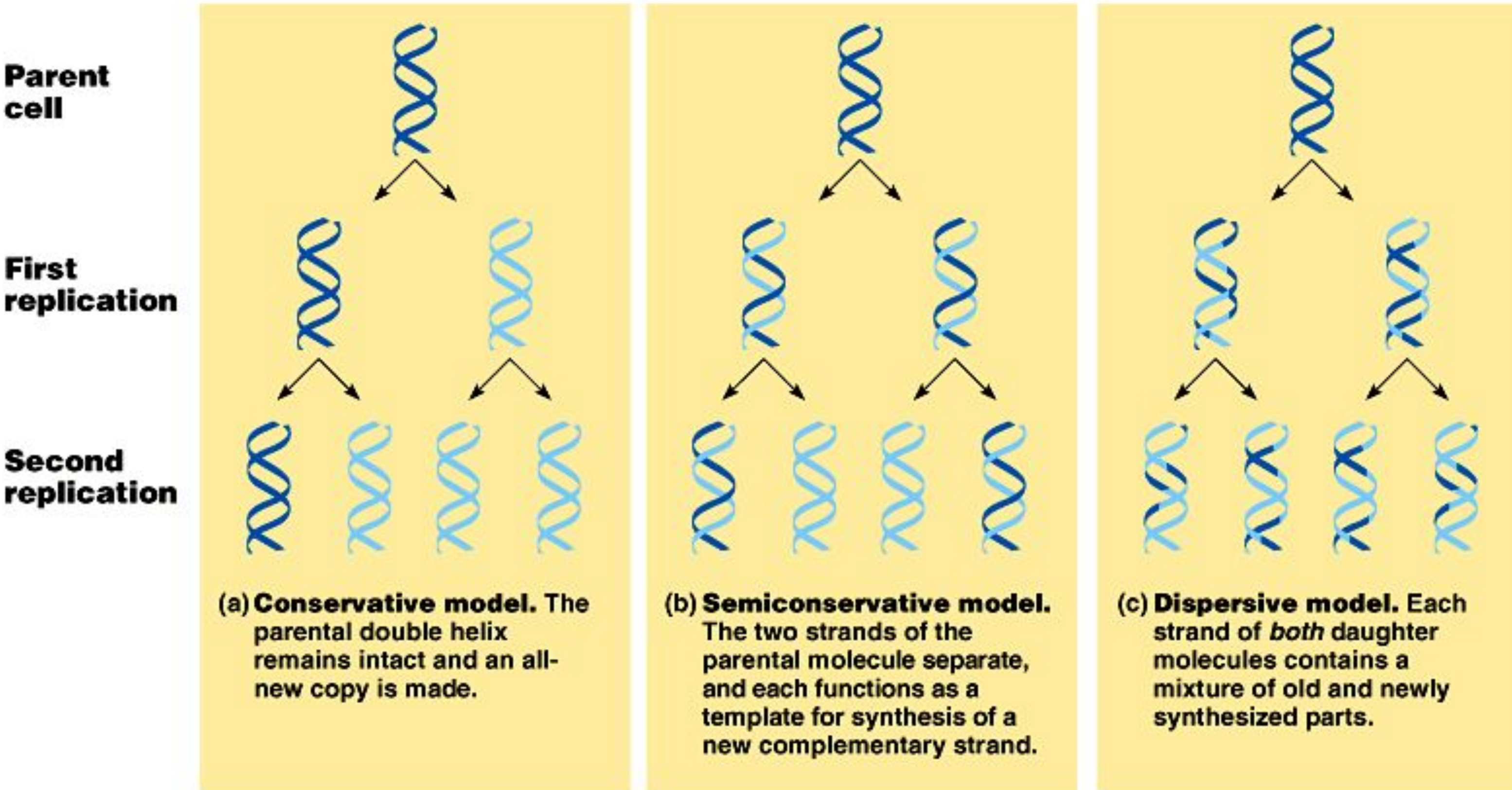


Collaborated at Cambridge, England.

Impact

- Article in *Nature*
  - "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copy mechanism for the genetic material"
    - Second paper 2 months later describes semiconservative replication and that mutations must change bases in DNA (information encoded in the bases and their order)
- DNA became the genetic material…

DNA Replication



(a) **Conservative model.** The parental double helix remains intact and an all-new copy is made.

(b) **Semiconservative model.** The two strands of the parental molecule separate, and each functions as a template for synthesis of a new complementary strand.

(c) **Dispersive model.** Each strand of *both* daughter molecules contains a mixture of old and newly synthesized parts.