

Genetics → Study of gene function

From molecules of DNA to the gene pool of a population.

DNA contains ALL of the information for building an organism.

Genes → Functional units of DNA.

Individuals inherit genes.

Individuals contribute to the gene pool of a population.

Interactions between genes and the environment determine what organisms become.

Genetic analysis requires an observable trait that is easily monitored.

Phenotype → Characteristics of an organism.

Phenotypes change throughout the life of an organism as its genes interact with the environment.

Phenotype is determined by the genotype.

Genotype → The set of genes an organism inherits.

(Very little change over time)

Geneticists study partial genotypes and partial phenotypes of organisms. (i.e.) Study the effect of one or a few genes.

A particular phenotype can be caused by more than one genotype.

Genetic analysis is greatly simplified by using an easily followed phenotype in an organism that is practical to study.

(e.g.) mice vs. elephants

Wild Type→genotypic or phenotypic characteristics of natural populations or standard laboratory strains.

An individual that is not wild type is a variant or mutant.

Mutation is the basis for variation within a species and is the raw material for evolution.

Developmental Noise→Random molecular events in cells of a developing organism that cause phenotypic variations.

THUS: Genotype determines phenotype BUT...
expression of the information in the genotype is influenced by the environment, developmental noise, and other genes.

For Genetic Analysis:

1. A phenotype that is easy to identify.
2. A phenotype that is not highly influenced by the environment.
3. Try to determine the influence of a single gene.

Mendelian Analysis→Analyzing hereditary information using Mendel's techniques and principles.

Gregor Mendel→(1860s) Conducted quantitative and systematic studies of inheritance. Proposed the concept of the gene.

Particulate Inheritance→Characteristics determined by discrete units that are inherited intact.

Mendel's Peas→An individual pea plant produces both pollen and eggs. Peas can self-pollinate or be cross-pollinated.

Each pea (individual) results from a separate fertilization event.

Self-pollination (self)→Pollen and egg from the same flower. Both parents have the same genotype.

Cross-pollination (cross)→Pollen from one plant fertilizes an egg from another plant. Parents have different genotypes.

Pure Line→All offspring produced by selfing or crossing individuals within the same line have the same phenotype.

P→Parental generation. (Individuals in the first cross of a particular series of experiments)

F₁→First Filial Generation. (Progeny from the first cross)

F₂→Second Filial Generation. (progeny from selfing individuals from the F₁ generation)

Dominant→The phenotype that is expressed in the F₁ progeny when two pure lines are crossed.

Recessive→The phenotype that is covered up by the presence of the dominant phenotype. (Reappears in F₂)

Mendel used reciprocal crosses (Figures 2.4 and 2.5).

Experiment 1

P pure breeding purple flowers X pure breeding white flowers (Cross)
↓
F₁ All purple flowers

Reciprocal crosses gave the same result.
Purple is dominant and white is recessive.

F₁ X F₁ (Self)
↓
F₂ 3:1 ratio of purple:white

Recessive phenotype remained as a separate heritable trait.
Mendel studied 7 distinct characteristics (Table 2-1).

Experiment 2

P yellow X green (cross)
↓
F₁ All yellow (self)
↓
F₂ 3:1 yellow:green (self)
↓
F₃ ???

1/3 yellow F₂ always gave rise to yellow.
2/3 yellow F₂ gave 3:1 yellow:green ratio.
1/1 green F₂ always gave rise to green.

Underlying the 3:1 F₂ phenotypic ratio is a 1:2:1 genotypic ratio of YY:Yy:yy

Mendel proposed that each gene is present twice (gene pair).

Experiment 2 Again

P YY (yellow) X yy (green) (cross)
↓
F₁ All Yy (yellow) (self)
↓
F₂ 1 YY (yellow)
2 Yy (yellow)
1 yy (green)

Test Cross → Crossing to a recessive individual.

F₁ Yy X yy
↓
1:1
(Yy) Dominant : Recessive (yy)

Autosomes → all chromosomes other than the sex chromosomes.

Alleles → Different forms of the same gene (Y and y).

Homozygous → Both alleles of a gene pair are the same.
YY (dominant) or yy (recessive)

Heterozygous → Different alleles of a gene pair. Yy (dominant)
YY and Yy have different genotypes but the same phenotype.

Monohybrid Cross → Analyzing one characteristic (gene).

Dihybrid Cross → Analyzing two genes simultaneously.

Trihybrid Cross, etc...

Mendel's First Law → The two members of a gene pair segregate from each other into gametes; so that one half of the gametes carry one member of the gene pair and the other half of the gametes carry the other member of the gene pair.

Dihybrid Cross → Usually has 4 possible phenotypes.

- 1) Both dominant
- 2) One dominant, one recessive
- 3) One recessive, other dominant
- 4) Both recessive

Phenotypic ratio of F₂ progeny is 9:3:3:1 (Figure 2-9)

Independent Assortment → Different genes assort independently from one another.

Mendel's Second Law → During gamete formation the segregation of alleles of one gene is independent of the segregation of alleles of another gene.

Probability → The number of times an event is expected to happen divided by the number of opportunities for an event to happen. (i.e.) $p = x/y$

Dice → $p(\text{of a 3}) = 1/6$

Gametes → if heterozygous Aa: $p(A) = 1/2$, $p(a) = 1/2$

Product Rule → The probability that two independent events will occur simultaneously is the product of their probabilities.

$p(\text{of two 4s}) = (1/6)(1/6) = 1/36$

RrYy: $p(\text{gamete with 2 recessive alleles}) = (1/2)(1/2) = 1/4$

Sum Rule → The probability of either one of two independent events is the sum of the probabilities.

$p(\text{of two 4s or two 5s}) = 1/36 + 1/36 = 1/18$

RrYy: $p(\text{gamete with 2 recessive or 2 dominant alleles}) = 1/4 + 1/4 = 1/2$

Probability or chance governs the transmission of genes.

Punnett Square (Figure 2-10)

- 1) List the possible gametes from one parent on one side and the possible gametes from other parent on the other side.
- 2) Fill in gametic combinations in the squares.
- 3) Determine genetic ratios (phenotypic or genotypic) by counting squares.

Branch Diagram

- 1) List probabilities for one event.
- 2) List probabilities of second event next to those of the first.
- 3) Use the product rule to determine genetic ratios.

P	RRYY	X	rryy	(cross)	
		↓			
F ₁		↓	RrYy	(self)	R=round peas
		↓			r=wrinkled peas
F ₂		?			Y=yellow peas
					y=green peas

<u>Composition of F₂</u>	<u>Product rule result</u>
3/4 (Y-)	9/16 (R-Y-) round, yellow
3/4 (R-) (RR,Rr,rR)	3/16 (R-yy) round, green
3/4 (Y-)	3/16 (rrY-) wrinkled, yellow
1/4 (rr)	1/16 (rryy) wrinkled, green

F₂ phenotypic ratio of a dihybrid cross is 9:3:3:1.

Use the product rule directly for trihybrid, etc...

F₁ AaBbCcDdEe X AaBbCcDdEe

$$p(\text{AAbbCcDDEe}) = (1/4)(1/4)(1/2)(1/4)(1/2) = 1/256$$

$$p(\text{A-bbC-D-E-}) = (3/4)(1/4)(3/4)(3/4)(3/4) = 81/1024$$

Pedigree Analysis

Used in human genetics (Family tree)

Symbols used in pedigree analysis (See figure 2-16).

Autosomal Recessive Disorders (Figure 2-17)

Mendelian inheritance of an autosomal recessive disorder is revealed by the appearance of the phenotype in male () and female () progeny of unaffected individuals.

(e.g.) PKU, cystic fibrosis, albinism

Dominant disorders (Figure 2-20)

Mendelian inheritance of an autosomal dominant disorder show affected and in each generation and also show affected and transmitting the condition to sons and daughters in equal proportions.

(e.g.) achondroplasia (dwarfism)

D- (dwarfism)

dd (normal)

Interferes with bone growth during development.

Mitosis and Meiosis → Division of the nucleus and its contents (chromosomes). Eukaryotic cells only.

Cell Cycle (4 stages)

Mitosis (M); Gap1 (G1); DNA synthesis (S); Gap2 (G2)

Cells with two chromosome sets are diploid (2n).

Cells with one chromosome set are haploid (1n).

Mitosis (Figures 3-2, 3-3)

- 1) Somatic cells (non-gametes).
- 2) Produces genetically identical daughter cells from one progenitor.
- 3) Single division (2 cells).
- 4) Chromosomes duplicated during interphase (time between mitoses).
- 5) 4 continuous stages (Fig. 3-2).
 - A) Prophase
 - B) Metaphase
 - C) Anaphase
 - D) Telophase

Prophase

Chromosomes consisting of 2 sister chromatids condense.

Chromatids are still joined at the centromere.

Nucleoli disappear (RNA synthesis).

Nuclear membrane breaks down.

Nuclear spindle starts to form.

Metaphase

Nuclear spindle becomes prominent.

Chromosomes move to equatorial plane.

Centromere of each chromosome attaches to spindle fibers.

Anaphase

Pairs of sister chromatids separate and migrate to opposite poles (V-shaped structures).

Telophase

Nuclear membrane reforms around each daughter nucleus containing one complete set of chromosomes.

Nucleoli reappear; spindle disappears.

Cytoplasm divided in two by a new cell membrane.

Meiosis (Figures 3-2, 3-4)

- 1) Production of genetically different gametes.
- 2) Two cell divisions.
- 3) Produces 4 meiotic products.

Meiosis I (MI)

Homologous chromosomes separate. The two members of each homologous pair are called homologs.

Prophase I

Chromosomes become visible.

Homologous chromosomes pair (synapsis).

Synapsis requires a synaptonemal complex (protein, DNA, RNA)

Number of homologous pairs of chromosomes is $1n$.

Crossing over (X-over) occurs which is an exchange of DNA between homologous chromosomes.

Visualized by cross-shaped structures (chiasmata).

Metaphase I

Each pair of homologs are positioned at the equatorial plane. The two centromeres of a homologous pair attach to spindle fibers of opposite poles.

Anaphase I

Members of each homologous pair separate and move to opposite poles. Centromeres don't divide because replication is not completed through the centromere until Anaphase II.

Telophase I

2 daughter cells form. Nuclear membrane may reform.

Interphase

No DNA synthesis. Number of chromosomes in each cell has been reduced by 1/2 ($2n \rightarrow 1n$)

Meiosis II (MII)

Similar to mitosis where the number of chromosomes in the original and product cells are the same.

Centromeres divide \rightarrow separation of sister chromatids.

Net result of meiosis is the generation of 4 haploid ($1n$) gametes.

Mendel's laws are explained by meiosis.

Chromosome Theory of Inheritance \rightarrow Mendel's genes precisely paralleled the behavior of chromosomes (Figure 3-5).

Sex Linkage \rightarrow Inheritance pattern of genes on sex chromosomes.

In many organisms the gender is determined by the combination of sex chromosomes.

Sex chromosomes also carry genes unrelated to male and female development.

X-linked recessive inheritance \rightarrow Many more than .

Drosophila eye color phenotype correlated with chromosome differences in microscope. (Supported chromosome theory)

Thomas Hunt Morgan \rightarrow Nobel prize (1934)

Experiment 1 → Drosophila eye color (Figure 2-15)

P	red (W.T.)	X	white-eyed	
		↓		
F ₁	All	&	red	red (dominant) white (recessive)
		↓		
F ₂	3:1 red:white		BUT:	$\frac{\text{red}}{2} : 1$ $\frac{\text{white}}{\text{all}}$

Experiment 2 → Test Cross

F ₁	Red-eyed	(X ^{w+} X ^w)	X	white-eyed	(X ^w Y)
			↓		
1:1:1:1	red	:white		:red	:white
	(X ^{w+} X ^w)	(X ^w X ^w)		(X ^{w+} Y)	(X ^w Y)

Experiment 3 → Reciprocal cross of experiment 1 (Figure 2-15)

P	X ^w X ^w (white)	X	X ^{w+} Y (red)
		↓	
F ₁		1:1	red :white X ^{w+} X ^w X ^w Y
		↓	
F ₂	1:1:1:1	red :white	:red :white (X ^{w+} X ^w) (X ^w X ^w) (X ^{w+} Y) (X ^w Y)

Morgan's Explanation

(XX) → 2 chromosomes with eye color genes.

(XY) → 1 chromosome with eye color genes.

X-Linked Recessive (Humans)

Many more than show the recessive phenotype.
(If rare, almost all affected people are)

If rare, none of the offspring of an affected are affected, but all daughters are carriers.

carriers will pass the condition to 1/2 of her sons and 1/2 of her daughters will be carriers.

(e.g.) color blindness, hemophilia, Duchenne's muscular dystrophy (fatal X-linked)

X-Linked Dominant (Humans)

Affected pass condition to all daughters, never to sons.

Affected pass condition to 1/2 of sons and 1/2 of daughters.
(e.g.) hypophosphatemia (Vitamin D deficient rickets)

Y-Linked Inheritance (Humans)

No human phenotype other than maleness has been proven to be Y-linked.

Hairy ear rims? (See Figure 2-33)

X-Inactivation → Mammals inherit 2 X chromosomes. Early in development one of the 2 Xs is inactivated. If the functional X has a recessive allele, the recessive phenotype is expressed.

Barr Body → The inactive X (microscope).

Since the inactivation process is random, all are genetic mosaics. (i.e.) a mixture of cells having 2 genotypes corresponding to inactivation alternatives.

(e.g.) sweat glands (Figure 2-32)

Topography of Chromosomes → Cytological features

Chromosome size → Large variation within a genome.

Centromere → Structure that attaches to spindle fibers during mitosis and meiosis. (constricted appearance at metaphase)

Metacentric → Centromere in middle.

Acrocentric → Centromere off-center.

Telocentric → Centromere at one end.

Acentric → Chromosome fragment lacking a centromere.
Lost since no spindle fiber attachment.

Nucleolar Organizer → Clusters of ribosomal RNA (rRNA) genes (secondary constriction).

Euchromatin → Active DNA (lightly packed).

Heterochromatin → Inactive DNA (highly compacted).

- A. **Constitutive** → Always inactive, permanent feature of chromosomal region.
- B. **Facultative** → Present or absent at various times (active or inactive).

Banding Patterns → Stains result in characteristic banding patterns. (e.g.) Giemsa → G bands (Figure 3-32)

Polytene Chromosomes → Unusual feature of some flies. (Figure 3-33C)

Many rounds of replication, 1024 chromatids, characteristic banding pattern.

Balbiani rings or “puffs” are active regions of RNA synthesis. (i.e.) active DNA

Chromosome Structure

E. coli → 4,200 kb → 4.2 million bp

Human cell → 6 million kb → 6 billion bp

2m DNA in 0.006mm = 6µm nucleus.

Each chromosome is one DNA duplex.

Human body → sun and back 50 times!!!

Chromatin → Chromosomal DNA and protein.

Nucleosomes → 10 nm fiber (diameter) (Figure 3-38B)

DNA and chromosomal proteins called histones.

First level of packaging (beads on a string).

Each nucleosome contains an octamer of 2 subunits each of the histones H2A, H2B, H3, and H4.

DNA wrapped \square twice around histone octamer.

Solenoid → (30 nm fiber) A coil of nucleosomes.

Stabilized by histone H1 that runs down center of the structure.

Supercoil → (700 nm fiber) Seen during Mitosis and Meiosis (Figure 3-41)

Held together by a topoisomerase scaffold at scaffold attachment regions (SARs).

Replication & transcription of Chromatin

Nucleosomes do not dissociate. Probably loosen up to prevent steric hindrance of transcription and replication machinery.

Sequence Organization

1. Single copy protein coding genes.
2. DNA present in more than 1 copy.
 - A. Functional sequences
 1. gene families (e.g.) globin, actin, rRNA
 2. noncoding (e.g.) regulatory regions, telomeres
 - B. Sequences with no known function (20%)

(e.g.) VNTRs → Variable Number Tandem Repeats
In humans VNTRs are 1-5 kb sequences consisting of repeats 15-100 nt long. Used in DNA fingerprinting.

3. Spacer DNA

Unique single copy genes are embedded in a diverse assembly of repetitive DNA.

Background Information

Genes specify the structure of proteins.

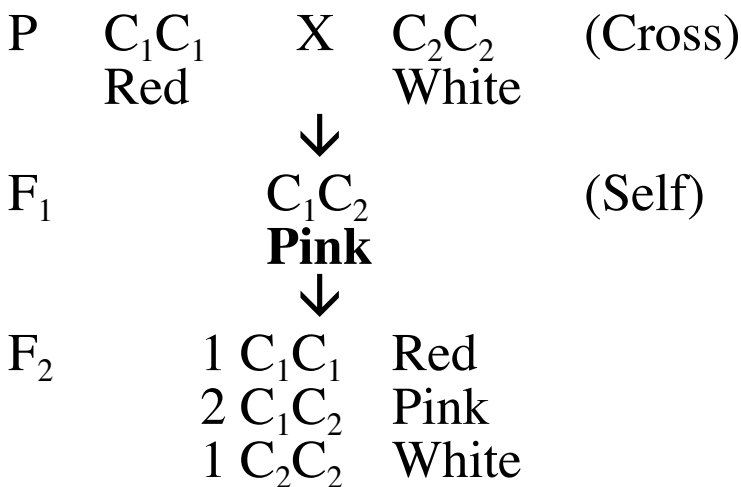
Genes do not act in isolation. (i.e., the gene products (protein) interact with one another).

Different alleles of a gene specify different forms of the corresponding protein. (e.g., active, inactive, etc.)

Cellular processes occur by pathways in which enzymes act in a series.

Incomplete Dominance → Heterozygotes have intermediate phenotypes of the two homozygotes.

(e.g.) Four-O-Clock Flower Color



Codominance → Heterozygote has phenotype of both homozygotes. (e.g.) Human Hemoglobin

$Hb^A Hb^A$ Normal Red Blood Cells (RBCs).

$Hb^S Hb^S$ Abnormal “sickle” shaped RBCs, anemia (often fatal).

$Hb^A Hb^S$ No anemia, RBCs form sickles at low [oxygen].

- 1) Hb^A is dominant in regard to anemia.
- 2) Hb^A is incompletely dominant in regard to cell shape.
- 3) Hb^A and Hb^S are codominant in regard to hemoglobin molecules.

Multiple Alleles → A gene with more than two alleles.
(e.g.) Human ABO Blood Groups (3 alleles)

<u>Genotype</u>	<u>Blood Group</u>
$I^A / I^A, I^A / i$	A
$I^B / I^B, I^B / i$	B
I^A / I^B	AB
i / i	O

I^A and I^B are fully dominant in $I^A i$ and $I^B i$ and codominant in $I^A I^B$.

Lethal Alleles → Alleles of a gene that kill an organism. May be lethal if homozygous or even heterozygous.

Lethality may occur early during embryonic development, after birth, or later in life.

Many human lethals result in spontaneous abortions.

Mouse coat color

Dark coat → Normal (W.T.); Yellow coat → Variant

P Dark (AA) X Yellow ($A^Y A$)
↓

F₁ 1:1 Dark:Yellow

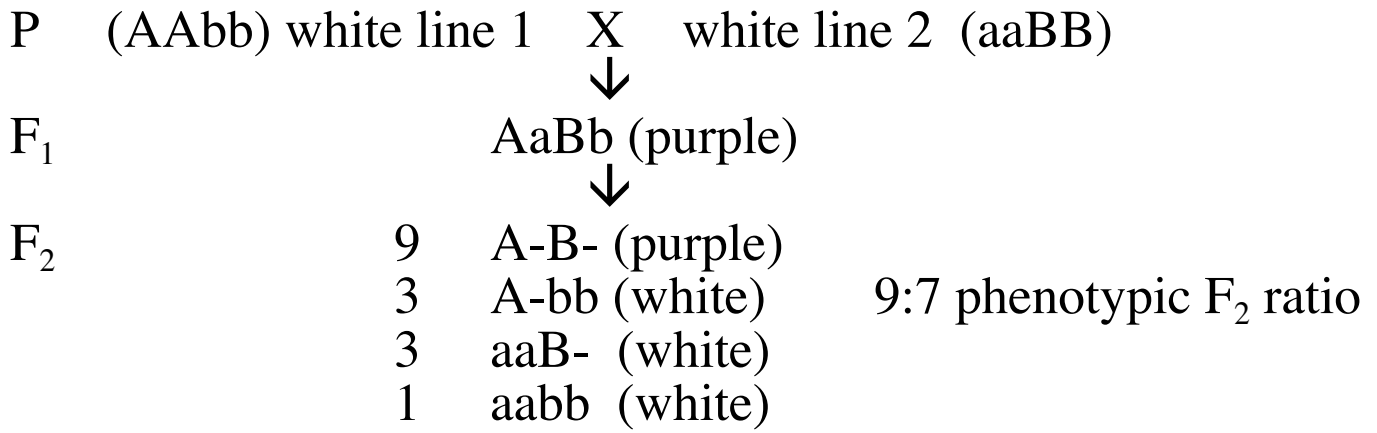
P Yellow ($A^Y A$) X Yellow ($A^Y A$)
↓

F₁ 2:1 Yellow:Dark (Suggests no homozygous yellow).

1/4 AA (Dark)
1/2 $A^Y A$ (Yellow)
1/4 $A^Y A^Y$ (Die Before Birth)

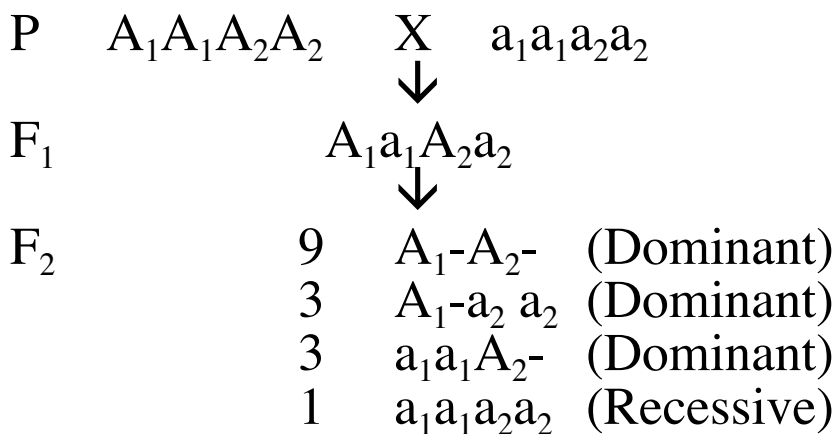
Semilethal → Lethal in some but not all individuals (environment).

Complementation → Dominant phenotype requires at least one dominant allele of each of two genes. Homozygosity for the recessive allele of either gene results in the recessive phenotype. (e.g.) Pea petal color (biochemical pathway)



If the same phenotype is observed in independently isolated organisms, complementation tests can determine if the phenotype is caused by alleles of the same or different genes. 9:7 F₂ ratio if different genes, all recessive if the same gene.

Duplicate Genes → Genes present more than once in the genome. One WT allele of either gene → dominant phenotype.



15:1 phenotypic ratio in F₂

Pleiotropic → Allele that causes more than one phenotype. (e.g.) mouse coat color (Yellow when heterozygous, lethal when homozygous)

Gene Linkage

Each chromosome can contain 1000s of genes. These linked genes segregate together during meiosis. (Figure 5-1)

Mendelian ratios are NOT observed.

Meiotic Recombination → generates haploid genotypes (gametes) that differ from the haploid parental genotypes. (Figures 5-4, 5-5)

Interchromosomal Recombination → If genes assort independently, crosses between a heterozygote and a tester strain generate:

50% Parental type gametes
50% Recombinant gametes
1:1:1:1 phenotypic ratio
(Figure 5-6)

Genes close together on the same chromosome do not assort independently.

pr ⁺ pr	vg ⁺ vg	X	pr pr	vg vg	(Test Cross)
		↓			
pr ⁺ vg ⁺	1339		pr ⁺	red eye (normal)	
pr vg	1195		pr	purple eye	
pr ⁺ vg	151		vg ⁺	normal wings	
pr vg ⁺	<u>154</u>		vg	vestigial wings	
	2839				

This is not a 1:1:1:1 ratio.

Parental Classes → Original arrangement of alleles on the two chromosomes. (Two most frequent classes)

Intrachromosomal Recombination → New combinations generated during meiosis when non-sister chromatids cross over (X-over) between the genes under study. (Figures. 5-2, 5-3, 5-7)

Recombinant Classes → observed less frequently than parentals. (Figure 5-8)

X-overs generate two reciprocal products or classes that are about equal in frequency.

Recombinant frequency (RF) significantly < 50% suggests linkage.

RF \square 50% suggests that the genes are unlinked on separate chromosomes (χ^2 Test).

Linkage Mapping

To separate linked genes the X-over must occur between them.

The greater the distance between linked genes, the greater the chance of a X-over between them.

The RF between linked genes is used to map their relative distance apart on the chromosome.

RF = 0.01 (1%) = 1 map unit (m.u.)

Gene Locus → Region on a chromosome where alleles of a certain gene are found.

$pr^+ vg^+ / pr\ vg$	X	$pr\ vg / pr\ vg$	
	↓		
	$pr\ vg / pr\ vg$		165
	$pr^+ vg^+ / pr\ vg$		191
	$pr\ vg^+ / pr\ vg$		23
	$pr^+ vg / pr\ vg$		<u>21</u>
			400

$\frac{44 \text{ recombinant}}{400 \text{ total}} \times 100 = 11\% = 11 \text{ m.u.}$

$11\% < 50\%$ pr and vg are linked

Given a genetic distance in m.u. we can predict frequencies of progeny from a test cross.

$pr^+ vg^+ / pr\ vg$ X $pr\ vg / pr\ vg$

If 11 m.u.

$pr\ vg / pr\ vg$	44.5%
$pr^+ vg^+ / pr\ vg$	44.5%
$pr\ vg^+ / pr\ vg$	5.5%
$pr^+ vg / pr\ vg$	5.5%

Three-Point Testcross

Map units are additive, but to order 3 genes we need to perform a three-point testcross. (Figure 5-10)

Double X-overs → Smallest class because two X-overs required.

Compare parental types with double X-overs. The gene that is switched is in the middle.

(Figures 5-12, 5-13)

P	v^+	v^+	cv	cv	ct	ct	X	v	v	cv^+	cv^+	ct^+	ct^+
							↓						
F ₁		v	v^+	cv	cv^+	ct	ct^+						(Test Cross)
							↓						
F ₂			v	cv^+	ct^+								580
			v^+	cv	ct								592
			v	cv	ct^+								45
			v^+	cv^+	ct								40
			v	cv	ct								89
			v^+	cv^+	ct^+								94
			v	cv^+	ct								3
			v^+	cv	ct^+								5
													1448

- 1) Consider v and cv (neglect ct for now)
 $v\ cv^+$ & $v^+\ cv$ parentals; $v\ cv$ & $v^+\ cv^+$ recombinants
 $RF = (45 + 40 + 89 + 94) / 1448 = 0.185 = 18.5\%$

- 2) Now consider v and ct (neglect cv)
 $v\ ct^+$ & $v^+\ ct$ parentals; $v\ ct$ & $v^+\ ct^+$ recombinants
 $RF = (89 + 94 + 3 + 5) / 1448 = 0.132 = 13.2\%$

- 3) Now consider cv and ct (neglect v)
 $cv^+\ ct^+$ & $cv\ ct$ parentals; $cv^+\ ct$ & $cv\ ct^+$ recombinants
 $RF = (45 + 40 + 3 + 5) / 1448 = 0.064 = 6.4\%$

Now construct the linkage map

v 13.2 ct 6.4 cv

But: $13.2 + 6.4 \neq 18.5$ WHY?

Because we didn't count the double X-overs between v & cv when we really needed to count them twice.

$$\begin{aligned} \text{RF} &= (45 + 40 + 89 + 94 + 3 + 3 + 5 + 5)/1448 \\ &= 0.196 = 19.6\% \end{aligned}$$

Interference → A X-over in one region of the chromosome decreases the chances that a X-over will occur in an adjacent region.

Calculate the frequency and number of double recombinants expected if there is no interference. (i.e., use the product rule)

$$\text{Expected frequency} = (0.132)(0.064) = 0.0084$$

$$\text{Expected number} = (0.0084)(1448) = 12$$

Interference (I) = $1 - \text{c.o.c.}$ (coefficient of coincidence)

$$I = 1 - \frac{\text{observed number of double X-overs}}{\text{expected number of double X-overs}}$$

$$I = 1 - 8/12 = 4/12 = 1/3 = 0.33 = 33\%$$

Obvious departures from 1:1:1:1 ratios are easy to recognize, smaller departures require the χ^2 test.

χ^2 Test → Tells us how often observations deviate from expectations purely on the basis of chance.

If linkage is not obvious, hypothesize that the genes are unlinked (Null Hypothesis) and calculate χ^2 .

$$\chi^2 = \text{total of } \frac{(O-E)^2}{E} \text{ for all classes}$$

Class	O	E	$(O-E)^2$	$\frac{(O-E)^2}{E}$
AB	150	130	400	3.08
ab	135	130	25	0.19
Ab	110	130	400	3.08
aB	<u>125</u>	<u>130</u>	25	<u>0.19</u>
	520	520		$\chi^2 = 6.54$

- 3) Calculate degrees of freedom (df).
df = (number of classes - 1) = 4 - 1 = 3
- 4) Find p from table (Table 4-1, page 126).
- 5) Accept null (unlinked) if $p > 0.05$
Reject null (linked) if $p < 0.05$

This is another example of a three-point testcross.

$a^+ a b^+ b c^+ c$	X	$a a b b c c$
	↓	
$a^+ b^+ c$	788	
$a b c^+$	802	
$a^+ b c$	72	
$a b^+ c^+$	68	
$a^+ b^+ c^+$	128	
$a b c$	122	
$a b^+ c$	9	
$a^+ b c^+$	<u>11</u>	
	2000	

Construct a linkage map and calculate interference.

First determine the middle gene a

1) Consider a and b (neglect c)
 $a^+ b^+$, $a b$ → parentals; $a^+ b$, $a b^+$ → recombinants
 $RF = (72 + 68 + 9 + 11) / 2000 = 160 / 2000 = 0.08 = 8\%$

2) Consider a and c (neglect b)
 $a^+ c$, $a c^+$ → parentals; $a^+ c^+$, $a c$ → recombinants
 $RF = (128 + 122 + 9 + 11) / 2000 = 270 / 2000 = 0.135 = 13.5\%$

3) Consider b and c (neglect a)
 $b^+ c$, $b c^+$ → parentals; $b^+ c^+$, $b c$ → recombinants
 $RF = (72 + 68 + 128 + 122 + 9 + 9 + 11 + 11) / 2000 = 430 / 2000 = 0.215 = 21.5\%$

c 13.5 a 68.0 b

$I = 1 - (O/E)$ $E = (0.08)(0.135)(2000) = 21.6$
 $I = 1 - (20/21.6) = 1 - 0.926 = 0.074 = 7.4\%$

Prokaryotes → Eubacteria and Archaeobacteria

Divide by binary fission resulting in genetically identical progeny. (Figure 7-1)

Single, circular chromosome.
No mitosis, meiosis, nucleus.
Haploid.

Bacteriophage (Phage) → bacterial virus.
Not free living → parasitize bacteria to replicate.

Conjugation → Transfer of DNA from one cell to another via direct cell to cell contact.

Requires the fertility (F) factor → small, circular DNA element that can replicate (mini chromosome).

Cells with F are F⁺; cells without F are F⁻.

F⁺ donor cells produce pili which attach to F⁻ recipient cells.
Donor transfers a copy of F to the recipient. A copy of F always remains in donor (replication). (Figure 7-5 A & B)

F can integrate into the chromosome by X-ing over. (**Hfr strain**).

Hfr (high frequency of recombination) → strains can transfer chromosomal genes that can recombine with recipient chromosome generating recombinants. (Figure 7-6 A & B)

F' → Integrated F can recombine out of chromosome and carry host genes in addition to F. F' can transfer bacterial genes to recipient cell generating recombinants.

Determining gene order from gradient of transfer → (p. 217)

Hfr met⁺ arg⁺ aro⁺ his⁺ str^S X F⁻ met⁻ arg⁻ aro⁻ his⁻ str^R

1. Determine the gene that is closest to the origin by performing an interrupted mating experiment (met⁺).
2. Then with a natural gradient of transfer experiment plate on minimal + all supplements except methionine. (i.e.) select for met⁺ recombinants

3. Screen for other genes by replica plating.

100% met⁺

60% arg⁺

20% aro⁺

4% his⁺

Gene Order
met arg aro his

Higher Resolution Mapping (Figure 7-13)

Hfr leu⁺ arg⁺ met⁺ str^S X F⁻ leu⁻ arg⁻ met⁻ str^R

----->-----

1. select for last gene (i.e.) leu⁺
Thus, every transferred fragment has all 3 genes.
2. screen for the other genes by replica plating

1 map unit=1% X-over in the interval

5% leu⁺ arg⁻ met⁻ (5 map units)
5% leu⁺ arg⁺ met⁻ (5 map units)
90% leu⁺ arg⁺ met⁺

And, rare quadruple X-overs leu⁺ arg⁻ met⁺
(determines the middle gene)

Product rule (0.05)(0.05)=0.0025=0.25%

Bacterial Transformation → Conversion of one genotype to another by introducing chromosomal DNA.
Requires recombination (similar to Hfr x F⁻ cross).

If two genes are close to each other both can be transferred on the same DNA fragment.
Limited use for genetic mapping.

Virulent Phages → Always lytic

Temperate Phages → Lytic or lysogenic

Lysogeny → Phage infected bacteria not leading to lysis

Lysogenic strains carry a prophage (phage DNA with lytic functions turned off).

Can be integrated or as a separate circle. UV light or chemicals induce prophage to enter the lytic cycle.

Transduction → Phage can transfer bacterial genes from one cell to another by packaging bacterial DNA by mistake.

Generalized Transducing Phage → Can carry any part of the bacterial chromosome.

Specialized Transducing Phage → Can only carry a specific chromosomal region.

Generalized Transduction

When a cell is infected by a non-lysogenic phage the bacterial chromosome is broken up. Phage can package pieces of bacterial DNA instead of phage DNA.

(e.g.) P1 (Figure 7-26)

Transducing phage ejects bacterial donor DNA into a recipient strain → merodiploid → recombination.

Linkage data from P1 transduction

Genes must be close enough for P1 to transduce them in a single piece of DNA.

- 1) (Donor) P1 $\text{met}^+ \text{arg}^+$ X $\text{met}^- \text{arg}^-$ (Recipient)
- 2) Select for met^+ (plate on minimal with arginine)
- 3) Screen for the % of met^+ that are also arg^+ .

Strains transduced to $\text{met}^+ \text{arg}^+$ are cotransductants.

The greater the cotransduction frequency the closer the genes are to each other.

(Donor) P1 $\text{a}^+ \text{b}^+ \text{c}^-$ X $\text{a}^- \text{b}^- \text{c}^+$ recipient
Select for a^+ then screen for $\text{b}^{+/-}$ and $\text{c}^{+/-}$.

<u>Genotype</u>	<u># Colonies</u>
$\text{a}^+ \text{b}^+ \text{c}^+$	7
$\text{a}^+ \text{b}^+ \text{c}^-$	20
$\text{a}^+ \text{b}^- \text{c}^+$	46
$\text{a}^+ \text{b}^- \text{c}^-$	27
	<hr/> 100

The cotransduction frequency of a and b is 27%

The cotransduction frequency of a and c is 47%

Draw the linkage map

Specialized Transduction

(e.g.) □

Integration by a single X-over between gal and bio.
Useful for moving specific genes. (i.e.) gal or bio
(Figures 7-30, 7-31a)

Mapping genes on *E. coli* Chromosome

- 1) First use Hfr strains to map genes within about 10-15 min.
- 2) P1 transduction within the interval established by Hfr conjugation.

Conjugation, transformation and transduction require a double X-over to replace DNA in the bacterial chromosome.

(Figure 7-35)