

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 (WT)→genotypic or phenotypic characteristics of natural populations or standard laboratory strains.

An individual that is not WT 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, the genotype determines the phenotype; however, 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 (Cross) white flowers
		↓	
F ₁	All purple flowers		

Dihybrid Cross→Usually has 4 possible phenotypes.

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

Phenotypic ratio of F_2 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: $p(\text{gamete with 2 recessive or 2 dominant alleles}) =$

$(1/2)(1/2) + (1/2)(1/2) = 1/4 + 1/4 = 1/2$

Probability or chance governs the transmission of genes.

Punnett Square (Figure 2-10)

P RRYY X rryy (cross)
↓ R=round peas
F₁ RrYy (self) r=wrinkled peas
↓ Y=yellow peas
F₂ ? y=green peas

- 1) List the gametes from one parent on one side.
- 2) List the gametes from other parent on the other side.
- 3) 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.

Composition of F₂

Product rule result

	3/4 (Y-)	9/16 (R-Y-) round, yellow
3/4 (R-)	(YY, Yy, yY)	
(RR, Rr, rR)	1/4 (yy)	3/16 (R-yy) round, green
	3/4 (Y-)	3/16 (rrY-) wrinkled, yellow
1/4 (rr)	(YY, Yy, yY)	
	1/4 (yy)	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

To calculate genotypic frequency

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

To calculate phenotypic frequency

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

Pedigree Analysis

Used in human genetics (Family tree)

Symbols used in pedigree analysis (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 males and females in each generation and also show affected males and females 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 (1 cell gives rise to 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

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Prophase

Chromosomes consisting of 2 sister chromatids condense.

Chromatids are still joined at the centromere.

Nuclear membrane breaks down.

Spindle fibers start to form.

Metaphase

Spindle fibers become prominent.

Chromosomes move to equatorial plane.

Centromere of each chromosome attaches to a spindle fiber.

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.

Spindle fibers disappear.

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 (1 cell gives rise to 4 cells).

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).

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

Number of chromosomes is $2n$.

Number of chromatids is $4n$.

Crossing over (X-over) occurs → an exchange of DNA between homologous chromosomes (i.e. between non-sister chromatids).

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.

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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 males than females.

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

Thomas Hunt Morgan \rightarrow Nobel prize (1934)

Chromosome Theory Proof → Bridges & Morgan
Experiment 3 revisited

P	X^wX^w (white female)	X	$X^{w+}Y$ (red male)
		↓	
F ₁	$X^{w+}X^w$ (red female)	&	X^wY (white male)

PLUS: rare exceptional progeny (1/2000)

X^wX^wY (white female)	&	$X^{w+}0$ (red male)
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Exceptional chromosome arrangements were observed in a microscope.

Non-Disjunction → Failure of chromosomes or sister chromatids to separate in meiosis.

Exceptional progeny arise due to non-disjunction during meiosis. (Figure 3-13)

The number of X chromosomes dictates the gender of Drosophila. (Different than in humans)

2 Xs → female 1X → male

$X^{w+}0$ → sterile male because the Y chromosome is necessary for male fertility.

X-Linked Dominant Inheritance

Affected males transmit phenotype to all daughters, never to sons.

Human Sex Linkage → Pedigree Analysis.

The presence of the Y chromosome determines maleness in humans. (See Table 2-3)

X-Linked Recessive (Humans)

Many more males than females show the recessive phenotype.

(If rare, almost all affected people are male)

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

Female 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

X-Linked Dominant (Humans)

Affected males pass condition to all daughters, never to sons.

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

Y-Linked Inheritance (Humans)

Very few human phenotypes have been proven to be Y-linked.

X-Inactivation → Female 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 females 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.

Euchromatin→Active DNA (lightly packed).

Heterochromatin→Inactive DNA (tightly packed).

- A. **Constitutive**→Always inactive, permanent feature of chromosomal region (e.g. centromeres, telomeres, inactive X chromosome)
- 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.

Balbani 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.

(Figure 3-37)

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 ≈ twice around histone octamer.

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

Stabilized by histone H1 that runs down the center.

Supercoil → (700 nm fiber) Seen during Mitosis and Meiosis (Figures 3-35 and 3-41)

Held together by a 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.

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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.

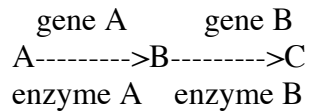
Background Information

Genes specify the structure of proteins.

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

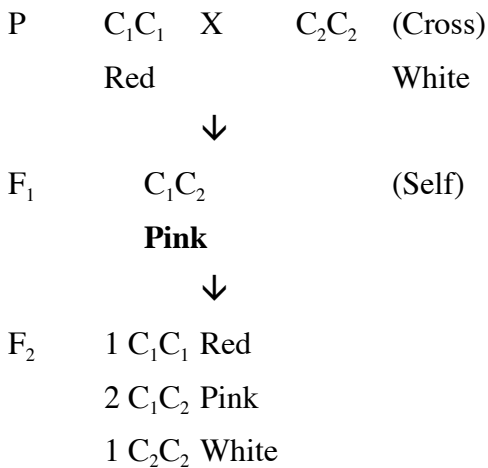
Different alleles of a gene specify different forms of the corresponding protein. (e.g. active, inactive, etc.)

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 regarding anemia.
- 2) Hb^A is incompletely dominant regarding cell shape.
- 3) Hb^A and Hb^S are codominant regarding 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 $I^A I^B$.

AB→Universal acceptor (no Ag on cell surface)

O→Universal donor (no Ab anti A or 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 lethal genes result in spontaneous abortions.

Mouse coat color

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

P Dark (AA) X Yellow ($A^Y A$)

↓

F_1 1:1 Dark:Yellow

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

↓

F_1 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).

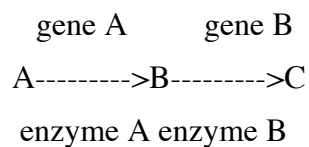
- Duchenne muscular dystrophy → lethal in early adulthood
- Cystic fibrosis → lethal in early adulthood
- Huntington's → lethal in late adulthood

Pleiotropic → Allele that causes more than one phenotype.

(e.g.) mouse coat color (Yellow when heterozygous, lethal when homozygous)

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.

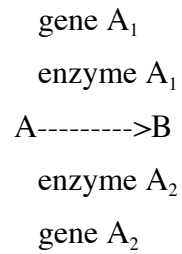
(e.g.) Pea petal color (biochemical pathway)



P	(AA bb) white line 1	X	white line 2 ($aaBB$)	
			↓	
F ₁			AaBb (purple)	
			↓	
F ₂		9	A-B- (purple)	
		3	A-bb (white)	9:7 phenotypic F ₂ ratio
		3	aaB- (white)	
		1	aabb (white)	

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.



P	$A_1A_1A_2A_2$	X	$a_1a_1a_2a_2$	
		↓		
F ₁	$A_1a_1A_2a_2$			
		↓		
F ₂	9	A_1-A_2- (Dominant)		
	3	$A_1-a_2 a_2$ (Dominant)		
	3	$a_1a_1A_2-$ (Dominant)		
	1	$a_1a_1a_2a_2$ (Recessive)		

15:1 phenotypic ratio in F₂

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)

Recombinant Classes → observed less frequently than parentals.

(Figure 5-8)

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)

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

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

RF \approx 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.

Problem 1

$pr^+ vg^+ / pr vg$	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

44 recombinant x 100 = 11% = 11 m.u.

400 total

11% < 50% pr and vg are linked

Problem 2

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 ⁺	<u>5</u>
				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) = $1 - \text{c.o.c. (coefficient of coincidence)}$

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

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

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

$I = 1 - (O/E) \quad E = (0.08)(0.135)(2000) = 21.6$

$I = 1 - (20/21.6) = 1 - 0.926 = 0.074 = 7.4\%$

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 Strains (**high frequency of recombination**)

-Can transfer chromosomal genes to F⁻ recipient.

-DNA fragment can recombine with chromosome and generate recombinants. (Figure 7-6 A & B)

Determining gene order from gradient of transfer

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

Gene order → leu arg met

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 $met^+ arg^+$ X $met^- arg^-$ (Recipient)
- 2) Select for met^+ (plate on minimal with arginine)
- 3) Screen for the % of met^+ that are also arg^+ .

Strains transduced to $met^+ arg^+$ are cotransductants.

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

(Donor) P1 $a^+ b^+ c^-$ X $a^- b^- c^+$ recipient

Select for a^+ then screen for $b^{+/-}$ and $c^{+/-}$.

<u>Genotype</u>	<u># Colonies</u>
$a^+ b^+ c^+$	7
$a^+ b^+ c^-$	20
$a^+ b^- c^+$	46
$a^+ b^- c^-$	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.) λ

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)