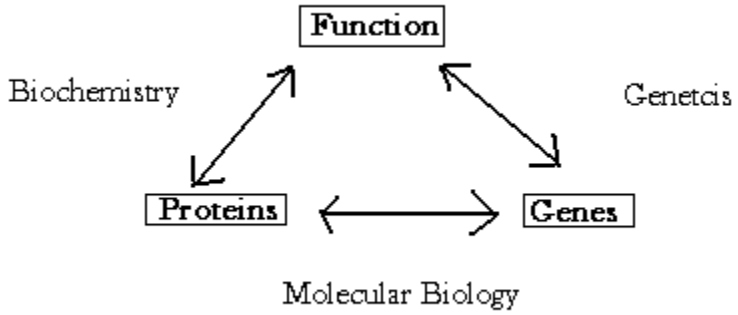
Genetics I: Mendel, Mitosis and Meiosis

One way to study biological function is to take an organism/cell and separate it into components, such as proteins, and then study the individual components.

Relationship between genes, proteins and function:



Biochemistry – The study of one component (e.g. one protein) in the absence of the rest of the organism

Genetics – The study of the organism in the absence of one component (e.g. one gene). The study of " mutants" – organisms which lack one or more components with respect to the "wild type" or normal phenotype.

I. Mendel's First Law: Particles of Inheritance

~ 1860's: Gregor Mendel (a monk) wanted to understand what factors controlled heredity.
-Before Mendel, heredity was though of as "blending." Offspring looked like a blend of both parents.
-Early "experiments" done to study heredity were done with farm animals and plants.
-Mendel studied inheritance in pea plants.
-Mendel studied peas because:

- peas were easy to grow
- could easily control parentage
- self-pollination also possible
- had several traits with easily observed variations

Mendel could obtain true-breeding strains (all progeny looked like parents)

§ Mendel's experiments

-Started with two pure-breeding strains of pea plants with distinct traits.

F_o (parental): Round Peas x Wrinkled Peas

↓

F_1 (1^{st} filial generation) ALL ROUND

The wrinkled trait had disappeared! Mendel observed no blending in the F_1 progeny. He then self-crossed some F_1 plants (peas are able to self-pollinate).

F_1 round x F_1 round

↓

F_2 (2^{nd} filial generation) some round and some wrinkled progeny

-The wrinkled trait had reappeared in the F2 progeny!

-The results were the same whether he had self-crossed some F1 plants or had crossed two F1 plants

Mendel's Conclusion: This was not blending; this was due to some discrete nature of inheritance.

-He looked at the F2 data produced from the F1xF1 cross and found:

F2: 5474 round: 1850 wrinkled

2.96:1 ratio

-Mendel said it looked like a 3:1 ratio of round:wrinkled peas. Mendel repeated the experiments many times and found similar results: ratios such as 3.01:1, 3.15:1, 2.95:1 etc. Based on these results, Mendel formulated the notions of particles of inheritance and made a model.

Mendel's model (hypothesis):

-Each pea plant (such as round or wrinkled) had particles of inheritance, which he called **gametes**. Each plant had two copies of these particles.

-He assigned the symbols for particles of inheritance as follows:

Round particles (R) and Wrinkled Particles (r)

F_o: round (RR) x wrinkled (rr)

↓

F1 Rr round "wins" in F1

Mendel suggested that the F1 plants produced gametes as follows:

Gametes from F1 parent #1:

Gametes from F1 Parent #2

	R	**r**
R	**RR**	**Rr**
r	**rR**	**rr**

He then said that the gametes in were separated in the F1 generation and then came together in the F2.

He crossed two F1 parents to produce F2 progeny.

F1xF1 F1 round (Rr) x F1 round (Rr)

F2: RR(round) Rr(round) rR(round) rr(wrinkled)

3/4 round:1/4 wrinkled

From these experiments, Mendel made a hypothesis

Mendel's Hypothesis (Law) #1
-There exists discrete particles for inheritance that segregate during gamete (egg or sperm) formation. These particles occur as pairs in organisms but are reduced to ones in gametes.

-Mendel tested Law #1 by making predictions.

Experiment #1: Mendel did an F2 x F2 cross (self-crossed some F2 plants)

a) F2 wrinkled x F2 wrinkled =
F3 all wrinkled (F3(rr))

b) F2 round x F2 round =
1/3 of the time all F3 progeny were round (RR(F2) x RR(F2) = All RR(F3))
2/3 of the time 3:1 ratio of round to wrinkled (Rr(F2) x Rr(F2) = RR, Rr, rR ,rr (F3))

Experiment #2: Mendel then did some **testcrosses**:

a) Rr(F1) x rr(F_0) =
½ round RR : ½ wrinkled rr

b) RR(F1) x rr(F_0) =
all round Rr

-If the F1 is Rr, then a testcross with F_0 rr would yield ½ round:1/2 wrinkled. If the F1 is RR then a testcross with the F_0 rr would yield all round.
-From his results, Mendel's model was validated. Mendel's concepts of particles of inheritance, however, were too abstract for most people at the time.

§

§2. Genetic Definitions

gene: a discrete particle of inheritance controlling a trait (pea shape or pea appearance)

allele: an alternative form of a gene (R or r)

genotype: a pair of alleles that a particular individual possesses (RR, Rr or rr)

homozygote: an individual with two copies of the same allele (RR or rr)

heterozygote: an individual with two copies of two different alleles (Rr): refers to genotype

phenotype: a trait exhibited by an allele that distinguishes one individual from another (round vs. wrinkled); result of an assay

dominant phenotype 1 is dominant to phenotype 2 if the heterozygote shoes phenotype 1

recessive: phenotype 2 is recessive to phenotype 1 if the heterozygote shows phenotype 1

For example, round is dominant to wrinkled and wrinkled is recessive to round.

Use dominant and recessive with respect to phenotype and alleles. The phenotypes associated with the mutations, but not the mutations themselves, are dominant and recessive.

§3. Chromosomes: Mitosis and Meiosis

About the same time, cytologists were making observations about cells.

Cytologists observed that when cells were about to undergo cell division, materials in the cell nucleus picked up a staining dye and became visible under the microscope.

They called the se materials chromosomes (colored things):

-Chromosomes come in matched pairs (2n)
-during **mitosis** (a feature of growth and development), the number of chromosomes is preserved, as they duplicate during the cell growth cycle (2n → 4n)
-They pairs of chromosomes are lined up in the middle of the cell before the nucleus divides.

In **Meiosis** (the process by which gametes (eggs and sperm) are formed in germ cells)

1) The number of chromosomes is halved (2n → n)
2) The gametes have n# of chromosomes

During the cell growth cycle in germ cells, the chromosomes are duplicated (2n → 4n). In the first meiotic division, the homologous chromosomes line up next to each other and then the pairs are separated (2 cells with 2n chromosomes). In the second meiotic division, the sister chromatids of each chromosomes are separated (resulting in 4 cells with n chromosomes).

2n chromosomes → mitosis → 2n chromosomes
2n chromosomes → meiosis → n chromosomes

Cytologists found that gametes separate and come together during formation of the **zygote**. When two gametes (n = gamete) fuse: n+ n = 2n

This suggests chromosomes have the same behavior as Mendel's particles of inheritance. Perhaps chromosomes and genes are one and the same. So…. A new idea was developed.

Chromosomal Theory of Inheritance

~1900: Behavior of chromosomes parallels behavior of particles of inheritance (segregation and combination), therefore, inheritance has a chromosomal basis and therefore, genes are chromosomes.
~1935: Barbara McClintock, working with corn, showed that some genes can be rearranged on chromosomes and also that some genes can be mobile within the chromosome

§4. Mendel's Second Law: Independent Assortment of Different Genes

a) Dihybrid (Two-gene) cross:

F_0: Round, Green (RRGG) X Wrinkled, Yellow (rrgg)

↓

F1: all Round, Green (RrGg) (double heterozygote)

Therefore, round is dominant, wrinkled is recessive
Green is dominant, yellow is recessive

b)testcross

RrGg X rrgg

↓

Gametes from parent 1: **RG, Rg, rG, rg**
Gametes from parent 2: **rg**

Gives 4 peas: **RrGg** (round green), **Rrgg** (round yellow), **rrGg** (wrinkled Green) and **rrgg** (wrinkled yellow)

Ratio of 1 : 1 : 1 : 1

The ratios of the phenotypes suggest that these chromosome pairs were assorting independently during meiosis. According to Mendel's law, the R and G genes assort independently if they are on different chromosomes. But what would happen if these genes were on the same chromosome?

Peas have only 7 pairs of chromosomes. If the chromosome theory of inheritance is correct, then peas should only have 7 traits. What if peas exhibited 8 traits???

Genetics II: Linkage and the Chromosomal Theory

An individual has two copies of each particular inheritance (gene). These two copies separate during the formation of gametes and come together when the two gametes combine to from a zygote.

Continue with Mendel's Second Law from Genetics I

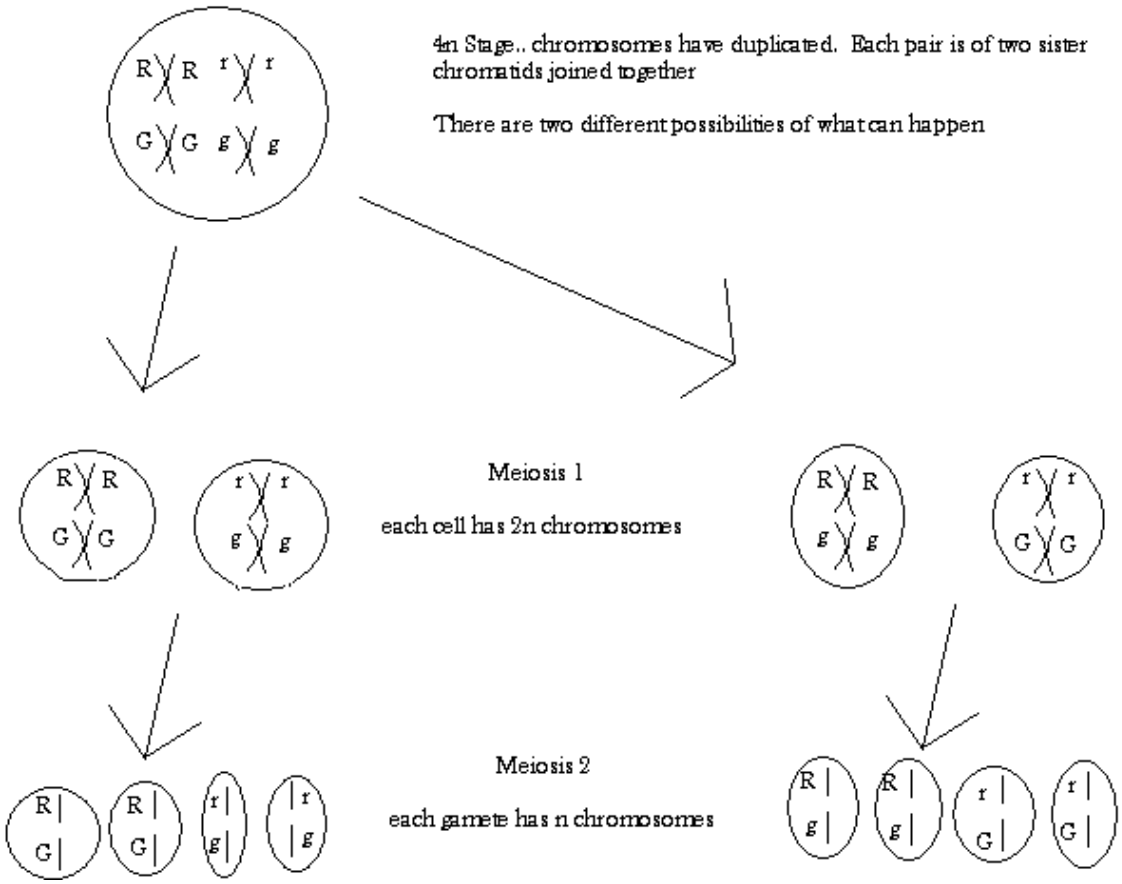
Traits assort independently only if the R and G gene loci are on different chromosomes. A locus is a place on a chromosome where a gene resides.

Based on these results, the chromosomal theory predicts that the gene for the round trait and the gene for the green trait are on separate chromosomes.

According to the chromosome theory:

If the R and G genes are on different chromosomes:

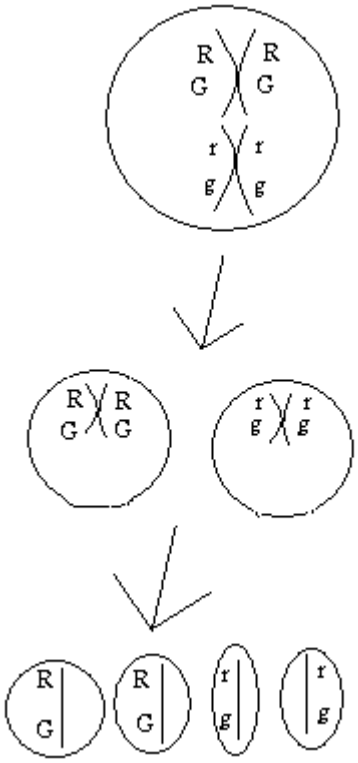
Look at F1 hybrid cells nucleus: RrGg



If genes are on different chromosomes that expect gametes to form in a 1 : 1 : 1 : 1 ratio

RG 25% : rg 25% : Rg 25% : rG 25%

But if the R and G genes are on the same chromosome



If genes are on the same chromosome, expect gametes to form in a 1:1 ratio

RG 50% : rg 50% : Rg 0% : rG 0%

Since these last two (Rg rG) do not form.

According to Mendel the traits should assort independently:
RG 25% : rg 25% : Rg 25% : rG 25%
According to chromosomal theory, the traits should assort with chromosomes
RG 50% : rg 50%

Actually, a third result occurs when the two genes are on the same chromosome.
You also see the two other classes of progeny at a lower frequency

RG, rg are parental types while Rg and rG are non-parental types occurring at a lower frequency

How does this happen??

Through a process called recombination

§2. Recombination

Recombination was first studied using the fruit fly Drosophila Melanogaster by Thomas Morgan (~1910)

Look at cross of two pure breeding strains of flies

F_0 : male $\frac{+\ +}{+\ +}$ X female $\frac{b\ vg}{b\ vg}$

↓

F1: All $\frac{b\ vg}{+\ +}$

\+ = wild type phenotype (body and wings)

b = allele for black body

vg = allele for vestigial (short) wings

Take F1 heterozygote and do a testcross:

$\frac{b\ vg}{+\ +}$ X $\frac{b\ vg}{b\ vg}$

↓

EXPECT : $\frac{b\ vg}{b\ vg}$ $\frac{+\ +}{b\ vg}$ $\frac{b\ +}{b\ vg}$ $\frac{b\ vg}{b\ vg}$

A 1 : 1 : 1 : 1 ratio

However, the progeny was found in the following ratios:

944 : 965 : 206 : 185

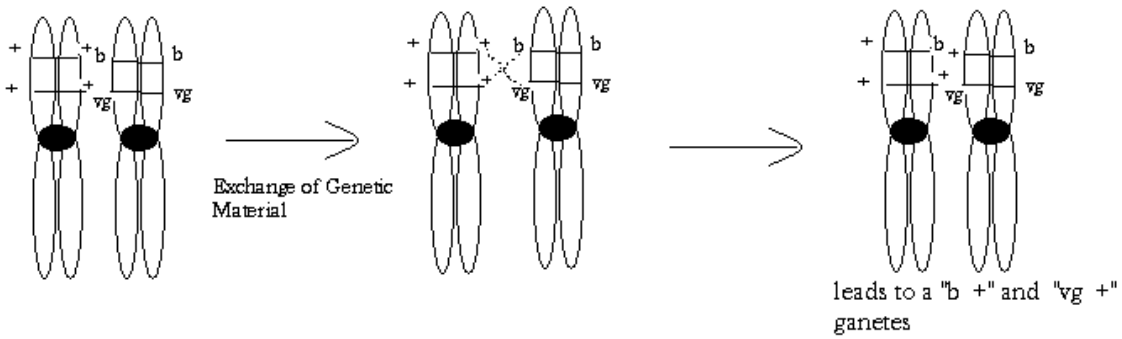
The flies represented by the numbers 944 and 965 are the parental types. The flies represented by the numbers 206 and 185 are the non-parental types (recombinants).

-If the b and the vg genes were on different chromosomes, you would expect a 1 : 1 : 1 : 1 ratio for the genotypes
-If the b and the vg genes were on the same chromosome, you would expect a 1:1:0:0 ratio of the genotypes.

These results; however, indicate that some recombination must have taken place to produce the non-parental (recombinant) types.

Recombination occurs during Meiosis where gametes are being produced.

In the 4n stage of Meiosis, crossing-over occurs between two different chromosomes, effectively exchanging genetic material…



-Recombination (cross-over event) occurs at some frequency. The non-parental types (recombinants) are produced when recombination occurs.

-Looked at cells undergoing meiosis and saw chromosomes touching each other to form a chiasma (cross over).

Was this proof that recombination occurred?

Produces four different gametes: + + b vg (parental) b + vg + (non-parental)
To prove that recombination occurred had to look at number of recombinants produced.

Recombination frequency (RF) is a measure of the probability of genetic exchange

$$\text{Frequency of Recombination} = \frac{\#\,nonparentals}{Total\#\,progeny} \quad \frac{recombinants}{recombinants + parentals}$$

$$\text{From the data : RF} = \frac{206 + 185}{944 + 965 + 206 + 185} = \frac{39}{2300} x100 = 17\%$$

This means that 17% of the time, there is recombination between genes on a chromosome.

If two genes are close recombination frequency is low - ~ 0%
If two genes are far apart, recombination frequency is high ~ 50%

-Genes are said to be linked on the same chromosome if recombination occurs between these genes (RF less than 50%)
-Genes are said to be unlinked if they are very far apart on a chromosome or a re on different chromosomes (RF ~ 50%)

§3. Genetic Maps

A Genetic Linkage Map shows the order of genes on a chromosome. The order is based on the recombination frequency data between the genes

~1911 Alfred Sturtevant, a student in T H Morgan's lab, used recombination frequency data to construct genetic linkage maps.
-He used RF values to assign distances between the genes in map units (m.u)

Example: b and vg genes on a chromosome

B VG (under the letters is the locus where the gene is found)
I----17 mu---I
Therefore the RF value tells us that the b and the vg genes are 17 map units apart on the same chromosome

Sturtevant found that the RF between the vg gene and another gene called cinnabar (cn) was 8 % VG CN.
I--8mu—I

Now there are two possibilities:

1) b vg cn RF between b and cn is 25%
I--------17mu---I---8mu--I

2) b cn vg RF between b and cn is 9%
I ---9mu-I----8mu---I
I----------17mu------I

Sturtevant did the cross and found that the recombination frequency between b and the cn genes was 9 % therefore map #2 is correct.

Sturtevant looked at other genes on the chromosome and made a more extensive genetic linkage map:

B cn vg lobe curved wings
I--------9%---------I----------8%-----I-----~5%------I--------~3%-------I

B = black wings, cn = cinnabar eyes, vg = vestigial wings, lobe = lobe eyes

Sturtevant inferred that genes resided on chromosomes. He was able to map genes on chromosomes by looking at RF data between genes.

§4. Sex-determination and Sex Linkage

Most plants and animals are diploid (2n) – have two copies of each chromosome.
N = # of chromosomes.

In the nucleus of the cells, the chromosomes are found in sets of identical pairs with the exception of 1 set of chromosomes.

Matched chromosomes are called autosomes.
Unmatched chromosomes are called sex chromosomes.

Humans have 22 pairs of autosomes and 1 pair of sex chromosomes (XX or XY).
In most organisms, the type of sex chromosome found in the cell determines the sex of the individual.

Look at Different Organisms

	Humans	Flies	Chickens	Worms
Males	XY	XY	ZZ	XO
Females	XX	XX	ZW	XX

In most cases the males are heterozygotes (XY) while females are homozygotes (XX).
Exception is in chickens

So, what makes a male a male?
Does XX cause femaleness or does Y cause maleness?
Depends on the organism.
In humans: XXY -phenotypically male
In flies : XXY -phenotypically female

In humans Y chromosome is the male determining factor.

Sex-linkage
Genes are found not only on autosomes but also on the sex-chromosomes (mainly on the X).
Connection between sex chromosomes and sex determination was strengthened by experiments on sex linkage.

Studies on Sex-Linkage

Flies:
F_0: White eye Male X Normal Eye Female

↓

F1: All normal eye flies (both male and female)
(white is recessive to normal eye color)

Take 2 F1 flies and do a cross:
Normal F1 male X Normal F1 Female

↓

F2: All daughters are normal
50% male normal, 50% male white-eyes

Conclusion: Gene for eye color is on X chromosome – results cannot be explained by simple dominance/recessiveness. Therefore the trait is Sex-linked.

Genetics III: Human Genetics

§ Review of Sex-linkage in Flies

Quick review: F1 Carrier females (m/+) pass mutant allele to half of offspring and normal allele to half of offspring, while F1 normal males (+/Y) pass on normal allele to all daughters, and Y allele to all males. Since all daughters on the F2 generation get the normal allele + from their father, they cannot have the mutation.

To have a chance of producing white-eyed females, mom has to be +/m or m/m and dad has to be m/Y

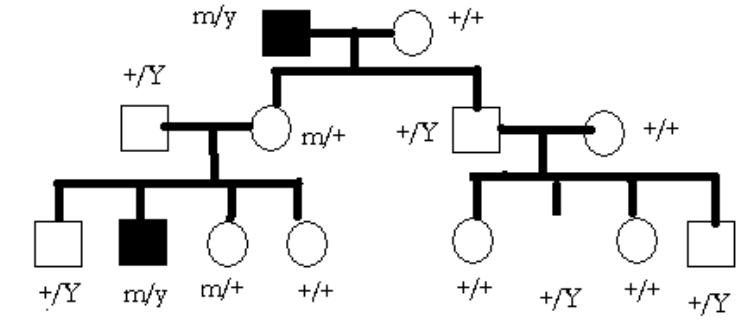
§2. Human Genetics

Pedigrees are used to show the inheritance of human genetic diseases.
A pedigree in which one can trace the pattern of inheritance of a genetic trait.

Modes of inheritance of genetic diseases

1)X-linked (sex-linked) inheritance (X-linked recessive trait)

a)Pedigree:

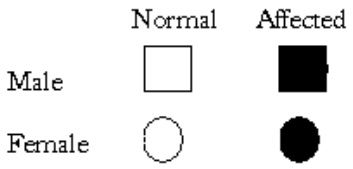


All females are phenotypically normal (but 1/2 are carriers)

1/2 sons are normal,
1/2 are colorblind

All Normal Progeny (sons and daughters)

Notation (symbols)



+ = normal allele carried on X chromosome

m = mutant allele carried on X chromosome

Y = Y chromosome (carries no allele)

In X-linked traits with a recessive phenotype, females are carriers. Carrier females have the mutant allele, but do not exhibit the trait.

b)Characteristics of an X-linked inherited trait:

1) Predominantly affects males – males only need 1 copy of mutant allele to show trait.
(trait is rare in females – they need to be m/m to show the trait – occurs if affected male marries carrier female)

2) Affected males (m/y) do not transmit trait to sons (do not transmit mutant allele)

3) The trait "skips" generations – Females are unaffected carriers – carry the mutant allele, but do not exhibit the trait, therefore they appear phenotypically normal.

4) Carrier females can transmit the trait (mutant allele) to ½ sons and ½ daughters.

5) Affected females transmit <u>mutant allele</u> to all sons and all daughters (daughters become carriers)

Why are males predominantly affected by sex-linked traits?

- Need only 1 mutant copy of gene to exhibit trait

Males: $\frac{m}{\quad}$ X^m affected males lack good copy of gene
Y

Femlaes: $\frac{m}{+}$ X^m carrier females still have good copy of gene
X^+

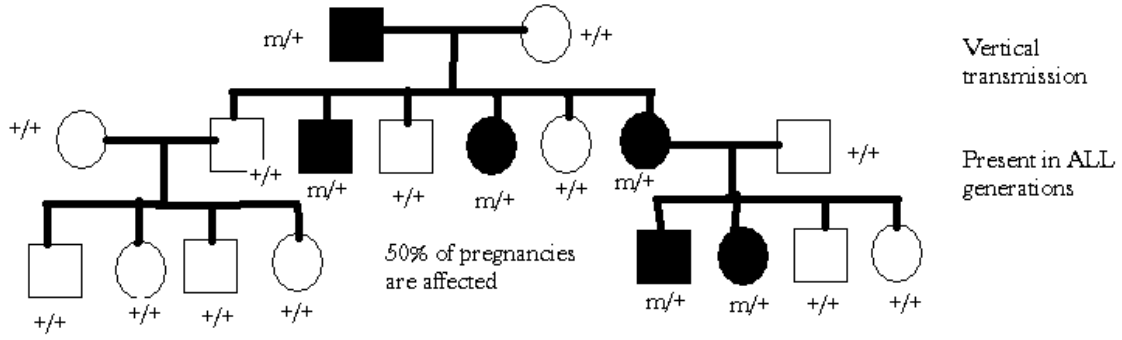
Examples: red-green color-blindness, hemophilia

Genes that are affected in these diseases are carried on X chromosomes.

2) <u>Autosomal Dominant Inheritance</u>

<u>Autosomes</u> (non sex chromosomes)
Humans have 22 pairs of autosomes and 1 pair of sex chromosomes

a) <u>Pedigree of an autosomal dominant trait</u>



b) <u>Characteristics of an Autosomal Dominant Trait</u> (e.g. Huntingtons Disease)

1) Heterozygotes are affected – only 1 copy of allele necessary to be affected with trait

2) Trait is present in every generation (does not "skip")

3) Affects males and females equally

4) Affected individuals m/+ transmit trait to 50% of progeny. If affected is m/m ALL children get the trait.

5) Unaffected individuals never transmit trait.

Huntington's Disease – brain degeneration – occurs 40-50 years old.

Huntington's disease is a disease that shows full penetrance – which means that if you have the mutant allele – always have the disease phenotype.

For most diseases however, if you are m/+ you will get disease with certain probability. This is due to incomplete penetrance – having mutant allele does not mean that you will always get the disease phenotype. Heterozygotes (m/+) show trait with probability $p < 100\%$

Examples of disease that show incomplete penetrance:

Colon cancer: ~80% of individuals with m/+ genotype will develop cancer

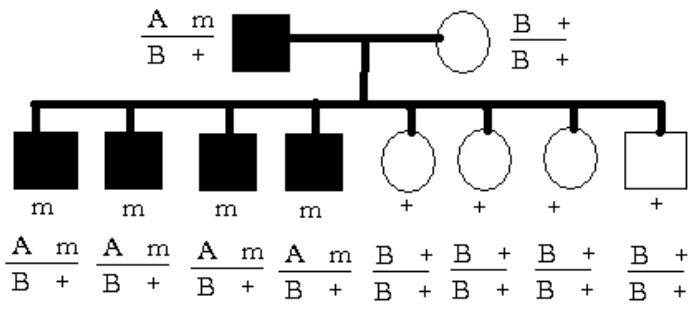
Type I diabetes (juvenile onset diabetes): shows ~30% penetrance

Breast Cancer: ~85% of individuals with mutant allele develop breast cancer.
Two genes implied: BRAC1, BRAC2 (BReast CAncer)
Genes found in chromosome #17
It is inherited in an autosomal dominant fashion.
It is an example of a sex-linked trait (evident in only one sex – prostate cancer is another example of a sex linked trait)

Baldness – example of a sex linked polygenic (many genes involved) trait – also androgen (hormone) dependent.

Use linkage to other genes to trace and find these mutant genes

Example: Pedigree showing the inheritance of a mutant gene (m)



A and B are MUTANT ALLELES of some gene wit hsequence differences (DNA spelling differences)

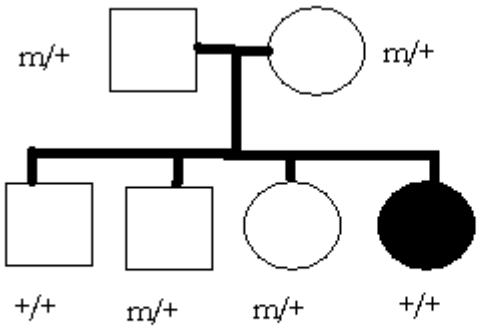
mutant allele A is transmitted with mutant allele m
mutant allele B is transmitted with wild-type allele +

Since phenotype of mutant allele A is always seen with phenotype of mutant allele of disease gene (m), we can say that A is <u>linked</u> to m, therefore it is close to it on the chromosome. Likewise, B must be linked to the wild-type allele.

If A is seen with wild-type phenotype or B is seen with the mutant (disease) phenotype, we can assume that <u>recombination</u> has occurred.

<u>3) Autosomal Recessive Inheritance</u>

a) pedigree:



e.g cystic fibrosis
sickle cell disease

parents are not affected, are carriers

1/4 wild type, 1/2 carriers, 1/4 affected

<u>b) characteristics of an autosomal recessive trait:</u>

1) Not present in every generation, in a large pedigree may be only 1 or 2 persons affected

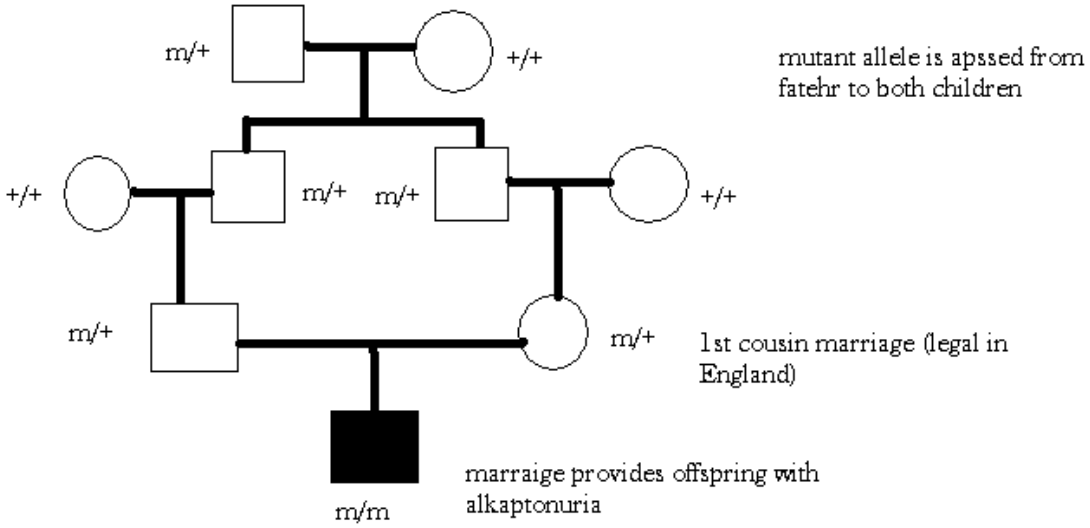
2) Parents not usually affected – ¼ of progeny are affected

3) Offspring of affected not usually affected (not usually transmitted to next generation

4) Affects both males and females equally

This type of inheritance was discovered by work done by:
Archibald Garrod (English Physician ~1900's)

Garrod studied patients with the disease alkaptonuria –
- phenotype of alkaptonuria is that person's urine turns black upon exposure to air
- studied patients with alkaptonuria and found that 8/17 were children of 1st cousin marriages (inbreeding)
- this led Garrod to believe that alkaptonuria was a genetic disease



-Inbreeding increases the chance that a disease allele (m) that is rare in the population, becomes heterozygous (m/m)
-Many rare diseases are the result of inbreeding;
e.g albinism cases are due to 1st cousin marriages.

§ 3. Biochemical Basis of Genetic Diseases

Garrod investigated why the urine of alkaptonurics turned black upon exposure to air.

- found that patients with alkaptonuria excreted large amounts of homogentisic acid (HGA), which turns black upon exposure to air.
- he hypothesized that HGA was produced from the breakdown of proteins, especially amino acids phenylalanine and tyrosine
- HGA oxidizes (upon exposure to air) and turns into a black substance

Garrod tested his hypothesis by feeding patients:

1) High protein diet → result: increased amounts of Hga in urine
2) Phenylalanine → result: increased HGA in urine

3) Tyrosine → result: increased HGA in urine
4) HGA → result: quantitive increase of HGA in urine.

Garrod proposed that there was a pathway in humans that involved the breakdown of amino acids and also suggested that the alkaptonuria (genetic disease) was due to a block in the breakdown of HGA:

Proteins →→Phenylalanine→Tyrosine→→HGA→XX Product Z
This is step blocked in patients with alkaptonuria

Garrod hypothesized that the disease was due to a <u>biochemical defect</u>. HGA builds up because the enzyme to breakdown HGA is missing in alkaptonuria patients.

Hypothesis: <u>Genetic Disease due to Biochemical Defect</u>.

Garrod realized that <u>genes</u> could encode <u>enzymes</u>, which create biochemical reactions.
He called diseases like alkaptonuria "Inborn errors of Metabolism"

Garrod's work led to the work of Beadle and Tatum who in 1942 developed the notion: <u>One Gene : One Enzyme</u>

<u>Genetics IV: Biochemical Genetics</u>

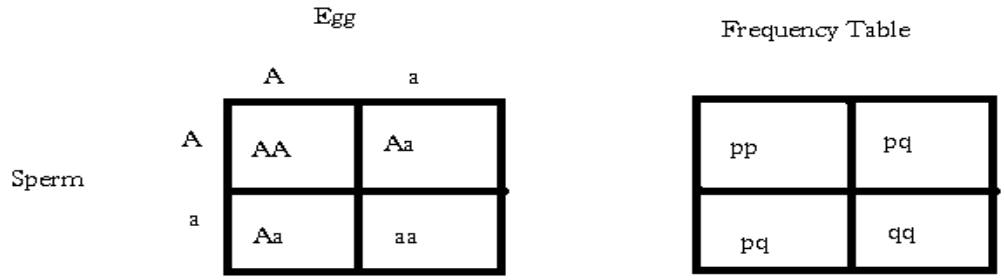
<u>§1. Population Genetics</u>

This field was advanced by laws proposed by two people <u>Hardy and Weinberg</u> (1908)

Suppose in a population there are 2 alleles for a given gene

<u>Alleles</u>:	A	a	(2 forms of gene)
<u>Frequency</u>:	p	q(1-p)	(p+q=1))only 2 alleles for gene)

How often an "A" allele will be in the egg (in female) = p
How often an "a" allele will be in the egg = q



$AA = p^2$
$Aa = 2pq$
$Aa = q^2$

AA	:	Aa	:	aa
p^2		$2pq$		q^2

This is the equilibrium distribution – called the Hardy-Weinberg equilibrium

If you know Allele Frequencies, you can calculate genotype frequencies:

$(p+q)^2 = 1 \rightarrow p^2 + 2pq + q^2 = 1$

At Hardy-Weinberg Equilibrium:
$AA = p^2$
$Aa = 2pq$
$Aa = q^2$

Assuming equilibrium, allele frequencies (and therefore genotype frequencies) do not change over time. Allele frequencies tend to "stay constant" in populations.

Example: cystic fibrosis (autosomal recessive disease)
Frequency of individuals in a population with cystic fibrosis is 1/2000

Let A = normal or wild type allele
a = cystic fibrosis (disease) allele
q^2 (affected individuals) = 1/2000 so $q = \sqrt{1/2000} = 1/44$ therefore $p = 43/44$

Frequency of Carriers: $2pq = 2(43/44)(1/44) \sim .044 = 1/22$
Therefore about 5% of population is carriers

Why are diseases like cystic fibrosis (cf) still prevalent in certain populations?

Heterozygotes carry mutant allele – may have a certain advantage, say increased resistance to cholera infection therefore "a" allele is still in the population.

§2. Biochemical Genetics

-Archibald Garrod united a genetic defect with an enzymatic defect.
-Biochemical Genetics uses experimental genetics to dissect biochemistry

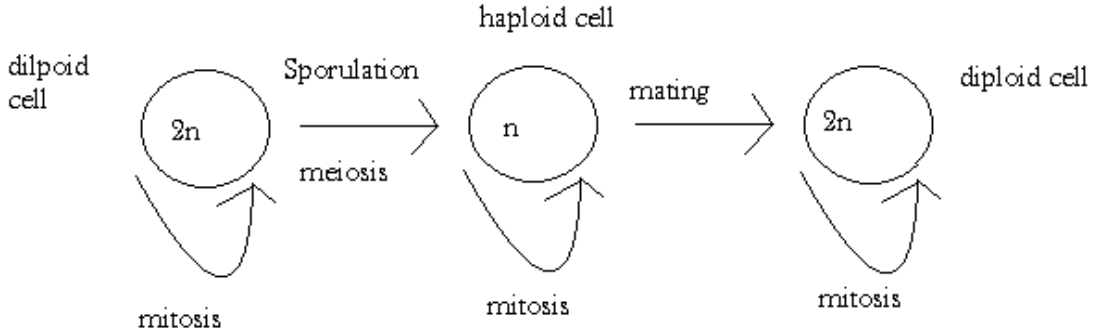
Experimental System:

Yeast: single celled fungus, eukaryote (has a nucleus)

Take a suspension of yeast cells and spread on a solid medium of agar in a petri dish, which contains nutrients. Over time these single cells grow on the agar into visible clumps called colonies. Each colony represents a pile of identical cells, each the descendant of the single cell that was plated on that position.

Life Cycle of the Yeast

Yeast can grow as diploid (2n) or haploid (n) n=16 chromosomes in yeast



Growth Requirements

-Yeast can grow on a minimal medium: agar containing: a carbon source (a fermentable sugar such as glucose, fructose or lactose), nitrogen, phosphorous, salts.

-Yeast has biosynthetic pathways in which they can make all of the biological components required from the simple nutrients provided in a minimal medium

-Yeast can also grow in rich medium – which contains complex nutrients and macromolecules which yeast can take up from the medium

-Yeast can switch off its own synthetic pathways and use what is available in the medium by regulating enzymes involved in pathways

-yeast can synthesize what it needs

-yeast can also take up nutrients from medium

-To understand these biosynthetic pathways we need to characterize yeast that lack certain enzymes, and thus cannot grow under certain conditions.

For Example: How would you isolate genes/proteins that are involved in amino acid synthesis (e.g synthesis of arginine)

- Find yeast that cannot synthesize arginine. Need to go on a mutant hunt (look for mutant yeast that cannot grow without arginine added to the growth medium

Mutant Hunt Strategy:

-plate on rich medium

-replica plate (transfer) yeast colonies to minimal medium

-look for colonies that cannot grow on minimal medium – this is a mutant that has some kind of defect in a biosynthetic pathway
-the way you design a mutant hunt will determine what mutants you will find
-once you obtain a collection of mutants that cannot grow on minimal medium without a supplement, need to determine which component (nutritional supplement) the mutants require for growth on minimal medium.

For example, look for a mutant strain that cannot synthesize arginine:
Plate on rich medium → transfer to minimal medium→ transfer to minimal medium + arginine

All cells grow on rich medium. All colonies except ones that are deficient in a biosynthetic pathway grow on minimal. Ones that are deficient in an arginine synthetic pathway can grow on minimal medium + arginine. (could also do selection on rich medium – arginine. Colonies that do not grow on this medium would be an arginine synthesis mutant)

If we tested growth of yeast on rich vs. minimal medium, we could obtain a variety of mutants called auxotrophs

Definitions:

Prototrophs: wild type strain that can grow on minimal medium
Auxotrophs: Mutant strain that has lost the ability to grow on minimal medium

The mutants that were isolated above that do not grow on minimal medium, but grow on minimal medium +arginine are called arginine auxotrophs

"aux" means needs help

But to analyze a large number of cells we need a better way to test growth on conditions and screen for mutants.

A technique called replica plating was developed by Esther Lederberg (used her facial compact cloth)

Replica Plating is when you transfer all colonies in the same arrangement between two plates by pressing the plates lightly on a piece of velvet.

You can do this type of screening to isolate loss of function mutants.

Two types of Assays:

- to look for colonies that have a desired property or function

1) Genetic Screen: Look at all colonies that grow and then look for cones that cannot grow on a particular medium. Screening for mutants that have "lost a function" – their ability to grow under certain conditions.
2) Genetic Selection: Look for colonies that can grow on a particular medium. Selecting for mutants that have "gained a function" – ability to grow under certain conditions (conditions that would normally not permit growth, like drug resistance)

To study mutants that have an inability to grow on arginine – need to make a collection of arginine auxotrophs – mutants that need only arginine to grow on minimal medium.

To obtain these mutants – need to use haploid yeast cells – have only 1 copy (n) of each gene. Cannot use diploid (2n) yeast because a mutation in 1 of the 2 genes in not enough for auxotrophy.

This is because the level of enzyme produced from 1 copy of the gene (half the normal levels) is sufficient for normal function

Look at haploid vs. diploid yeast:
Haploid: 1 mutation in arg gene leads to arg auxotrophy
Diploid: 1 mutation in arg gene does not lead to arg auxotrophy because other gene is wt

To be arg auxotrophic both copies of the arg gene need to be mutated; not common to find diploid yeast that has two mutations in the same gene

§3. Characterization of Mutants

Collect a large number of mutant yeast strains that are auxotrophic for arginine – call them Arg1, Arg2, Arg3 etc.

Then ask, are these mutants in the same gene or in different genes?

In order to do this, first test whether the mutations are causing a recessive phenotype.

1) Test of Recessivity:

- loss of enzyme function is usually recessive to wild type phenotype
- usually 50% of a gene product is enough to show a wt phenotype for an enzymatic defect – usually only 1 wt copy of gene is enough to show wt phenotype.

DO CROSS TO TEST RECESSIVITY: Cross 2 haploid strains: Arg1 with wt … if the resulting phenotype is wild type than Arg1 is recessive to wt.

Note: This test assumed that arg auxotrophy was recessive to wild-type phenotype. (It's possible to have a mutant allele that gives rise to a phenotype that is dominant to wt)

2) Test of Complementation\

Do a complementation test to determine if the two different mutations are in the same gene or in different genes. Cross two haploid mutants and look at phenotype of diploid.

a) if mutants are in the same gene: Arg1 and Arg2 mutations are the two haploid strains used, the resulting diploid will be unable to grow on minimal medium, since both mutations are in the same gene and the diploid therefore lacks a functional enzyme – the diploid can not grow without Arginine added to the medium.

**Arg1 and Arg2 are mutation in the same gene. MUTATIONS IN THE SAME GENE FAIL TO COMPLEMENT EACH OTHER!!!!

b) If mutations are in different genes: Arg1 and Arg2 mutations are the two haploid strains used, the resulting diploid can grow on minimal medium since both mutations are in different genes, the diploid has a functional copy of each enzyme; therefore the diploid can grow on minimal medium.

**Arg1 and Arg2 are mutations in different genes. MUTATIONS IN DIFFERENT GENES CAN COMPLEMNT EACH OTHER!!!!
Mutations in different genes complement each other, restores protrophy (each mutant rescues the others defect).

Can do a complementation test between different haploids and make a table:

	Wt	Arg1	Arg2	Arg3	Arg4
Wt	+	+	+	+	+
Arg1	+	-	-	+	+
Arg2	+	-	-	+	+
Arg3	+	+	+	-	-
Arg4	+	+	+	-	-

"+" means complementation
"-" means no complementation
Notice that All mutants are recessive to wild type
Mutants 1 and 2 fail to complement: therefore they are in the same gene
Mutants 3 and 4 fail to complement: therefore they are in the same gene

The resulting table suggests that there are two complementation groups or genes involved in arginine synthesis.

Complementation Groups:
Collection of mutants that fail to complement each other – this defines a gene.

There are two genes involved in arginine synthesis.