Chapter 9 ~ Linkage & Recombination Frequency

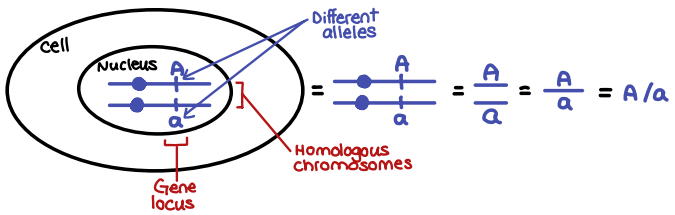
Introductions

- Mendel reported that the pairs of loci he observed segregated independently of each other
  - ex: The segregation of seed color alleles was independent of the segregation of alleles for seed shape.
- This observation was the basis for his Second Law (Independent Assortment) & contributed greatly to our understanding of heredity as single units.
  - However, further research showed that Mendel's Second Law did NOT apply to EVERY pair of genes that could be studied.
  - In fact, we now know that alleles of loci close together on the same chromosome tend to be inherited together.
  - This phenomenon is called "LINKAGE" & is a MAJOR exception to Mendels Second Law of Independent Assortment
- Unlinked genes are on different chromosomes or far apart on the same chromosomes, while linked genes are close (enough) together on the same chromosome.
- The random assortment of the different alleles of genes on different chromosomes depends upon the segregation & independent assortment of the chromosomes during meiosis I.
  - However, genetic recombination of different alleles of genes on the same chromosome can only occur by crossing over.
- When genes are located physically very near to each other on a particular chromosomes, they act as if they are linked & inherited together

- Researchers use linkage to determine the location of genes along chromosomes in a process called genetic mapping.
  - The concept of gene linkage is important to the natural process of heredity & evolution as well as our genetic manipulation of crops & livestock

Review of Genetic Nomenclature & Symboles

- A gene is a hereditary unit that occupies a specific position (locus) within the genome or chromosome & has one or more specific effects upon the phenotype of the organism and can mutate into various forms (alleles)
  - A genotype is the specific allelic composition of a cell or organism.
  - Normally only the genes under consideration are listed in a genotype, while the alleles at the remaining gene loci are considered to be wild type.
- A phenotype is the detachable outward manifestation of a specific genotype.
  - In describing a phenotype, usually only the characteristics under consideration are listed while the remaining characters are assumed to be wildtype (normal)



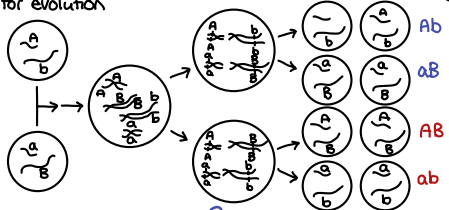
- Usually, gene names are unique & their corresponding symbols are unique letters or combinations of letters.
  - Ex: The "vermillion" gene in Drosophila is represented by the letter "V" while "vg" is the symbol for the "vestigial" gene & "vvl" is the symbol for the "ventral veins lacking" gene locus

NOTE: The same letter symbols may represent a different gene in another organism. Gene symbols & gene names are typically shown *italicized text*, but not always

- The normal, or wild type, form of a gene is usually symbolized by superscript plus sign, "+"
  - Ex: "a^+" "b^+" etc... it is sometimes abbreviated to just "+". A forward slash is occasionally used to indicate that the two symbols are alleles of the same gene, but on homologous chromosomes
- A typical mutant form of the gene, of which there can be many, can be symbolized by a superscript sign "-"
  - Ex: "a^-","b^-" or sometimes abbreviated to just "a" "b" etc (no superscript) Therefore, if the genotype of a diploid organism is given as a^+/a^-, it means there is a wild type allele & mutant allele of the "a" gene at the "a" locus. This may also be abbreviated to +/a
- In some species of diploids, the dominant allele is typically designated w/ the uppercase letter(s), while the recessive allele is given the lowercase letter(s).
  - Ex: Mendels peas → dominant Rough allele is "R", while the recessive smooth alleles is "r"

Recombination & Recombination Frequency

- The process of meiosis leading to a separation of chromosomes, as well as crossing over, is necessary for the understanding of the process of recombination
  - The term "recombination" is used in several different contexts in genetics. In reference to heredity, recombination is defined as a process that results in gametes w/ combinations of alleles that were not present in the gametes from the parental generation
- Recombination is important because it contributes to the genetic variation that may be observed b/w individuals within a population & that may be acted upon by selection for evolution



Inter/Intra-chromosomal Recombination

- Interchromosomal recombination occurs either through independent assortment of alleles whose loci are on different chromosomes.
- Interchromosomal recombination occurs through crossovers b/w loci on the same chromosomes
  - It is important to remember that in both of these cases, recombination is a process that occurs during meiosis (mitotic recombination may also occur in some species, but it is relatively rare
  - As an example of interchromosomal recombination, consider loci on two different chromosomes We know that if these loci are on diffent chromosomes there is no physical connection b/w them, so they are unlinked & will segregate independently as did Mendel's traits.
- The segregation depends on the relative orientation of each pairs of chromosomes at metaphase

  Since the orientation is random & independent of other chromosomes, each of the arrangements (& their meiotic products) is equally possible for two unlinked loci

Intrachromosomal recombination occurs through crossovers. Crossovers occur during prophase I of meiosis, when pairs of homologous chromosomes have aligned w/ each other in a process called synapsis.

Crossing over begins w/ the breakage of DNA of a pair of non-sister chromatids. The breaks occur at corresponding positions on two non-sister chromatids, & then the ends of non-sister chromatids are connected to each other resulting in a reciprocal exchange of double-stranded DNA.

Generally, every pair of chromosomes has at least one crossover during meiosis, but often multiple crossovers occur in each chromatid during prophase I

Because interchromosomal recombination occurs through independent assortment genes in this situation are always unlinked. Intrachromosomal recombination has instances of linked genes.

Inheriting Parental & Recombinant Gametes

- If we consider only two loci & the products of meiosis results in recombination, then the meiotic products (gametes) are said to have a recombinant genotype
- On the other hand, if no recombination occurs b/w the two loci during meiosis, the products retain their original combinations & are said to have non-recombinant, or parental genotype.
- The ability to properly identify parental & recombinant gametes is essential to apply recombination to experimental examples.
  - To properly identify recombinant & parental gametes from an individual, you need to know the genotype of its parents (the P gen)
  - This is most easily demonstrated in a dihybrid.

    If for two genes, one parent has the genotype

    A/A B/B → AB } they can only produce one type of gamete

    Similarly, if they are

    a/a b/b → ab } they can also only produce 1 type of gamete

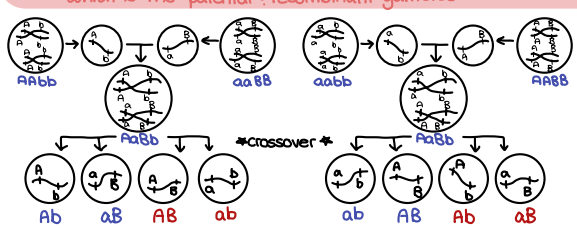
    However if those two gametes (AB & ab) combine, they create an individual (F1) w/ a genotype written as A/a B/b.

    It can be easier to keep track of the parental combinations of gametes by keeping them together when writing the genotype, for this ex: AB/ab
- The above dihybrid individual can produce four different gametes:
  1. AB
  2. ab
  3. Ab
  4. aB

  The parental gametes are those that the individual from their parents, in this case AB & ab. Ab & aB are recombinant gametes & are evidence of a recombination event happening, resulting in a different combination of alleles

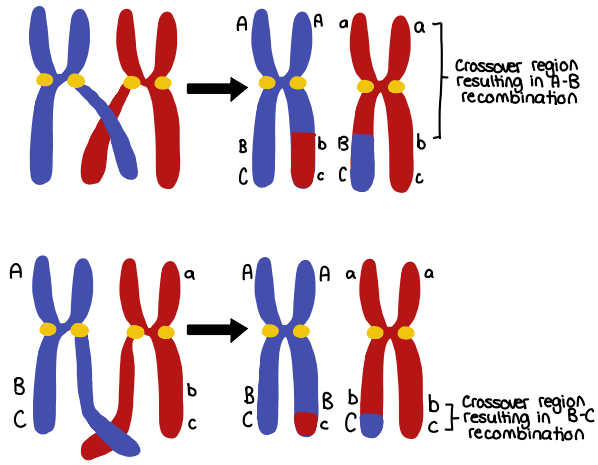
the P gen has one parent homozygous for both dominant alleles, and the other homozygous for both recessive alleles.

NOTE: this will not always be the case. In some instances one parent will be homozygous, with one gene dominant & the other gene recessive (A/A b/b) and the other parent will be the opposite (a/a B/B) This situation will change, which is the parental & recombinant gametes



Cont'd... Recombination & Recombination Frequency

Cont'd Inheriting Parental & Recombinant Gametes



Recombination Frequency

Recombination Frequency (RF) is a calculation to define the number of parental & recombinant gametes. The equation is as follows:

$$\text{Recombination Frequency} = \left(\frac{\text{No. recombinant progeny}}{\text{Total no. of progeny}}\right) \times 100\%$$

Through identifying & defining parental & recombinant gametes, you can calculate the RF and from there, decide the degree of linkage

Based upon the equation & independent assortment, you can see that the recombination frequency cannot be higher than 0.50 If alleles are assorting independently, there will be a random distribution of alleles in the progeny 50% will be recombinant gametes & 50% will be parental gametes, making the RF approximately 0.50.

If a gene is linked, you will see a higher percentage of parental gametes making the RF <0.50.

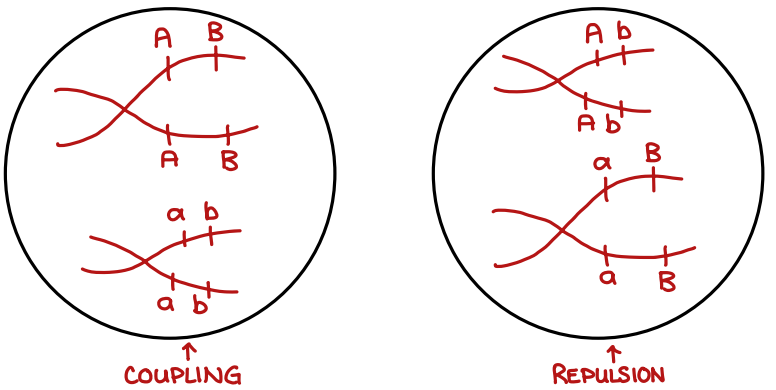
You will never see recombinant gametes more than parental, and in no situation will recombination frequency be higher than 0.50, except slightly w/ regards to standardize experimental error.

If you calculate a recombination frequency higher than 0.50 you should ensure you have accurately defined parental & recombinant gametes

Coupling & Repulsion (Cis & Trans) Configuration

- Just by looking at an organism that is heterozygous at two loci, you cannot tell how the mutand & wild type alleles are arranged. Both mutant alleles could be on one homologous chromosome, & both wild type alleles could be on the other
  - ex: a^-b^-/A^+A^+

  This is known as coupling (or ions) configuration.
- When one wild type allele & one mutant allele are on one homologous chromosome, & the opposite is on the other, this is known as repulsion (or trans) configuration
  - ex: A^+b^-/a^-B^+
- The way to determine the orientation to look at the parents of that cross if you know the genotypes of them.
  - If the parents are homozygous for both genes and one shows dominant phenotypes & the other shows both recessive phenotypes, then you know that the individual you are looking at is in coupling configuration.
  - If one parent has one dominant and one recessive phenotype & the other has the opposite, then you know the individual is in repulsion configuration



Unlinked Genes VS Partial Linkage VS Complete Linkage

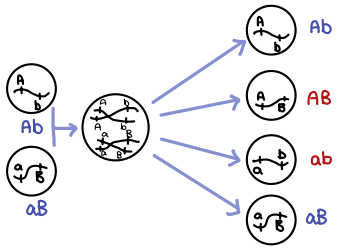
- When comparing any two genes, they can be varying distances apart.
  - Their RF allows us to categorize them into the degree of linkage.
  - The amount of linkage can be placed on a sliding scale

Linkage Description	Recombination Frequency
Unlinked	Approximately 50% or more than 35%
Partial linkage	More than 0% to 30%
Complete linkage	0%

Unlinked Genes

- Unlinked genes appear to segregate & show independent assortment.
  - There will be a random & even distribution of gamet types, & an RF of 0.50 is the expectation
  - This situation occurs in two instances: either when the genes are on completely different chromosomes, or when they are far enough apart on a single chromosome that crossover are so numerous that alleles are distributed randomly
- Either way, because the alleles are assorting independently, you should observe an equal number of recombinant & parental gametes, w/ an RF near ~0.50

Note: Because of real-life variability this value can be anywhere from ~0.40 to ~0.60



Complete Linkage:

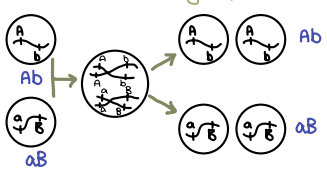
Having considered unlinked loci, let us turn to the opposite situation, in which two loci are so close together on a chromosome that the parental combinations of alleles always segregate together

This is because the physical distance b/w the two loci is so short that crossover events become extremely rare.

Therefore, the alleles at the two loci are physically attached to the same chromatid and will nearly always segregate together into the same gamete

In this case, no recombinants will be present following meiosis, & the recombination frequency will be 0.00

This is complete linkage & is rare, as the loci must be so close together that crossovers are virtually impossible to detect



Partial Linkage

- It is also possible to obtain recombination frequencies between 0% & 50% which is a situation we call incomplete (or partial) linkage
- Incomplete linkage occurs when two loci are located on the same chromosome, but the loci are far enough apart so that crossovers occur b/w them during some, but NOT all, meioses
  - Genes on the same chromosome are said to be syntenic regardless of whether they are completely or incompletely linked or unlinked
  - Thus all linked genes are syntenic, but not all syntenic are linked

Because the location of crossovers is essentially random for any given base pair of the chromosome, the greater the distance b/w two loci, the more likely a crossover will occur b/w them

Furthermore, loci on the same chromosome, but sufficiently separated from one another, will on average have multiple crossovers b/w them, & they will behave indistinguishably from physically unlinked loci.

A recombination frequency of 50% is therefore the maximum RF that can be observed, and indicative of loci that are either on separate chromosomes, or sufficiently seperated on the same chromosome

Experimental Determination of Recombination Frequency

Consider a complete experiment in which our objective is to measure RF

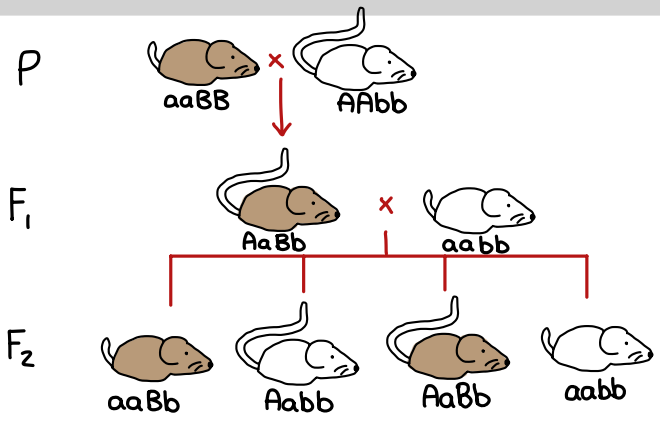
We need at least two alleles for each of 2 genes, & we must know which combinations of alleles were present in the parental gametes

The simplest way to do this is to start w/ pure-breeding lines w/ contrasting alleles at two loci

Ex: Could cross short-tailed (aa), brown mice (BB) w/ long-tailed (AA), white mice (bb)
Thus aaBB = short-tailed & brown while AAbb are long-tailed & white

we know that the parental gametes = aB or Ab NOT AB or ab
ALL progeny = dihybrids (AaBb)

We DO NOT know at this point if the 2 loci are on different chromosomes or whether they are on the same chromosomes or how close they are to eachother



We can then infer unambiguously the genotype of the gametes produced by the dihybrid individual, and therefore calculate the recombination frequency b/w these two loci

Ex: If only two phenotypic classes were observed in the F2 (ie short tails & brown fur (aaBb) & white fur w/ long tails (Aabb))

we would know that the only gametes produced following meiosis of the dihybrid individual were of the parental type: aB & Ab & the RF would therefore be 0%. Alternatively we may observe multiple classes of phenotypes in the F_2 ratio

Tail Phenotype	Fur Phenotype	# of progeny	Gamete from Dihybrid	Genotype of F2 from Test Cross	(P)arental or (R)ecombinant
Short	Brown	48	aB	aaBb	P
Long	White	42	Ab	Aabb	P
Short	Brown	13	ab	aabb	R
Long	White	17	AB	AaBb	R

$$RF = \frac{\#\text{ recombinant offspring}}{\text{Total offspring}}$$

$$RF = \frac{13 + 17}{48 + 42 + 13 + 17}$$

$$= 0.25$$

RF is below 0.30, we can say that the tail length gene & fur colour gene are partially linked

The recombination events that may be detected will occur during meiosis in the dihybrid individual

If the loci are completely or partially linked, then prior to meiosis, alleles aB will be located on one chromosome & alleles Ab will be on the other chromosome.

These are the parental gametes based on our knowledge of the genotypes of the gametes that produce the dihybrid.

Thus, recombinant gametes produced by the dihybrid will have the genotypes

AB or ab

Now that we have identified the parental & recombinant gametes, how do we determine the genotype of the gametes produced by the dihybrid individual?

The most practical method is to use a test cross in other words, to mate AaBb to an individual w/ only recessive alleles at both loci (aabb)

This will give a different phenotype in the second generation for each of the four possible combinations of alleles in the gametes of the dihybrid

	AB	Ab	aB	ab
ab	Aa Bb	Aa bb	aa Bb	aa bb
Phenotype	Long Brown	Long White	Short Brown	Short White
Recomb. or Parental	R	P	P	R