Chapter 11 ~ Recombination Mapping of Gene Loci

Introductions

- Genetic mapping = linkage mapping
  - -supplies geneticists w/ the evidence that a trait or disease which is passed from one gen to the next is linked to one or more genes.
  - -Genetic maps also provide information regarding which chromosome contains the gene in question & where the gene lies on that chromosome
- We will examine use of RF data to construct genetic maps
- Discuss the limitations of this technique (based on events during meiosis)
- 2 loci could show
  - -independent assortment (unlinked, RF ~ 50%)
  - -partial linkage (RF < ~35%)
- Linked = 2 genes on same chromosome (syntenic)
- Unlinked = Genes are far apart on same chromosome / different chromosomes (non-syntenic)
- We will learn how to:
  - -Construct genetic map using 2 pt & 3 pt crosses

Genetic Map Based on RF in Drosophila

MUTANT		WILD TYPE
Short aristae	0	Long aristae
Black body	48.5	Grey body
Cinnabar eyes	57.5	Red eyes
Vestigial wings	65.5	Normal wings
Brown eyes	104.5	Red eyes

Genetic Mapping

A genetic map (recombination map) is a representation of the linear order of gene (or loci) & their relative distances determined by crossover frequency along a chromosome.

The fact that such linear maps can be made supports the concept of genes being arranged in a fixed, linear order along a single duplex of DNA for each chromosome

We can use recombination frequencies to produce genetic maps of all the loci along each chromosome & ultimately in the whole genome

Calculating Map Distances:

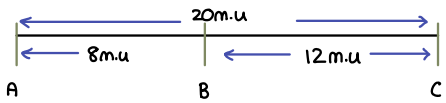
- Combine the concepts of linkage & calculating RF to construct genetic maps shows the relative location of 2 or more genetic traits
  - -Usually we analyze the offspring in a particular cross & tracks how many times 2 given genetic traits are inherited together
    - ↳ Ex: eye color & wing shape.
  - -The higher the % of progeny that inherit both traits together, the closer the genes responsible for the traits on the chromosome.
- Genetic maps = Based on rates of recombination

The units of genetic distance = map units (mu) or centiMorgans (cM)

RF of two loci converted into cM

RF in % is ~ the same as map distance in cM

1mu = 1% Recombinance rate



Genetic distances measured w/ recombination rates are also approximately additive

The distance b/w gene A to B is 8 m.u & from B to C is 12 m.u

Therefore, the distance b/w A & C is 20 mu & gene B is located b/w genes A & C

NOTE: This approximation works well only for small distances (RF < 30%) but progressively fails at longer distances. This is b/c as the two loci get farther apart the RF reaches a max at 50% like it would for two loci assorting independently (not linke)

In fact some chromosomes are > 100cM long but such loci at the tips only have an RF of 50%.

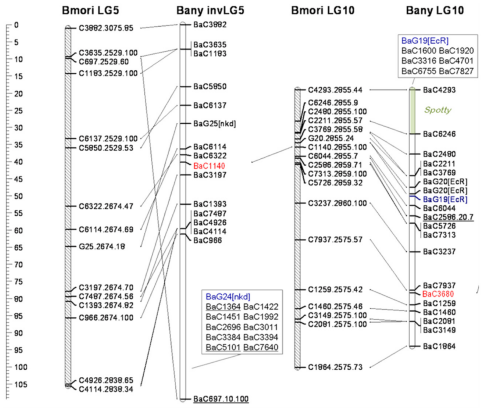
Calculating the map distance of the WHOLE chromosome of over 50cM comes from mapping of multiple loci dispersed along the chromosome, each w/ a value of less than 50%, with their total adding up to the value of 50

The method for mapping of these long chromosomes is described next

NOTE: The map distance of 2 loci alone doesn't tell us anything about the orientation of these loci relative to other features, such as centromers or telomers on the chromosome

Genetic Maps are an Approximation

- Genetic maps are useful for showing the order of loci along a chromosome but the distances are only a relative approximation.
- Correlation b/w RF & actual chromosomal distance is more accurate for short distances (↓RF) than long distances.
  - -Observed RF b/w 2 relatively distant markers tend to underestimate the actual number of crossovers that occured.
  - -This is b/c as distance b/w loci increases the possibility of a second crossover occuring increases b/w the loci
- Problem b/c double-crossovers produce gametes w/ the same genotypes as if no recombination events happened
- Have parental genotypes so appear as parental & aren't counted as recombinant
- Specific mathematical formulae can be adjusted for this & better estimates can be made



Map Distances Over Long Chromosomes

- Map distances are always calculated for one pair of loci at a time.
  - -However, by combining the results of multiple pairwise calculations, a genetic map of many loci on a chromosome can be produced
- A genetic map shows the map distance, in cM that separates any 2 loci & the position of these loci relative to all other mapped loci.
- The genetic map distance is roughly proportional to the physical distance
  - -Ex: The amount of DNA b/w 2 loci
  - -Ex: Arabidopsis, 1.0 cM corresponds to approximately 150,000 bp & contains ~ 50 genes

Exact # varies w/ organism & chromosomal position

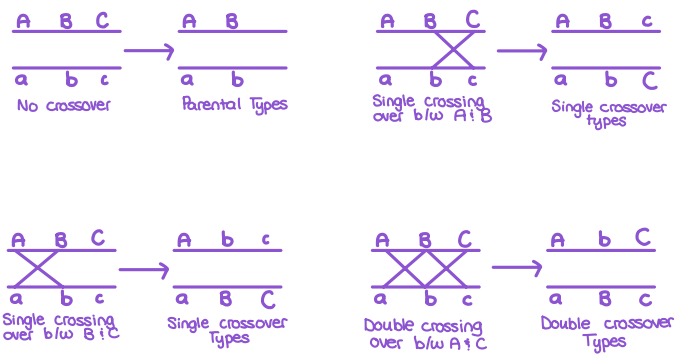
- Crossover hot spots → Areas w/ higher rates of recombination than other

Large regions of heterochromatin = Often areas w/ lower rates of recombination

- When a novel gene or locus is identified by mutation or polymorphism, crossing it in w/ previously mapped genes, and then calculating RF can determine it ~position on a chromosome
- Using novel gene & already mapped genes show complete or partial linkage w/ an existing locus, the RF indicate the ~position of the novel gene w/in the genetic map. Map-based cloning

Benefits of Genetic Maps

1. Track genes/alleles when breeding crops & animals
2. In studying evolutionary relationships b/w species
3. In determining the causes & individual susceptibility of some human diseases



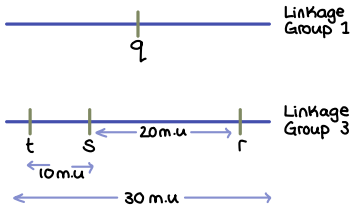
Two Point & Three Point Crosses

Gene maps can be created by using the info obtained through a series of test crosses where one of the parents is heterozygous for a different pair of genes & we can calculate the RF b/w pairs of genes. A test cross b/w 2 genes is called 2 point test cross.

Two Point Crosses

With Independent assortment → RF = 50%.
- Genes q & r are either located on different chromosomes / are VERY distant from each other on the same chromosome
  - ↳ Belong to different linkage groups
- Genes q & s are also in diff linkage groups
- Genes r & s RF = 20%. so = 20 m.u = linked
  - ↳ means these genes are seperated by 20 m.u
- Genes r & t RF = 10%. so = 10 m.u
  - ↳ To see if its 10 m.u to the left/right of r look @ distance s–t
  - ↳ If to the left then sum distance = 20m.u + 10m.u = 30m.u
  - ↳ If to the right of gene r then the distance b/w t–s will be less = 20m.u - 10m.u = 10m.u
- From the data we know that RF b/w s–t = 28%.
  - t lies to the left of r

Gene loci	RF (%)
q & r	50
q & s	50
q & t	50
r & s	20
r & t	10



Three Point Crosses

- Useful for mapping location of a new mutation relative to 2 mapped locis location
  - Pure breeding lines w/ contrasting genotypes are crossed = heterozygous at 3 loci
    - ↳ Trihybrid is then test crossed w/ tester homozygous recessive for all 3 genes
      - Determines combination frequency b/w each pair of genes
    - ↳ Punnet square it to predict possible outcomes
- Efficent method of mapping 3 genes at once = Three point cross
  - Allows order & distance b/w 3 potentially linked genes to be determined in a single cross experiment

Trihybrid x tester = 8 different gametes = 8 different phenotype combos

Next step → Identify alleles as recombinant or parental gametes
- Compare only 2 loci at one time to parental gametes
- Ex: Parents of Trihybrid = A/A, b/b, C/C & a/a, B/B, c/c
  - Parental gametes = aBc & AbC
  - By comparing you releave them as either recombinant or parental

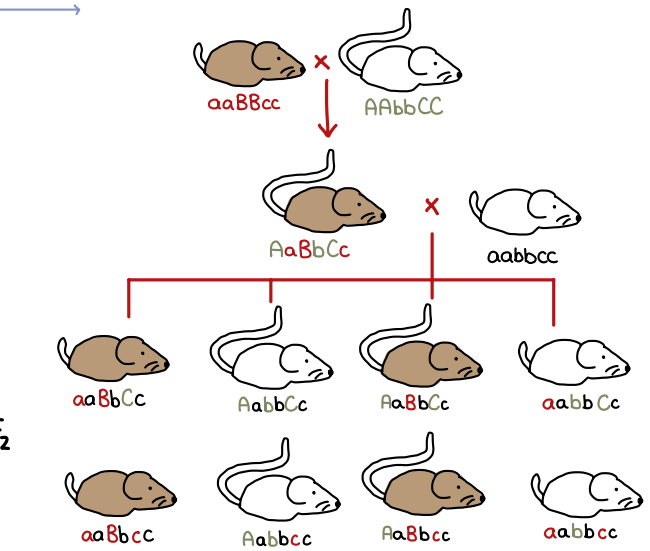
loci A,B – RF = $\frac{(1+16+12+1)}{120} = \frac{30}{120} = 25\%$

loci A,C – RF = $\frac{(1+5+1+5)}{120} = \frac{12}{120} = 10\%$

loci B,C – RF = $\frac{(5+15+12+5)}{120} = \frac{38}{120} = 32\%$ (not corrected for double crossovers)

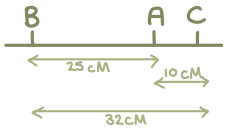
- Once classes of progeny are ID, RF can be calculated for each pair of loci individually
- Use these numbers to build the map, placing the loci w/ the largest RF on the ends

NOTE: Sum of distances b/w A-B & A-C (10% + 25% = 35%) is less than the distance calculated for B-C (32%.)



♂ / ♀	aBC	P2 AbC	abC	ABC	P1 aBc	Abc	abc	ABc
abc	aa Bb Cc	Aa bb Cc	aa bb Cc	Aa Bb Cc	aa Bb cc	Aa bb cc	aa bb cc	Aa Bb cc
Phenotype	Short tail Brown Long whis	Long tail White Long whis	Short tail White Long whis	Long tail Brown Long whis	Short tail Brown Short whis	Long tail White Short whis	Short tail White Short whis	Long tail Brown Short whis

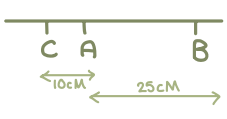
Tail Phenotype	Fur Phenotype	Whisker Phenotype	# of progeny n = 120	Gamete from Trihybrid	Genotype of F_2 from test cross	Loci A,B	Loci A,C	Loci B,C
Short	Brown	Long	5	aBC	aaBbCc	P	R	R
Long	White	Long	38	AbC (P2)	AabbCc	P	P	P
Short	White	Long	1	abc	aabbCc	R	R	P
Long	Brown	Long	16	ABC	AaBbCc	R	P	R
Short	Brown	Short	42	aBc (P1)	aaBbcc	P	P	P
Long	White	Short	5	Abc	Aabbcc	P	R	R
Short	White	Short	12	abc	aabbcc	R	P	R
Long	Brown	Short	1	ABc	AaBbcc	R	R	P



We deduce that the order must be BAC or CAB

IF we included the double recombinant classes (ABc & abC) (multiplied by 2) the calculation of RF b/w B–C is:

loci B,C – RF = $\frac{(5+16+12+5+2(1)+2(1))}{120} = \frac{42}{120} = 35\%$ (corrected for double crossover)



3 point crosses useful for:
1. Determining the order of 3 loci relative to each other
2. Calculating map distances b/w the loci
3. Detecting some of the double crossover events that would otherwise lead to an underestimation of map distance

Some double crossover events may NOT be detected:
EX: Crossover b/w
A & B
A & C

Parental gametes (AbC & aBc) are the result of no crossovers or double crossovers b/w 2 alleles

All 3 loci are linked = frequency expected to be relatively high

Recombinant gamest are the result of 1 crossover b/w 2 alleles
- abc
- aBC
- Abc
- ABC

Single crossovers = More common BUT more likely b/w loci A & B (25 cM) are farther apart than A & C (10 cM)
↳ Detect more recombinant gametes w/ the former

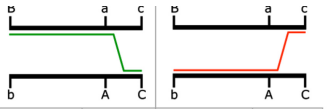
Cont'd...Two Point & Three Point Crosses

Cont'd...Three Point Crosses

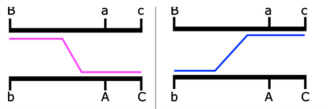
- If all 3 genes are unlinked, then we expect independent assortment & an equal number of all progeny types
- Recombinant genotypes that are a result of two recombination events will be rare.
  - Number will be different if all the linked genes are equal distances from each other, or if one pair is more linked than the other
- In the case where two genes are linked & one gene is unlinke the following applies
  - As in the example before, we will use the same parental gametes (AbC & aBC) but will assume the genes A & C are linked & B is unlinked
  - Linkage causes a higher prevlance of parental gametes, we expect there to be more parental organization of A & C and fewer recombinant organizations of A & C
  - Presence/Absence of parental B is NOT important here b/c it is unlinked & will assort independently

- Recombinant gametes that are the result of double crossover events (ABc & abC)
- Double crossovers b/w 3 linked genes like this is rare, so we don't expect to see many offspring from these recombination gametes

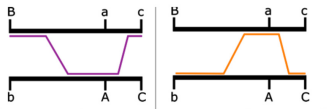
A & C have a stronger linkage than A & B



gamete	# of progeny	gamete	# of progeny
aBC	5	Abc	5



gamete	# of progeny	gamete	# of progeny
ABC	16	abc	12



gamete	# of progeny	gamete	# of progeny
ABc	1	abC	1

Coincidence & Interferance

- Map distance tells us about
  - Relative distance b/w genes
  - Proportion recombinant & non-recombinant gametes produced in a cross
- Double crossovers = underestimation of map distance

- Theoretically calculating the proportion of double recombinant gametes using the rule of probability (multiplication) & then multiply this theoretical probability the total number of progeny, we would the expected # of double crossover progeny.

- In reality much less observed in the progeny produced.
  - This is b/c the calculated number assumes crossover is independent of each other.
  - Crossovers are NOT independent one crossover may inhibit other crossover in the nearby vicinity on the chromosome so double crossovers become less frequent than expected

- The term interference is used to describe the degree to which one crossover interferes w/ other crossovers in the region at the chromosome in question.

We are able to calculate the interference using the formula:

$$\text{Interference} = 1 - \text{coefficient of coincidence}$$

Calculate the coefficent of confidence next:

$$\text{Coefficient of coincidence} = \frac{\#\text{ of observed double crossovers}}{\#\text{ of expected double crossovers}}$$