BIOL2416 GENETICS
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THREE-POINT TEST CROSSES

WHY?

1. To determine if three genes are linked in any way
2. To determine the linear order of three linked genes
3. To estimate the map distance between any of the linked genes (more accurate if genes are close together and if large numbers of progeny are scored)

WHAT?

Let's keep it simple and just discuss a test cross involving three autosomal recessive traits. One parent would be heterozygous for all three, the other parent would be homozygous recessive for all three. Let's say that all genes exhibit CIS coupling, so that in the heterozygous parent, all three dominant alleles are on one chromosome, and all recessive alleles are all on the other chromosome.

HOW?

For the above scenario:

P genotypes: ABC / abc x abc / abc

P phenotypes: ABC abc

Note we are using allele symbols to represent PHENOtypes. From this cross, you might get the following progeny phenotypes (baby flies):

Baby phenotype	**Looks like?**	**Observed # of babies**
ABC ("big-big-big")	1st parent	401
abc	2nd parent	389
ABc	Recombinant type	4
abC	Recombinant type	6
AbC	Recombinant type	75
aBc	Recombinant type	65
aBC	Recombinant type	35
Abc	Recombinant type	25
		Total = 1000 babies

Boy, that's a lot of recombinant types. Where did they come from? Let's take a closer look and see which traits came from which parents:

Baby phenotype	**Baby type**	**Which TWO traits were inherited TOGETHER from ONE of the parents ? (recall parents were ABC and abc)**	**Which trait was swapped / came from the other parent?**
ABC ("big-big-big")	Parental type	ABC	none
abc	Parental type	abc	none
ABc	Recombinant type	AB from ABC parent	c
abC	Recombinant type	ab from abc parent	C
AbC	Recombinant type	AC from ABC parent	b
aBc	Recombinant type	ac from abc parent	B
aBC	Recombinant type	BC from ABC parent	a
Abc	Recombinant type	bc from abc parent	A

1. HOW CAN YOU TELL IF THE GENES ARE LINKED?

You need to look at the actual numbers observed for the babies. If you see 8 phenotypic classes in the babies, where 2 classes are very numerous, 2 are very scarce, and the other 4 are in between, then you likely have three genes that are ALL LINKED ion the same chromosome:

Baby phenotype	**Type**	**Observed numbers**	**Relative frequency**
ABC ("big-big-big")	Parental type	401	many
abc	Parental type	389	many
ABc	Recombinant type	4	very few
abC	Recombinant type	6	very few
AbC	Recombinant type	75	in between
aBc	Recombinant type	65	in between
aBC	Recombinant type	35	in between
Abc	Recombinant type	25	in between

So these three genes are all linked on the same chromosome.

2. **HOW CAN YOU DETERMINE THE ORDER OF THE THREE LINKED GENES?**

Parental progeny is the most numerous, and recombinant progeny can only be produced by a single or a double crossover event between the linked genes. You can think of a double crossover as two single crossovers; the chance that one crossover occurs between the first two linked genes AND a second crossover

occurs between the second two linked genes becomes pretty slim (product rule!). So you can:

- ASSIGN the "VERY FEW" classes the label of "DOUBLE CROSSOVER" progeny (see table below).
- Now look at WHICH TRAIT was SWAPPED OUT in these DOUBLE-CROSSOVER babies. Yep, it was the C! Since only alleles in the middle can experience a double crossover, C must sit in the middle. The gene order is A - C – B (or B – C – A, same difference!)
- While you're at it, ASSIGN the in-between classes the label of "SINGLE CROSSOVER" progeny (see table below).

3. HOW CAN YOU ESTIMATE THE MAP DISTANCES BETWEEN THE LINKED GENES?

If all three gene are linked, you will have add up the single crossover recombinants for each pair of genes to the double crossover recombinants, then divide by the total and multiply by 100%. That's a mouthful. Let's break it down. First go back to the table and determine where each single crossover (between which 2 genes) is taking place. Remember that C is in the middle, so single crossovers will involve either C and A, or C and B:

Baby phenotype	**Type**	**Observed number**	**Due to what kind of Xover?**	**Which traits STAYED together?**	**Which trait was swapped / CROSSED OVER?**
ABC	parental	401	none	ABC	none
abc	parental	389	none	abc	none
ABc	Recombinant	4	**Double** (around C)	AB	c
abC	Recombinant	6	**Double** (around C)	ab	C
AbC	Recombinant	75	**Single** (between C and B)	AC	b
aBc	Recombinant	65	**Single** (between C and B)	ac	B
aBC	Recombinant	35	**Single** (between A and C)	BC	a
Abc	Recombinant	25	**Single** (between A and C)	bc	A

So the map distance between A and C is:

(# ALL double Xovers + # of all single crossovers INVOLVING A and C)(100) / (total) =

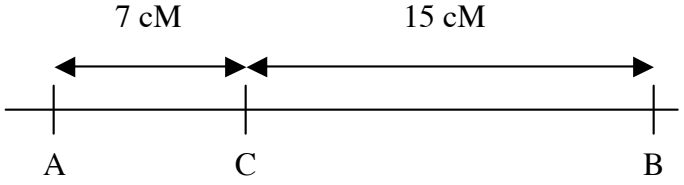
(4 + 6 + 35 + 25) (100) / 1000 = 7000 / 1000 = 7 map units or 7 cM between A and C

The map distance between C and B is:

(# ALL double Xovers + # of all single crossovers INVOLVING B and C)(100) / (total) =

(4 + 6 + 76 + 65) (100) / (1000) = 15000 / 1000 = 15 map units or 15 cM between C and B

Final Map:



4. HOW CAN YOU CALCULATE THE COEFFICIENT OF COINCIDENCE / INTERFERENCE?

Double crossovers do not tend to occur as often as expected from the observed rate of single crossovers, because a crossover event may interfere with a second crossover event nearby.

Coefficient of coincidence

= (observed % double crossovers) / (expected % double crossovers)

= 1 – interference (=1 if there is no interference = perfect match between observed and expected)

In our case:
expected % double crossovers =

= (recombination frequency between gene 1 and gene 2) / 100 x

(recombinantion frequency between gene 2 and 3) / 100

= (7/100)(15/100) = 0.0105 = 1.05%

observed % double crossovers = (4 + 6)/1000 = 0.01 = 1%

coefficient of coincidence = 1 / 1.05 = 0.95
interference = 1 – 0.95 = 0.05