Analysis of a tri-hybrid test cross (v.1.5)

Theoretical:

Given three loci, linked on a single chromosome in the following order

-----A-----B------C-----

Then, a cross between ABC//ABC x abc//abc tester involves

```
-----A-----B------C-----
-----A-----B------C-----
-----a-----b------c-----
```

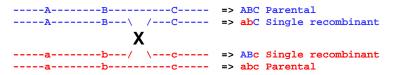
----a-----b-----c-----

Recombination may *not* occur at all (parental type), or may occur between A & B, or B & C (single crossovers) or between both: a double crossover

During Meiosis I, single cross-over between the A & B loci looks like:

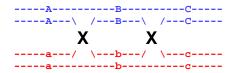
```
-----A-----B-----C----- => ABC Parental
-----A---\ /---B-----C----- => aBC Single recombinant
X
-----a---/ \---b------- => Abc Single recombinant
-----a---- => abc Parental
```

A single cross-over between the **B & C** loci looks like:



Each crossover *changes the phase* of one locus with respect to (wrt) its nearest neighbor. The frequency of either single crossover is proportional to the distance between loci

The frequency of a **double crossover** is the *product* of these frequencies: therefore a double-crossover is the *rarest* type:



A double crossover will reverse the phase of the middle locus

A A	_	-	
a	_		

Thus, when you compare the *most common* types (the **parental ABC** & **abc**) with the *rarest* (the **double-recombinants AbC** & **aBc**) the *cis / trans* **phase** of the *middle* locus is *reversed* wrt both *outside* loci Here: **B** is reversed wrt **A** & **C** (and **b** wrt **a** & **c**): *B* is the middle locus

Experimental:

Consider three loci E, F & G, linked on a single chromosome, but in an unknown order

The following cross is constructed:

```
EEFFGG x eeffgg (P) => EeFfGg x eeffgg (tester) (F1)
```

which produces the following counts among 1,000 F2 offspring The genotype of the offspring can be inferred directly from the phenotype

Genotype	Phenotype	Count	Class
E F G <mark>efg</mark>	'EFG'	370	Р
E F g <mark>efg</mark>	'EFg'	8	D
E f G <mark>efg</mark>	'EfG'	37	П
E f g <mark>efg</mark>	'Efg'	95	I
e F G <mark>efg</mark>	'eFG'	85	I
e F g <mark>efg</mark>	'eFg'	43	П
e f G <mark>efg</mark>	'efG'	12	D
e f g <mark>efg</mark>	'efg'	350	Р

(1) Identify the four pairs of phenotype classes: one parental class, which is most frequent, and which shows the same phase relationships as the parents two single recombinant classes, between the middle locus and each outside locus one double recombinant class, between the middle locus and both outside loci As this requires two events, this is the rarest class The two members of each recombinant pair should occur in approximately equal numbers, because each crossover produces two products (2) To determine gene locus order: Most common phenotypes EFG & efg are parental: no recombinants Rarest phenotype EFg & efG will be the double-recombinants: Compare phases of [EFG efg] vs [EFg efG] * The G locus changes phase ("flips") wrt E & F, therefore G is in the middle * ----*E*----*G*----*F*----(3) To determine the frequency of recombination between locus pairs, count all classes in which the phase changes from the parental types (i) for E & G EG & eg are parental types Eg & eG are recombinant types between E & G: (Class I): count all classes with this type # (EFg + efG + eFG + Efg)[(8 + 12) + (95 + 85)] / 1000 = **0.20 = 20% = 20 m.u. = 20 cM** (ii) for G & F GF & gf are parental types Gf & gF are recombinant types between F & G (Class II): # (EFg + efG + EfG + eFg)[(8 + 12) + (37 + 43)]/1000 = 0.10 = 10% = 10 m.u. = 10 cM(iii) for E & F EF & ef are parental types Ef & eF are recombinant types between E & F # (EfG + eFg + eFG + Efg) [(37 + 43) + (95 + 85)] / 1000 = 0.26 = 26% = 26m.u. = 26 cM(4) Map data look like The distance between E & F is greatest, so these are on the outside E----G--10cM--FE----F but 0.26 < (0.20 + 0.10) WHY? In the double recombinants, --A----B----C-- => ABC --A----b----C-- => AbC --a----B----c-- => aBc --a---b----c-- => abc so double recombinants resemble parental type

but each represents 2 cross-overs between outside (E & F) loci

(3.iii) Then the *corrected count* of crossovers between **E & F** [(37 + 43) + (95 + 85) + **(2)(8 + 12)**] = 0.3 = 30 m.u. = **30 cM**

(5) Interference occurs where one cross-over decreases the probability of others nearby: Compare the observed vs expected # double-recombinants

Interference (I) = 1 - (observed d.r.)/(expected d.r.) = 1 - [(10 + 10) / (0.20)(0.10)(1000)] In this case, I = 1 - 20/20 = 0 : # expected = # observed, & there is no interference

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