

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-----c----- => **Abc Single recombinant**
 -----a-----b-----c----- => **abc Parental**

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

-----A-----B-----C----- => **ABC Parental**
 -----A-----B---\ /---C----- => **abC Single recombinant**
X
 -----a-----b---/ \---c----- => **ABc Single recombinant**
 -----a-----b-----c----- => **abc Parental**

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-----B-----C-----
 -----A---\ /---B---\ /---C-----
X **X**
 -----a---/ \---b---/ \---c-----
 -----a-----b-----c-----

A double crossover will reverse the phase of the *middle* locus

-----A-----B-----C----- => **ABC**
 -----A-----b-----C----- => **AbC**
 -----a-----B-----c----- => **aBc**
 -----a-----b-----c----- => **abc**

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 efg	'EFG'	370	P
E F g efg	'EFg'	8	D
E f G efg	'EfG'	37	II
E f g efg	'Efg'	95	I
e F G efg	'eFG'	85	I
e F g efg	'eFg'	43	II
e f G efg	'efG'	12	D
e f g efg	'efg'	350	P

- (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**)
 $[(8 + 12) + (95 + 85)] / 1000 = 0.20 = 20\% = 20 \text{ m.u.} = 20 \text{ cM}$

(ii) for **G & F**

GF & **gf** are parental types
Gf & **gF** are recombinant types between **F & G** (Class II):
 # (**EFg** + **efG** + **EFg** + **efG**)
 $[(8 + 12) + (37 + 43)] / 1000 = 0.10 = 10\% = 10 \text{ m.u.} = 10 \text{ cM}$

(iii) for **E & F**

EF & **ef** are parental types
Ef & **eF** are recombinant types between **E & F**
 # (**EfG** + **eFG** + **eFG** + **Efg**)
 $[(37 + 43) + (95 + 85)] / 1000 = 0.26 = 26\% = 26 \text{ m.u.} = 26 \text{ cM}$

- (4) **Map data** look like
 The distance between **E & F** is greatest, so these are on the outside

E-----20 cM-----**G**---10 cM---**F**
E-----26 cM-----**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**
 $[(37 + 43) + (95 + 85) + (2)(8 + 12)] = 0.3 = 30 \text{ m.u.} = 30 \text{ cM}$

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

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