Chapter 6

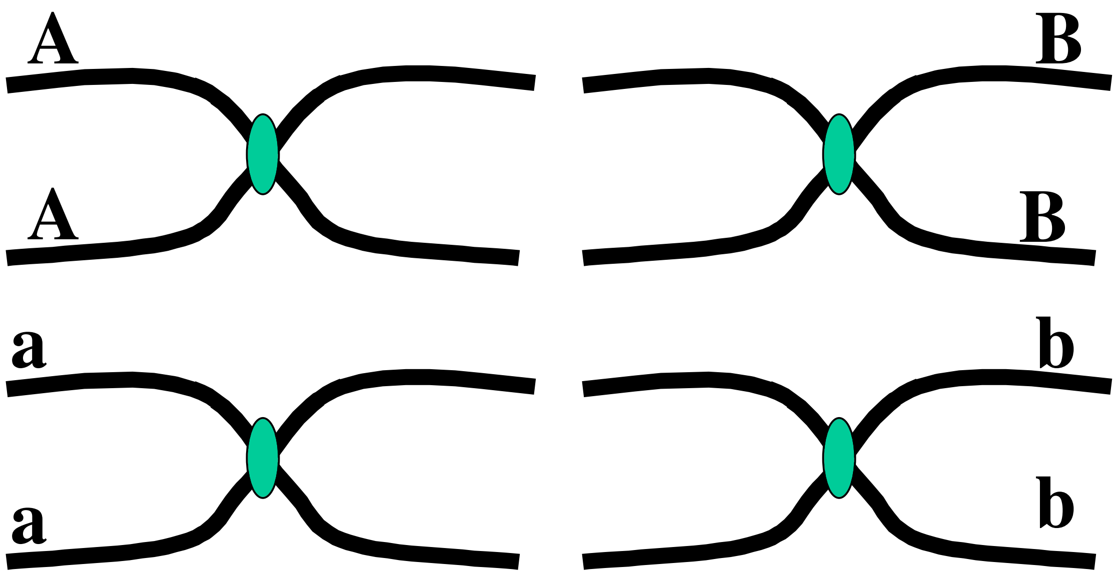
Linkage & Chromosome Mapping

Chromosome, NOT genes are the unit of inheritance

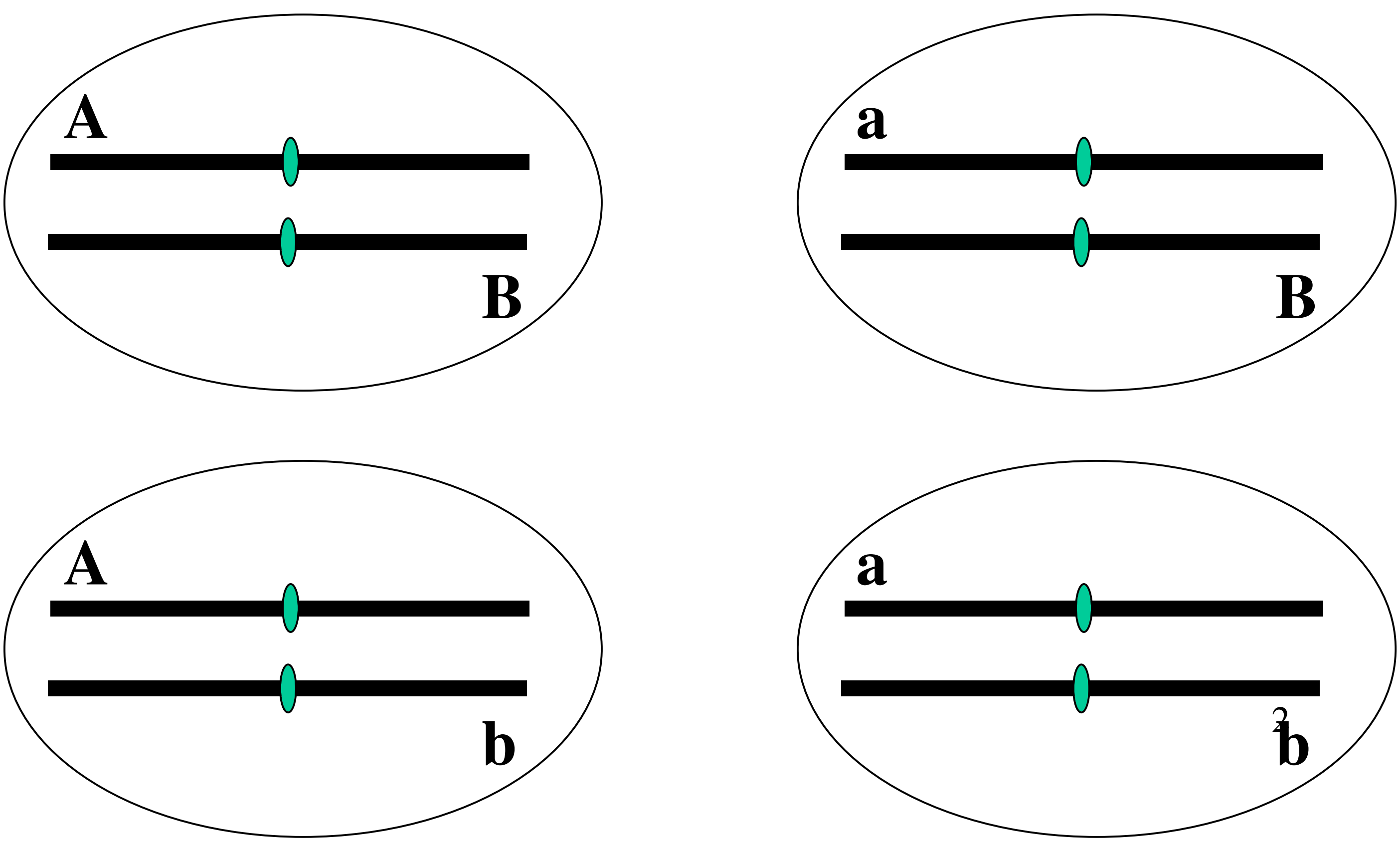
Crossing Over -- Prophase I

- ✓ Increases genetic variation
- ✓ Related to distance
- ✓ Chromosome maps

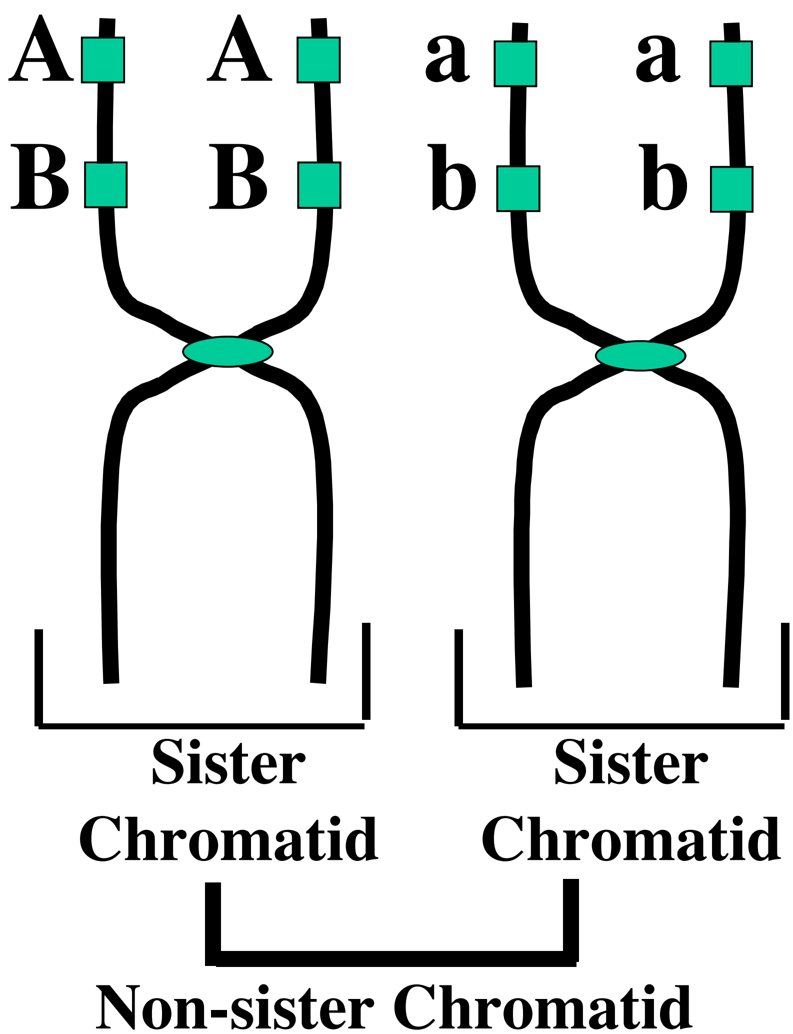
Independent Assortment: 2 genes on 2 different homologous pairs of chromosomes.
Figure 6.1a Page 138



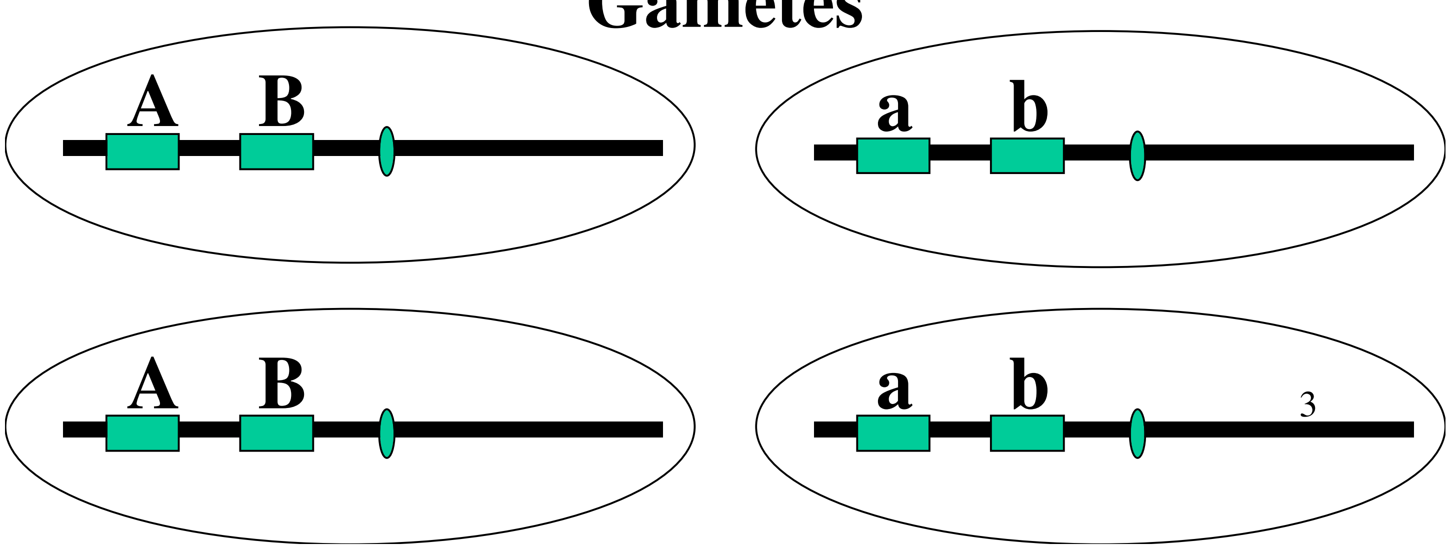
Gametes



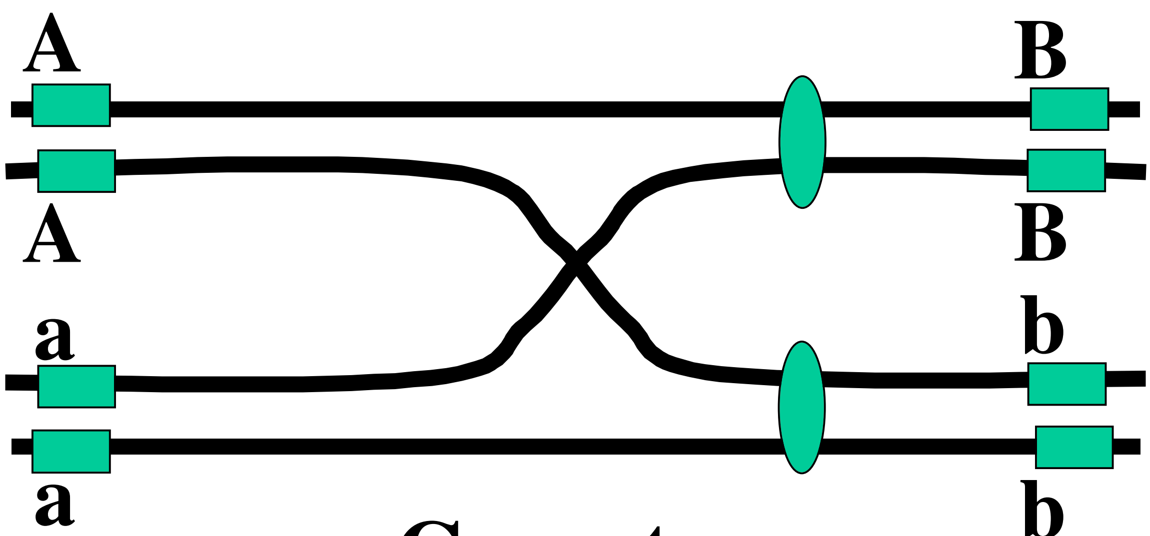
Linkage: 2 genes on a single pair of Homologs: No exchangeFig. 6.1b Page 138



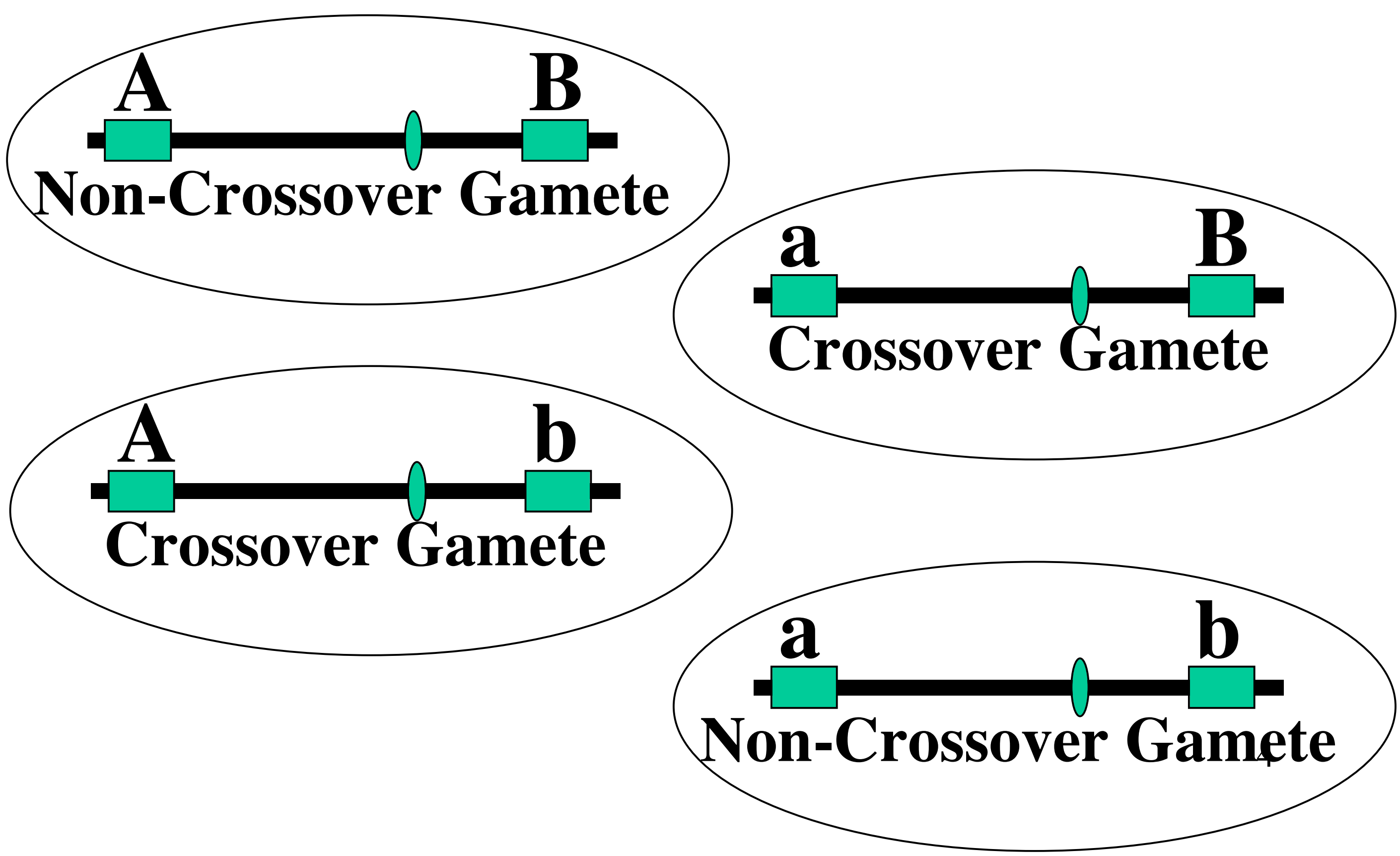
Gametes



Linkage: 2 genes on a single pair of Homologs: Single exchange
Fig. 6.1c Page 138



Gametes



- ✓ **Frequency of crossing over for any two loci on the same chromosome is proportional to the distance between them.**

- ✓ **Complete linkage -- Only parental gametes.**

- ✓ **As distance increases, proportion of recombinant gametes ⇑ and the porportion of parental gametes ⇓.**

- ✓ **Frequency of recombinant gemetes approaches but cannot exceed 50%.**

- ✓ **50% recombinant gametes = 1:1:1:1 (2 parental : 2 recombinant) -- This would be indistinguishable from 2 non-linked, independently assorting loci.**

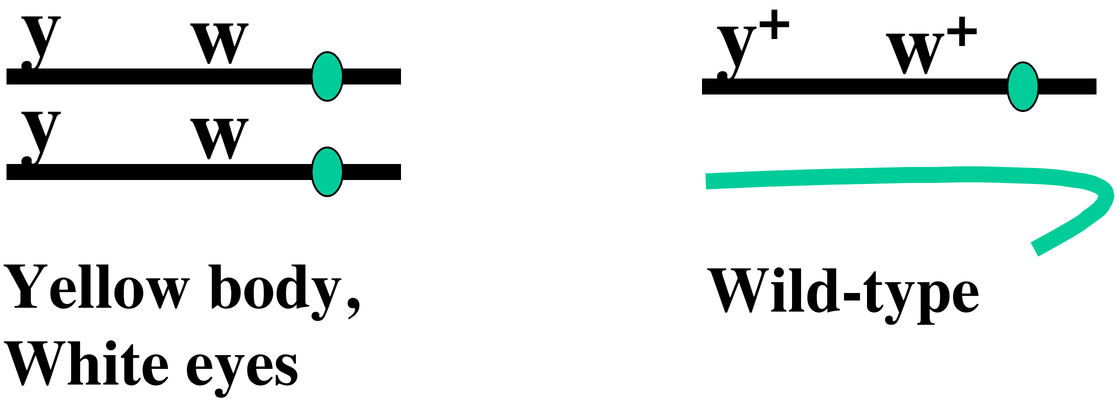
Incomplete Linkage, Crossing Over & Genetic Mapping

- ✓ **Complete Linkage is rare -- Crossing over is common.**

- ✓ **Drosophila geneticists**
  - ✓ **Thomas A. Morgan**
  - ✓ **Alfred A. Sturtevant**

  - ✓ **By examining single trait crosses, deduced mode of sex-linked inheritance.**

  - ✓ **Puzzling results with 2 sex-linked traits.**



F_1 Females -- wild-type

F_1 Males -- Expressed both mutant Traits

F_2 -- 98.7% Offspring had Parental Phenotype

1.3% either: Yellow body/Wild-type eyes
Or
Wild-type body / White eyes

Two Questions:

1. ***What is the source of gene separation*****?**

- ✓ **Chiasmata are the source and linked genes exist in a linear array.**

- ✓ **Morgan proposed the term: "Crossing-Over".**

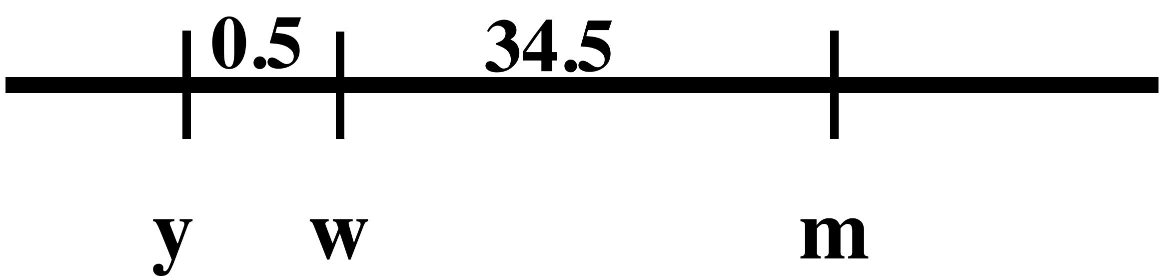
2. ***Why did frequency vary depending on genes examined?***

- ✓ **Answered by Sturtevant.**

- ✓ **Related to distance and this is additive.**

1. **Yellow, White 0.5%**
2. **White, Minature 34.5%**
3. **Yellow, Minature 35.4%**

1% recombination = 1 map unit = 1 centimorgan



By 1923 Morgan & Sturtevant had shown:

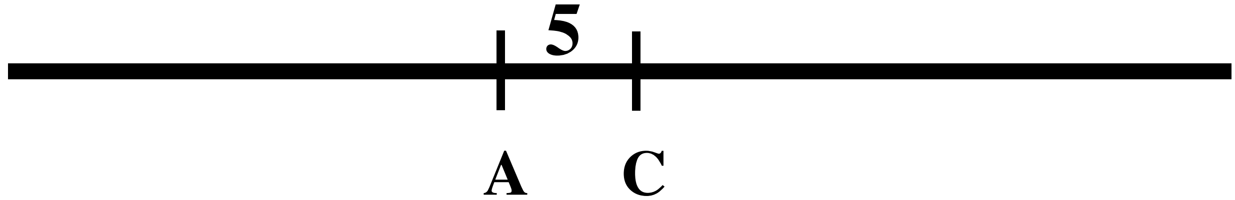
- ✓ **Linkage & Crossing over not restricted to sex-linked genes**
- ✓ **In *Drosophila*, crossing over occurs only in females**
- ✓ **Substantiated chromosomal theory of inheritance**

Two Pont Mapping

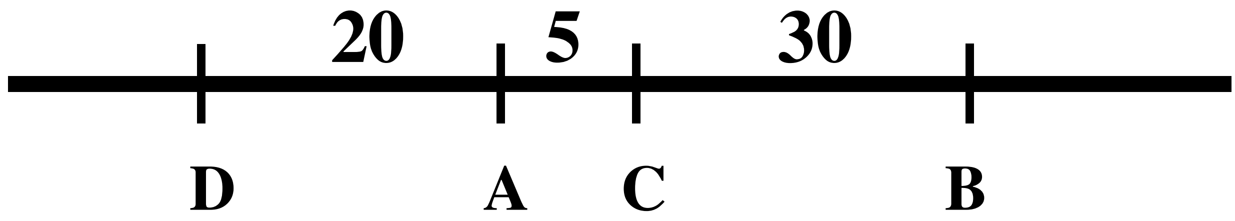
A ---> B	=	35
A ---> C	=	5
A ---> D	=	20
B ---> C	=	30
B ---> D	=	55
C ---> D	=	25

***Question*: What is the correct order of these genes and the distance between each pair?**

Step I: Find and map the smallest distance.



STEP II: Continue this process until the map is complete and all distance add up to observed recombination frequencies.



G = Yellow **g = Green**
R = Round **r = Wrinkled**

A plant of unkown genotype & phenotype is test crossed with a ggrr plant and you obtain the following progeny:

Yellow, Wrinkled	**88**
Yellow, Round	**12**
Green, Wrinkled	**8**
Green, Round	**92**

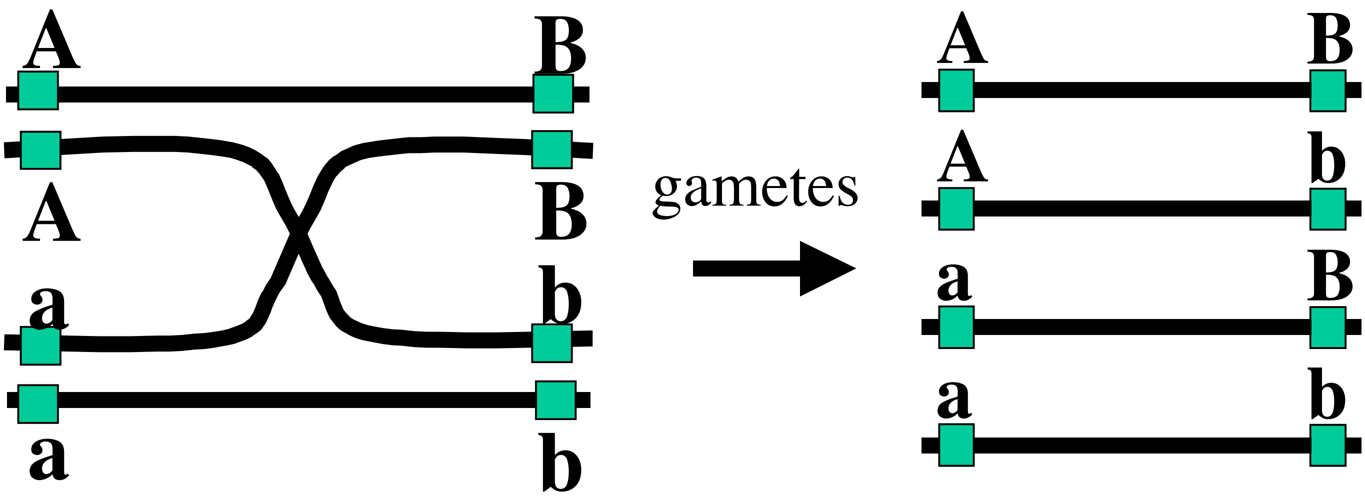
Questions:

1. What is the phenotype of the unknown plant?
2. What is the genotype of the unknown plant?
3. What is the distance between the two genes?
4. Cis of Trans configuration?

Yellow, Wrinkled	88	NCO
Yellow, Round	12	SCO
Green, Wrinkled	8	SCO
Green, Round	92	NCO

1. Genotype of unknown plant: Gr/gR
2. Phenotype: Yellow, Round
3. Distance between 2 genes:
   (12 + 8) / 200 = 0.1 X 100 = 100 mu
4. Configuration = Trans

Single Cross-Over Event -- Pg. 143

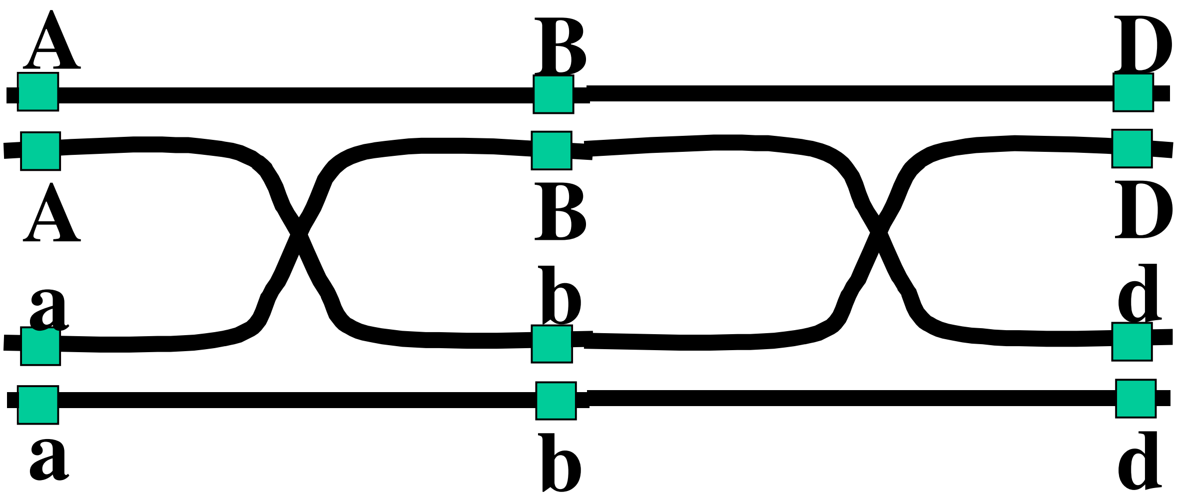


- ✓ **If only a single exchange -- theoretical limit of crossing over = 50%**

- ✓ **2 genes on the same chromosome ≥ 50 mu, a cross over can theoretically be expected to occur between them in 100% of the tetrads.**

- ✓ **For several reasons, this limit is never reached (discussed later).**

Multiple Cross Overs -- Pg. 144



Product Law:

A ---> B = 20%   B ---> D = 30%

Double Cross Over = 0.2 X 0.3 = 0.06 =6%

3 Criteria for successful 3-point mapping

1. Individual producing recombinant gametes must be heterozygous at all 3 loci.
2. Must be able to determine genotype of all gametes by examining phenotypes of offspring.
3. Must have a large number of offspring.

3 Autosomal Linked Genes in Maize

bm = Brown mid rib

v virescent seedling

pr = purple aleurone

What genotypes do you need to map these 3 genes?

Answer:

- **1 individual heterozygous for all 3 loci**
- **1 individual homozygous recessive for all 3 loci.**

You don't know:

- **Arrangement of mutant alleles (cis/trans)**
- **Gene order**
- **Distances between genes**

Offspring Phentoype			Observed
+	v	bm	230
pr	+	+	237
pr	v	+	82
+	+	bm	79
+	v	+	200
pr	+	bm	195
pr	v	bm	44
+	+	+	42
			1109

Step I: Make an assumption about gene order:

- **Assume order to be: pr v bm**

Therefore, the cross is:

+ v bm			X	pr v bm		
pr + +				pr v bm		

Step II: Determine the correct gene order by looking at the noncrossover and double crossover phenotypic classes.

Offspring Phentoype			Observed	
+	v	bm	230	NCO
pr	+	+	237	NCO
pr	v	+	82	SCO
+	+	bm	79	SCO
+	v	+	200	SCO
pr	+	bm	195	SCO
pr	v	bm	44	DCO
+	+	+	42	DCO

This is not the correct gene order so try another order.

Offspring Phentoype			Observed	
v	+	bm	230	NCO
+	pr	+	237	NCO
v	pr	bm	44	DCO
+	+	+	42	DCO

This *IS* the correct gene order! v pr bm

Step III: Calculate map distance between the 3 genes under study.

The distance between pv & v and between v & bm is equal to the % of all detectable exchanges occurring between them!

For any two genes, you must consider ALL appropriate SCO plus the DCO.

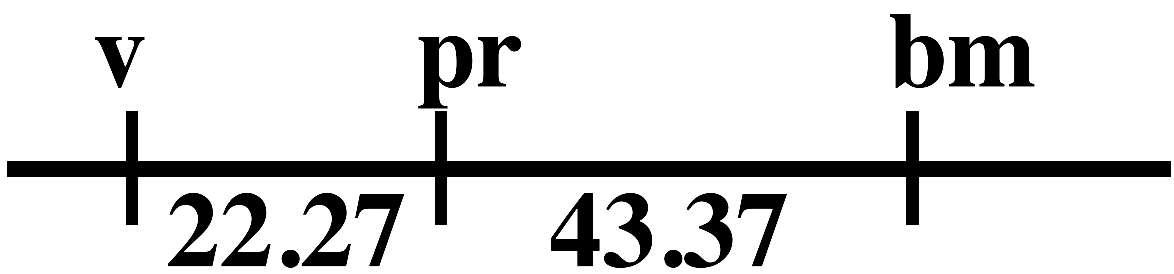
Now write all genotypes in the correct gene order!

Offspring Phentoype			Observed
v	+	bm	230
+	pr	+	237
v	pr	+	82
+	+	bm	79
v	+	+	200
+	pr	bm	195
v	pr	bm	44
+	+	+	42

Distance between v & pr:
(82 + 79 + 44 + 42) / 1109 X 100 = 22.27 mu

Distance between pr & bm:
(200 + 195 + 42 + 44) / 1109 X 100 = 43.37 mu

Step IV: Construct a chromosomal map



***Problem*: Singed bristles (sn), crossveinless wings (cv), and vermilion eye color (v) are due to recessive mutant alleles of 3 X-linked genes in *Drosophila*. When a female heterozygous for each of the three genes was testcrossed with a singed, crossveinless, vermilion male, the following progeny were obtained.**

singed, crossveinless, vermilion	**3**
crossveinless, vermilion	**392**
vermilion	**34**
crossveinless	**61**
singed, crossveinless	**32**
singed, vermilion	**65**
singed	**410**
wild-type	**3**

***Question*: (a) What is the correct genotype of the P1 male and female? (b) What is the correct order of these three genes?, and (C) what are the map distances between the three genes?**

sn	+	+	410	NCO
+	cv	v	392	NCO
+	+	v	34	SCO
sn	cv	+	32	SCO
+	cv	+	61	SCO
sn	+	v	65	SCO
sn	cv	v	3	DCO
+	+	+	3	DCO

Check to see if this is the correct order by looking At NCO and DCO.

The correct gene order is cv sn v

+	sn	+	410	NCO
cv	+	v	392	NCO
+	+	v	34	SCO
cv	sn	+	32	SCO
cv	+	+	61	SCO
+	sn	v	65	SCO
cv	sn	v	3	DCO
+	+	+	3	DCO

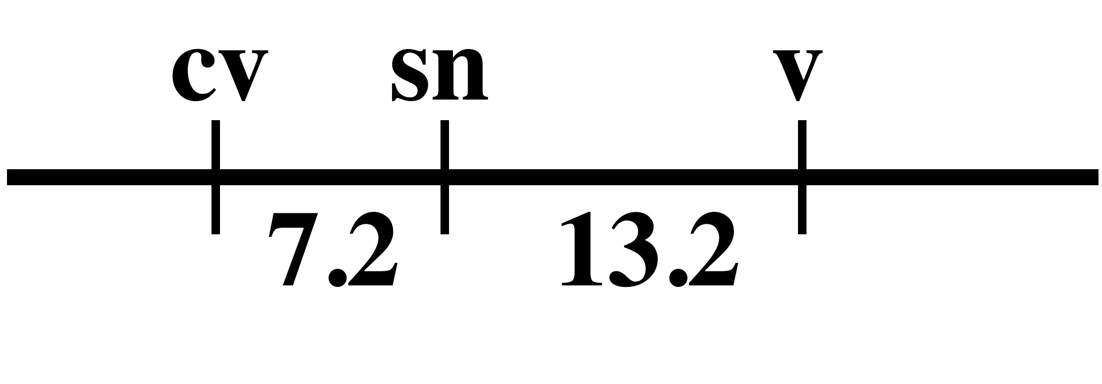
Calculate distance between each pair of genes and construct a chromosomal map:

<u>Distance between cv & sn:</u>

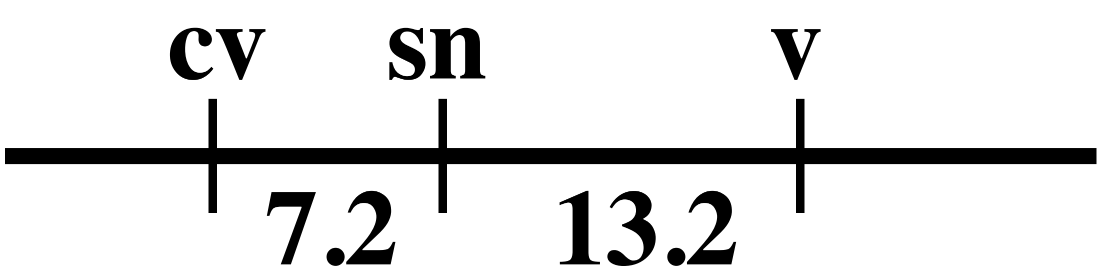
(34 + 32 + 3 + 3) / 1000 X 100 = 7.2 mu

<u>Distance between sn & v:</u>

(61 + 65 + 3 + 3) / 1000 X 100 = 13.2 mu



***Interference*: Reduction in the number of DCO events relative to what is expected based on the map distances.**



Observed DCOs = 6 / 1000 = 0.006

Expected DCOs = Multiply frequency of recombination between each pair of genes.

$DCO_{(EXP)}$ = 0.072 X 0.132 = 0.0095

<u>Coefficient of Coincidence (C):</u>

C = Observed (DCO) / Expected (DCO)

C = 0.006 / 0.0095 = 0.632

Interference (I) = 1 - C

I = 1 - 0.632 = 0.368

I = 1.0 : INTERFERENCE IS COMPLETE and no DCOs observed.

I = + : POSITIVE INTERFERENCE -- Fewer DCO observed than expected.

I = - : NEGATIVE INTERFERENCE -- More DCO observed than expected.

In eukaryotes, positive interference most common

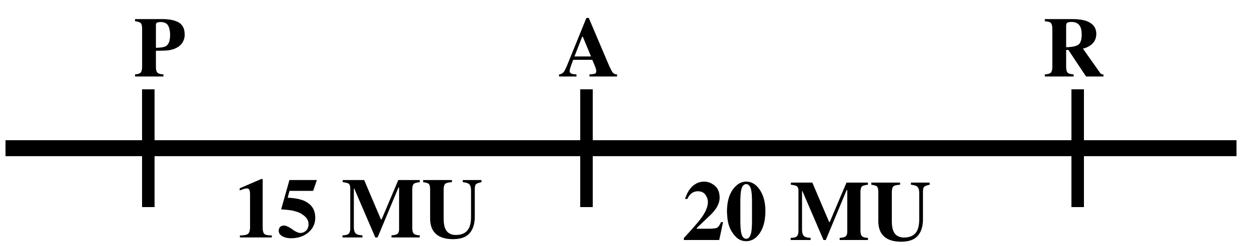
Why is 50% recombinant gametes the theoretical maximum?
Multiple strand exchanges Pp. 152
Figure 6.12

A. Two-Strand Double Exchange

B. Three-Strand Double Exchange

A. Four-Strand Double Exchange

Problem: **The father of Mr. Spock, first officer of the starship *Enterprise*, came from planet Vulcan; Spock's mother came from Earth. A vulcan has pointed ears (P) adrenals absent (A), and a right-sided heart (R). These three loci are autosomal, and they are linked as shown in the following map:**



Questions: **If Mr. Spock marries an Earth woman and there is no (genetic)interference, what proportion of their children will have:**

a. Vulcan phenotypes for all three characters?
b. Earth phenotype for all three characters?
c. Vulcan ears and heart but Earth adrenals?
d. Vulcan ears but Earth heart and adrenals?

P A R / p a r	X	p a r / p a r
P a R / p a r	(0.15 X 0.20) / 2 = 0.015	
p A r / p a r	(0.15 X 0.20) / 2 = 0.015	
P a r / p a r	(0.15 - 0.03) / 2 = 0.06	
p A R / p a r	(0.15 - 0.03) / 2 = 0.06	
P A r / p a r	(0.20 - 0.03) / 2 = 0.085	
p a R / p a r	(0.20 - 0.03) / 2 = 0.085	
P A R / p a r	(1 - 0.32) / 2 = 0.34	
p a r / p a r	(1 - 0.32) / 2 0.34	

a. Vulcan phenotypes for all three characters = 0.34

b. Earth phenotype for all three characters = 0.34

c. Vulcan ears and heart but Earth adrenals = 0.015

d. Vulcan ears but Earth heart and adrenals = 0.06

Sister Chromatid Exchange

- ✓ **Occurs, but its significance is unclear**
- ✓ **Agents that increase chromosome damage also increase the frequency of sister chromatid exchange.**
- ✓ **Frequency of sister chromatid exchange increases in the human autosomal recessive "*Bloom disease*"**

SOMATIC CELL HYBRIDIZATION AND HUMAN CHROMOSOME MAPS

- ✓ **Used to determine on which chromosome a particular trait is located.**
- ✓ **2 cells (mouse and human) can be induced to form a single hybrid cell with 2 nuclei -- *HETEROKARYON*.**
- ✓ **Culture heterokaryons**
- ✓ **Nuclei will fuse to form a *SYNKARYON*.**
- ✓ **After many generations, chromosomes from one of the two parental species are gradually lost.**

Linkage Analysis in Haploid Organisms:

- ✓ **Vegetative stage = Haploid**
- ✓ **Reproductive cells = Isogametes**
  - ✓ **Isogametes from 2 strains (+ and -) fuse to produce a diploid zygote**
  - ✓ **Diploid zygote undergoes meiosis to re-establish haploid condition**
  - ✓ **Haploid products are progenitors of vegetative stage.**

Neurospora **-- Meiosis occurs in *ASCUS*, initial haploid products are called *TETRADS*.**

- ✓ **Each cell divides mitotically to produce 8 haploid ascospores**
- ✓ **Ascospores refelct the sequence of formation**

- ✓ **Tetrad Analysis**
- ✓ **Mapping the centromere**

Five Combinations

First Division Segregation:

AA++

Second Division Segregation:

A+A+

+A+A

+AA+

A++A

Distance between gene and centromere

$$\frac{\text{1/2 second division segregant asci}}{\text{Total asci sored}}$$

Example: **65 1st division segregants**
70 2nd division segregants

What is the distance from the gene to the centromere?

$(0.5 \times 70) / 135 = 0.259 \times 100 = 25.9$ mu

<u>Ordered Tetrad Analysis:</u>

- ✓ **Tedious**
- ✓ **Distinguishes 1st and 2nd division segregants which is necessary to map gene in relation to the centromere.**

<u>Unordered Tetrad Analysis:</u>

- ✓ **Determine if 2 genes are linked**
- ✓ **Map distance between to loci.**

Examine 100 tetrads from the following cross:

a b X + +

TETRAD TYPE	P	NP	T
GENOTYPES	++ ++ ab ab	a+ a+ +b +b	++ a+ +b ab
#TETRADS	43	43	14

P = PARENTAL DITYPES
NP = NONPARENTAL DITYPES
T = TETRATYPES

✓ WHEN P = NP, THE TWO GENES ARE NOT LINKED.

THESE GENES ARE ON SEPARATE CHROMOSOMES!!!!

EXAMPLE 2:

P	NP	T
64	6	30

Because P ≠ NP, no independent assortment and hence, 2 genes are linked and can be mapped.

Exchange Frequency =

[(NP + 1/2T) / Total # Tetrads] X 100

{[(6 + 1/2(30)) / 100} X 100 = 21% or 21 mu