Linkage & Genetic Mapping in Eukaryotes

Ch. 6

LINKAGE AND CROSSING OVER

- In eukaryotic species, each linear chromosome contains a long piece of DNA
  - A typical chromosome contains many hundred or even a few thousand different genes
- The term linkage has two related meanings
  - 1. Two or more genes can be located on the same chromosome
  - 2. Genes that are close together tend to be transmitted as a unit

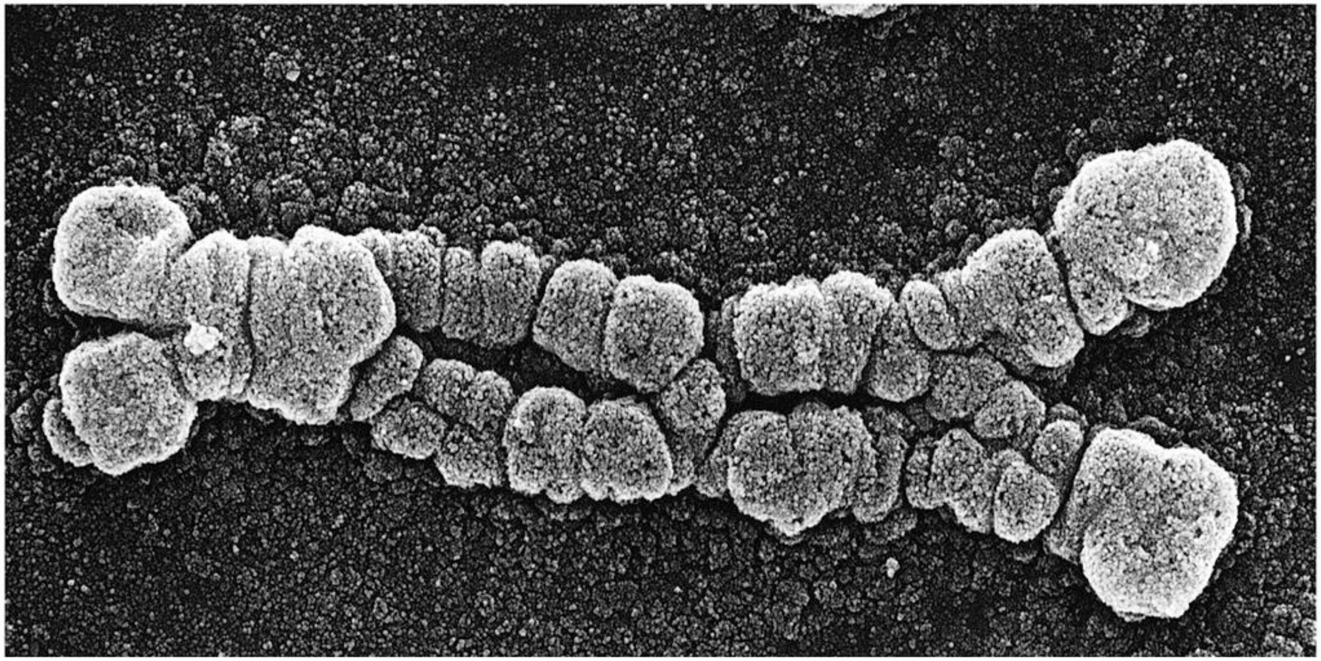
Linkage Groups

- Chromosomes are called linkage groups
  - They contain a group of genes that are linked together
- The number of linkage groups is the number of types of chromosomes of the species
  - For example, in humans
    - 22 autosomal linkage groups
    - An X chromosome linkage group
    - A Y chromosome linkage group
- Genes that are far apart on the same chromosome can independently assort from each other
  - This is due to crossing-over or recombination

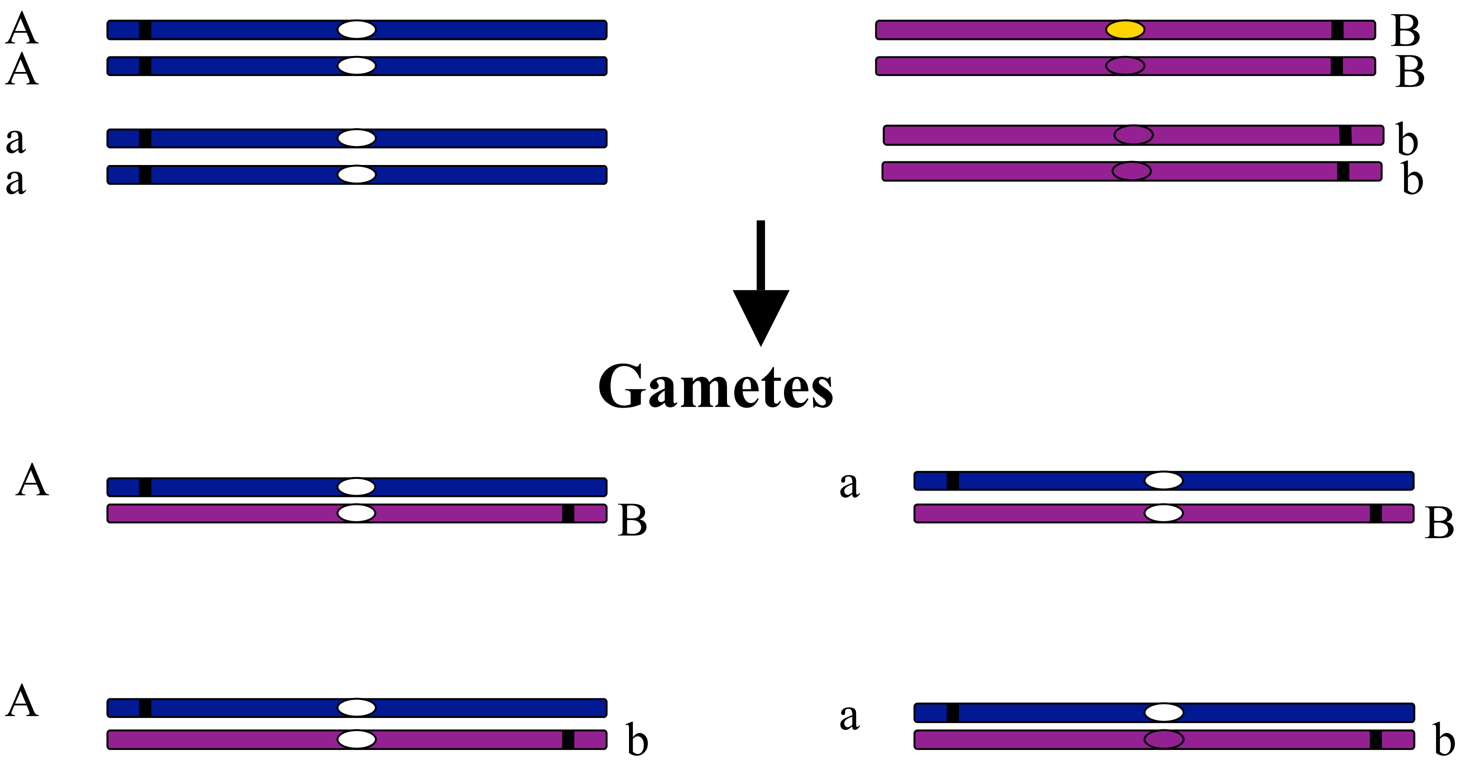
Linkage and Recombination

Genes nearby on the same chromosome tend to stay together during the formation of gametes; this is linkage.

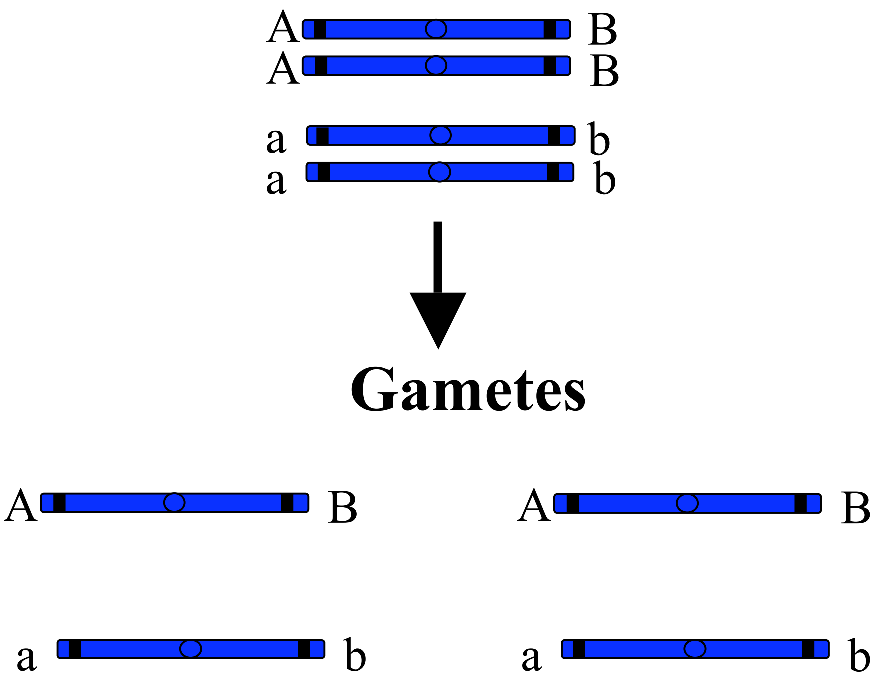
The breakage of the chromosome, the separation of the genes, and the exchange of genes between chromatids is known as recombination. (we call it crossing over)

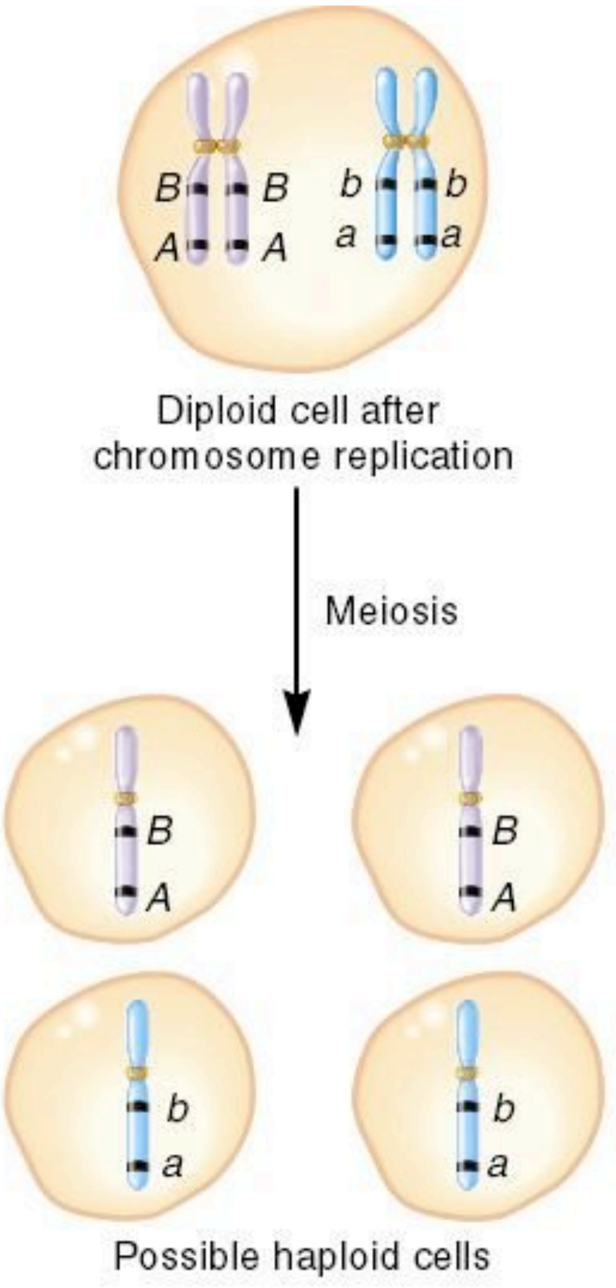


Independent assortment:
Genes on different chromosomes

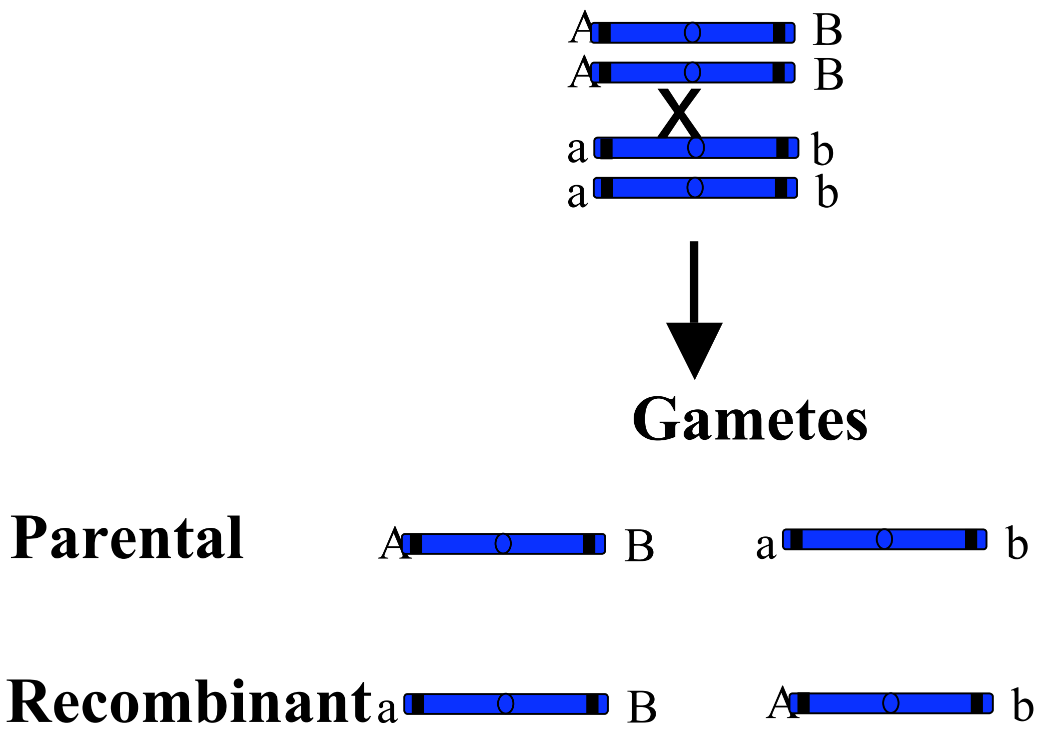


Linkage:
Two genes on same chromosome segregate together





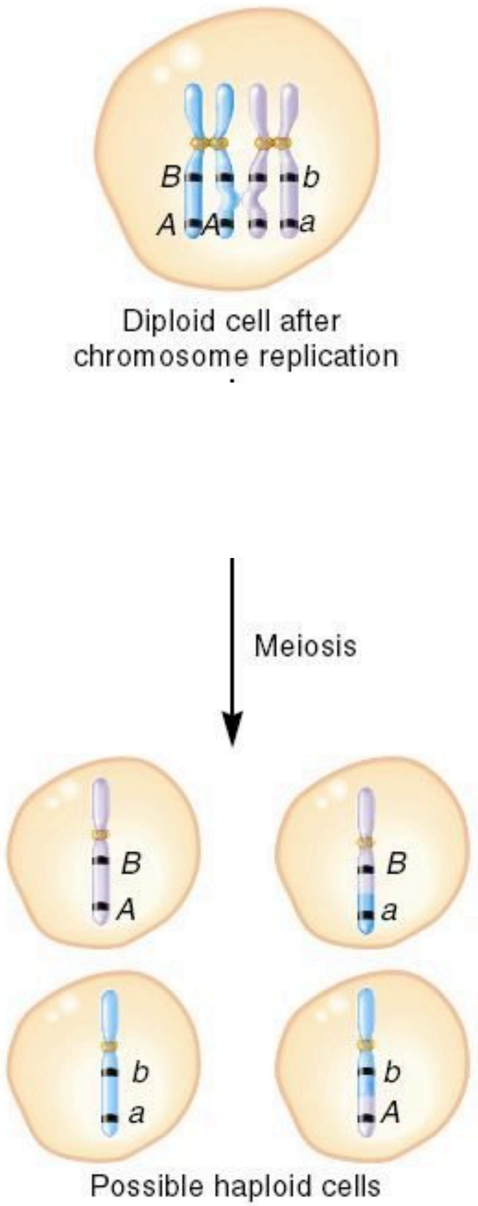
Linkage and Crossing over leads to separation of linked genes



Gametes

Parental

Recombinant



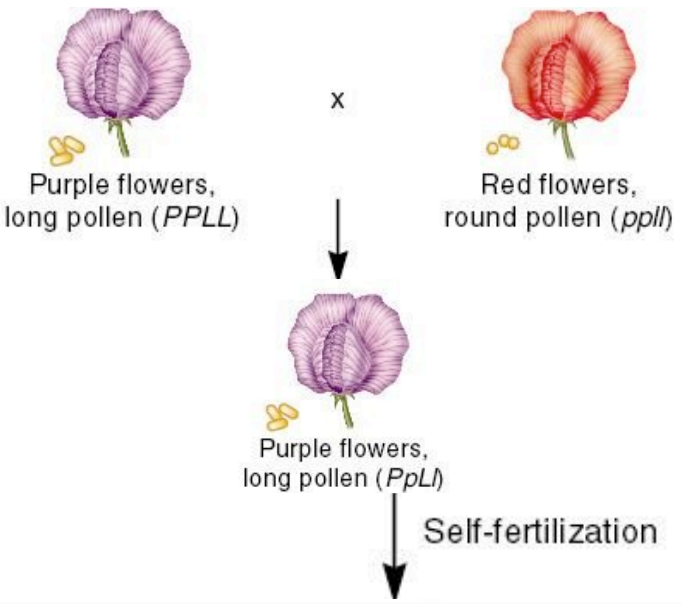
(b) Crossing over can reassort linked alleles.

Crossing Over Can Produce Recombinant Phenotypes

- In diploid eukaryotic species
  - linkage can be altered during meiosis as a result of crossing over (recombination)
- Crossing over
  - Occurs during prophase I of meiosis
  - Non-sister chromatids of homologous chromosomes exchange DNA segments

In 1905 Bateson and Punnett Discovered Two Traits That Did Not Assort Independently

- This is a dihybrid cross that is expected to yield a 9:3:3:1 phenotypic ratio in the F_2 generation



A much greater proportion of the two types found in the parental generation

F_2 offspring	Observed number	Ratio	Expected number	Ratio
Purple flowers, long pollen	296	15.6	240	9
Purple flowers, round pollen	19	1.0	80	3
Red flowers, long pollen	27	1.4	80	3
Red flowers, round pollen	85	4.5	27	1

Some genes on the same chromosome assort together more often than not

- In dihybrid crosses, departures from a 1:1:1:1 ratio of F1 gametes indicate that the two genes are on the same chromosome
  - Ex 1: For AaBb, four gametes = AB, Ab, aB, ab = 1:1:1:1 thus no linkage.
  - Ex 2: For AaBb, the observed four gametes are Ab aB only. The ratio is 2:0:2:0, thus linkage is present.
  - We use a testcross of F1 and the chi square analysis to determine whether genes are unlinked or likely to be linked

Chi square test pinpoints the probability that ratios are evidence of linkage

- Transmission of gametes is based on chance events
  - Deviations from 1:1:1:1 ratios can represent chance events OR linkage
  - Ratios alone will never allow you to determine if observed data are significantly different from predicted values.
  - The larger your sample, the closer your observed values are expected to match the predicted values.
- Chi square test measures "goodness of fit" between observed and expected (predicted) results
  - Accounts for sample size, or the size of the experimental population

Applying the Chi Square Test

- Framing a hypothesis
  - Null hypothesis – "observed values are not different from the expected values"
    - For linkage studies – no linkage is null hypothesis
    - Expect a 1:1:1:1 ratio of gametes or offspring from testcross
- Degrees of Freedom: for this example, DOF is the amount of classes which data can be grouped into, minus 1.
- We expect two classes for the gametes
  1. Parental class (genotype of parent cells)
  2. Non-parental (recombinant) genotype

DOF = classes (parental & recombinant) -1

For linkage analysis, the DOF will ALWAYS be 1.

Applying the chi square test to a linkage study

Genotype	Experiment 1	Experiment 2
A B	17	34
a b	14	28
A b	8	16
a B	11	22
Total	**50**	**100**

Class	Observed/Expected		Observed/Expected	
Parentals	31	25	62	50
Recombination	19	25	38	50

Applying the Chi Square Test

- We ALWAYS ALWAYS ALWAYS group the data into two PHENOTYPIC groups or classes for linkage analysis:

- Parental Class
  - AB and ab

- Recombinant (Non-parental) class
  - Ab and aB

Chi Square – Experiment 1 & 2

$$\chi^2 = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{number expected}}$$

(Parental) (Recombinant)

Experiment 1

$$\chi^2 = \Sigma \frac{(31 - 25)^2}{25} + \frac{(19 - 25)^2}{25} = 2.88$$

Experiment 2

$$\chi^2 = \Sigma \frac{(62 - 50)^2}{50} + \frac{(38 - 50)^2}{50} = 5.76$$

Degrees of Freedom = 2 (parental & recombinant classes) – 1 = 1

Chi Square – Experiment 1 & 2

- Experiment 1:
  - Based off of the Chi-square value of 2.88, we determine that it is less than the critical value of 3.84, so we CAN NOT reject the null hypothesis. Genes are not linked.
- Experiment 2:
  - A chi-square of 5.76 is greater than the critical value, so we CAN reject the null hypothesis. Genes are linked.

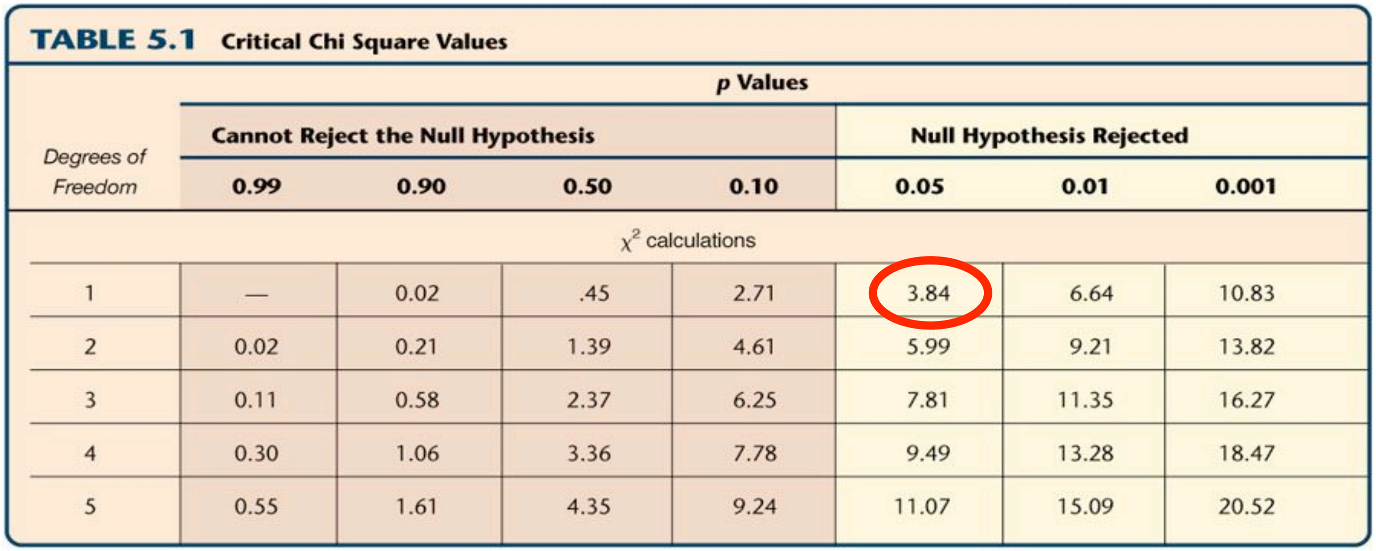


TABLE 5.1 Critical Chi Square Values

Degrees of Freedom	***p* Values**						
	Cannot Reject the Null Hypothesis				**Null Hypothesis Rejected**		
	0.99	**0.90**	**0.50**	**0.10**	**0.05**	**0.01**	**0.001**
	χ^2 calculations						
1	—	0.02	.45	2.71	3.84	6.64	10.83
2	0.02	0.21	1.39	4.61	5.99	9.21	13.82
3	0.11	0.58	2.37	6.25	7.81	11.35	16.27
4	0.30	1.06	3.36	7.78	9.49	13.28	18.47
5	0.55	1.61	4.35	9.24	11.07	15.09	20.52

χ^2 values that lie in the yellow-shaded region of this table allow you to reject the null hypothesis with > 95% confidence, and for recombination experiments, to postulate linkage.

Size Does Matter

- Sample size makes a difference
  - Larger the better
- Use real numbers not percentages
- P-value
  - The difference between expected and observed is statistically significant: $\leq .05$
  - Meaning difference is less than or equal to ± 5%
  - Null hypothesis is true and not due to chance

Example if testing for linkage

- **In corn the glossy trait (AA) gives glossy leaves and the ramosa trait (BB) determines branching of ears, a test cross produced the following results:**

Normal leaved and normal branches	**395**
Glossy leaved and ramosa branching	**382**
Normal leaved with ramosa branching	**223**
Glossy leaved with normal branching	**247**

    - **For a total of 1247 offspring.**
  - **Are the glossy and ramosa genes linked?**
    - **Null hypothesis: Genes are NOT linked**

Example if testing for linkage

- Expected values if 1:1:1:1 if NOT linked
  - 1247/2 =
    - 623.5
- Set up χ^2
  - $(777-623.5)^2/623.5 + (470-623.5)^2/623.5=$
    - 75.6
  - Degrees of freedom =
    - 1
  - Accept or reject?

Chi square values

$df = 1$

$\chi^2 = $ **75.5**

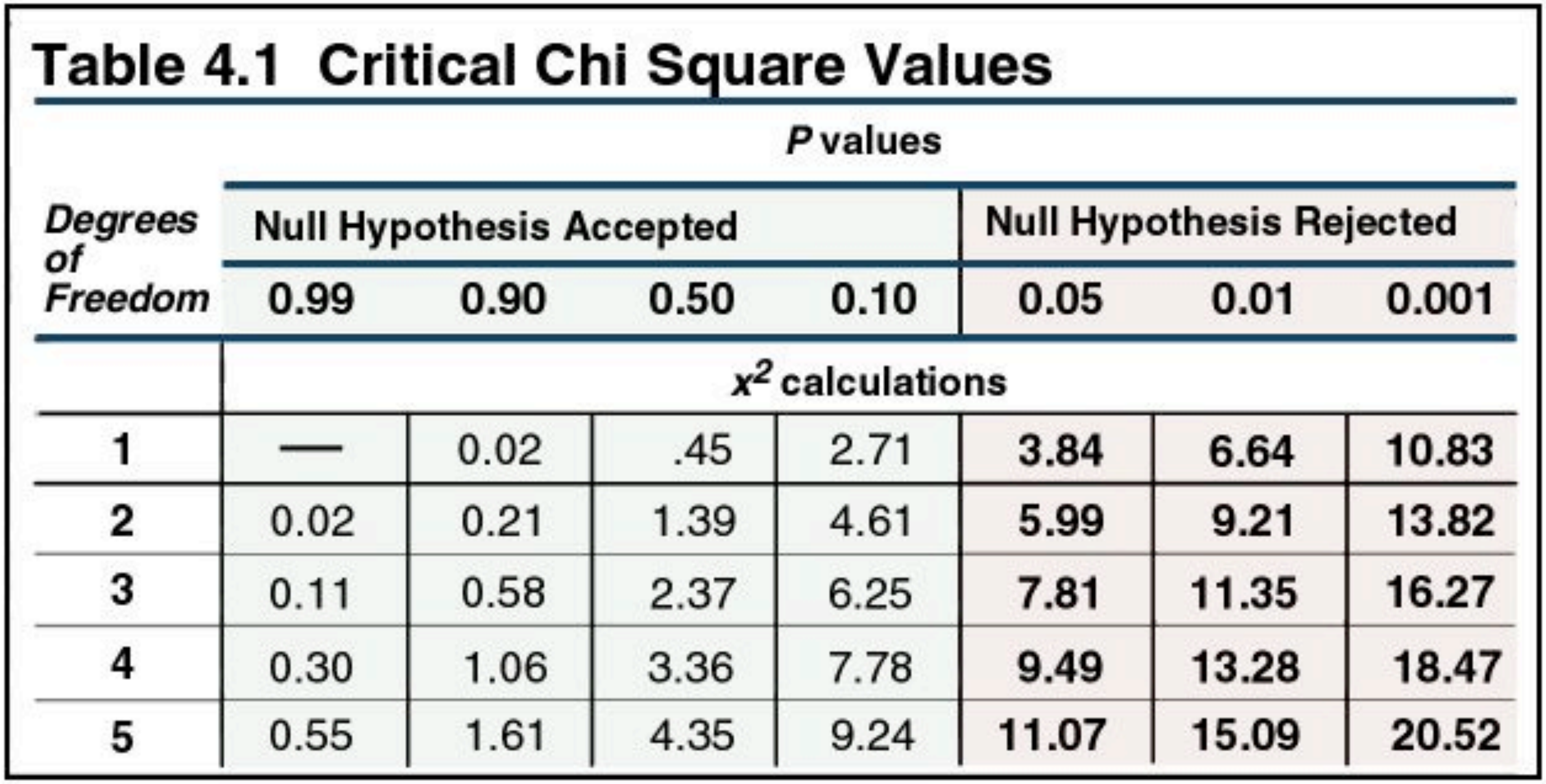


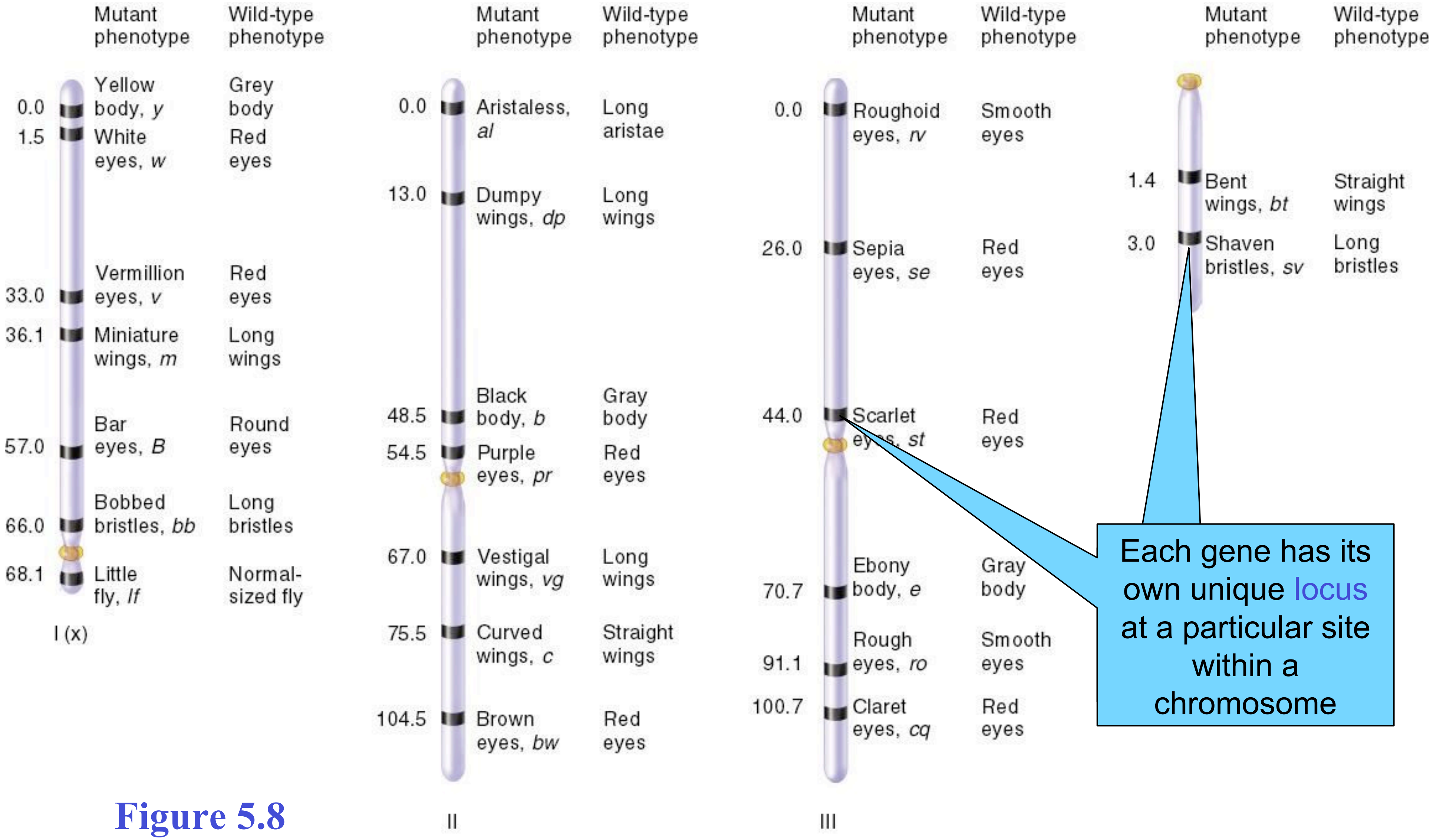
Table 4.1 Critical Chi Square Values

Degrees of Freedom	***P* values**						
	Null Hypothesis Accepted				**Null Hypothesis Rejected**		
	0.99	**0.90**	**0.50**	**0.10**	**0.05**	**0.01**	**0.001**
	x^2 calculations						
1	—	0.02	.45	2.71	**3.84**	**6.64**	**10.83**
2	0.02	0.21	1.39	4.61	**5.99**	**9.21**	**13.82**
3	0.11	0.58	2.37	6.25	**7.81**	**11.35**	**16.27**
4	0.30	1.06	3.36	7.78	**9.49**	**13.28**	**18.47**
5	0.55	1.61	4.35	9.24	**11.07**	**15.09**	**20.52**

- Reject
- Therefore: **genes are linked**

GENETIC MAPPING IN PLANTS AND ANIMALS

- Genetic mapping is also known as gene mapping or chromosome mapping
- Its purpose is to determine the linear order of linked genes along the same chromosome
- Figure 5.8 illustrates a simplified genetic linkage map of *Drosophila melanogaster*



Each gene has its own unique locus at a particular site within a chromosome

Figure 5.8

Genetic Maps

- Genetic maps are useful in many ways: they
  - 1. allow us to understand the overall complexity and genetic organization of a particular species
  - 2. improve our understanding of the evolutionary relationships among different species
  - 3. can be used to diagnose, and perhaps, someday to treat inherited human diseases
  - 4. can help in predicting the likelihood that a couple will produce children with certain inherited diseases
  - 5. provide helpful information for improving agriculturally important strains through selective breeding programs

Sturtevant, an undergrad student of Morgan’s (21 years old), invented a way to quantify the relationship between the rate of recombination and the distance between genes.

$$\frac{\#\ \text{recombinants}}{\#\ \text{total}} = \text{RF}$$

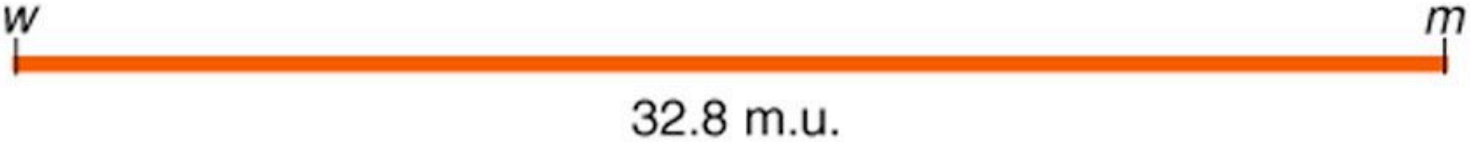
(RF=Recombination Frequency)

RF X 100 = % recombination

1% recombination = 1 map unit (mu)
= 1 cM (centiMorgan)

(a)

(b)



- Genetic maps allow us to estimate the relative distances between linked genes, based on the likelihood that a crossover will occur between them
- Experimentally, the percentage of recombinant offspring is correlated with the distance between the two genes
  - If the genes are far apart → more recombinant offspring
  - If the genes are close → fewer recombinant offspring
- Map distance = $$\frac{\text{Number of recombinant offspring}}{\text{Total number of offspring}} \times 100$$
  - The units of distance are called map units (mu)
    - They are also referred to as centiMorgans (cM)
  - One map unit is equivalent to 1% recombination frequency

Genetic Mapping and Testcrosses

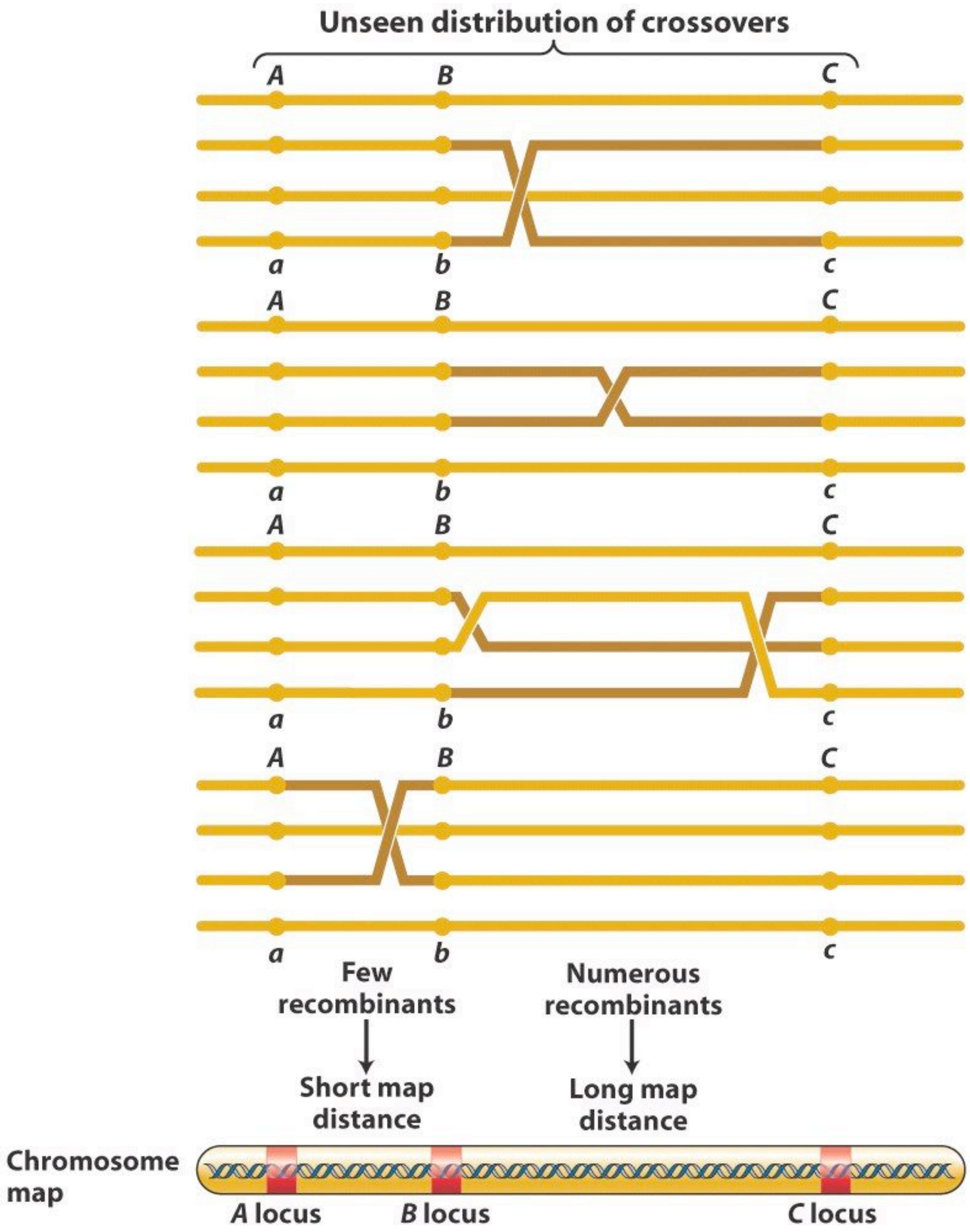
- Genetic mapping experiments are typically accomplished by carrying out a testcross
  - A mating between an individual that is heterozygous for two or more genes and one that is homozygous recessive for the same genes
- Genes that are located on DIFFERENT chromosomes show a recombination frequency of 50%
- Genes that are located FAR APART on the SAME chromosome show a recombination frequency of 50%
- The closer two genes are to each other on a chromosome, the small the recombination frequency will be
  - (approaches 0%).

The Hypothesis

- The distance between genes on a chromosome can be estimated from the proportion of recombinant offspring
  - This provides a way to map the order of genes along a chromosome

- Crossing Over creates recombinant offspring
  - An event where homologous chromosomes exchange parts, creating a new combination of gene alleles.
  - The exchange of genetic material between the two homologous chromosomes is termed <u>Recombination</u>.

- Example
  - Before Crossover:
    - Maternal Chromosome Genes: ABCD
    - Paternal Chromosome Genes: abcd
  - After crossing over:
    - Maternal Chromosome Genes: ABcd
    - Paternal Chromosome Genes: abCD

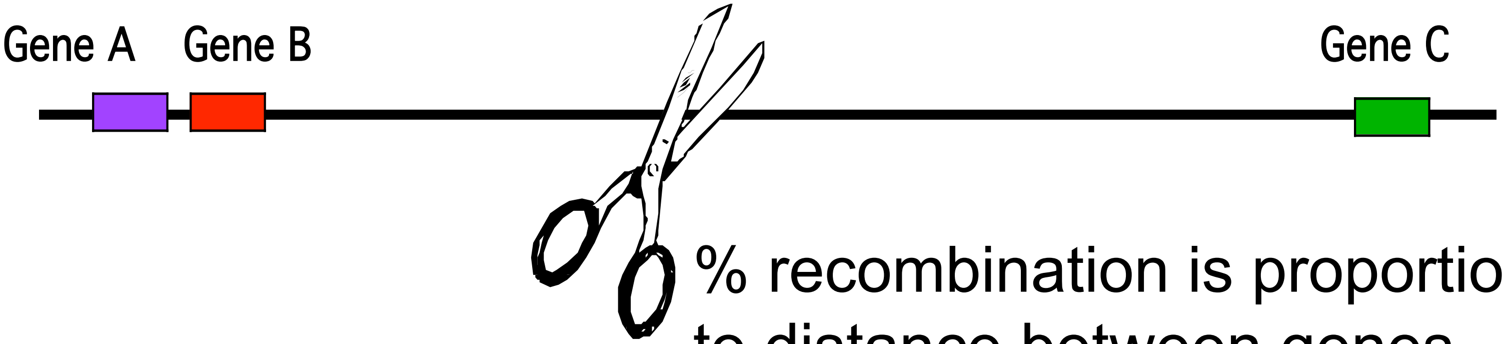


The frequency of recombination is proportional to the genetic distance

(based on the assumption that recombination is random)

Recombination and genetic distance

random cuts between (A and B) < (B and C)



% recombination is proportional to distance between genes.

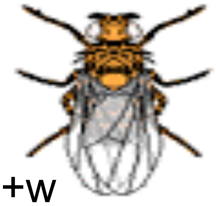
Quantification of the relationship between the rate of recombination between two genes and the distance between them:

recombinant offspring / total # offspring = recombination frequency between two genes

1% recombination = 1 map unit (m.u.) = 1 cM (centiMorgan)

Calculating the Distance Between y and w Genes

F1

♀  $\frac{X^{yw+}}{X^{y+w}}$ X ♂  $\frac{X^{y+w}}{Y}$

F2 males:

$X^{y\ w+}/Y$	4484	- Parental
X^{y+w}/Y	4413	- Parental
X^{y+w+}/Y	76	- Recombinant
$X^{y\ w}/Y$	53	- Recombinant
	9026	total

$$\frac{\text{\# recombinants}}{\text{total \#}} = \text{recombination frequency between two genes}$$

$$\frac{76 + 53}{9026} \times 100 = 1.43\%$$

= 1.43 map units between the y and w genes

Another example: Linkage in an autosomal gene

- **genotypes of F1 female revealed by test cross**
- **Parental class outnumbers recombinant class demonstrating linkage**

P ♀ $b\ c^+/b\ c^+$ × ♂ $b^+\ c\ /b^+\ c$

F_1 (all identical) $b\ c^+/b^+\ c$

Test cross ♀ $b\ c^+/b^+\ c$ × ♂ $b\ c\ /b\ c$

Test cross progeny

2934 $b\ c^+/b\ c$
2768 $b^+\ c/b\ c$

Parental classes $= \dfrac{2934 + 2768}{7419} \times 100 = 77\%$

871 $b\ c/b\ c$
846 $b^+\ c^+/b\ c$

Recombinant classes $= \dfrac{871 + 846}{7419} \times 100 = 23\%$

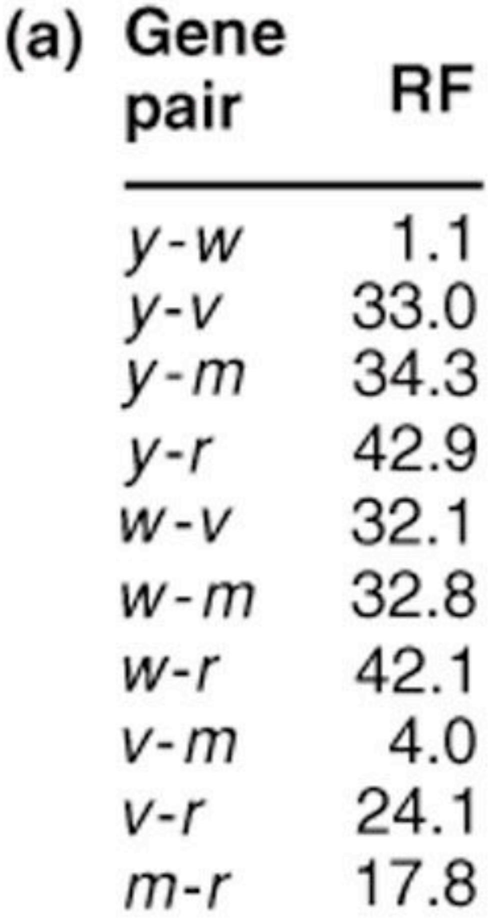
23 mu

Total 7419

Summary of linkage and recombination “rules”

- Genes close together on the same chromosome are linked and do not segregate independently
- Linked genes lead to a larger number of parental class than expected in double heterozygotes
- Mechanism of recombination is crossing over
- Chiasmata are the visible signs of crossing over
- Farther away genes are, the greater the opportunity for chiasmata to form
- Recombination frequencies reflect physical distance between genes
- Recombination frequencies between two genes vary from 0% to 50%

Mapping:
Locating genes along a chromosome



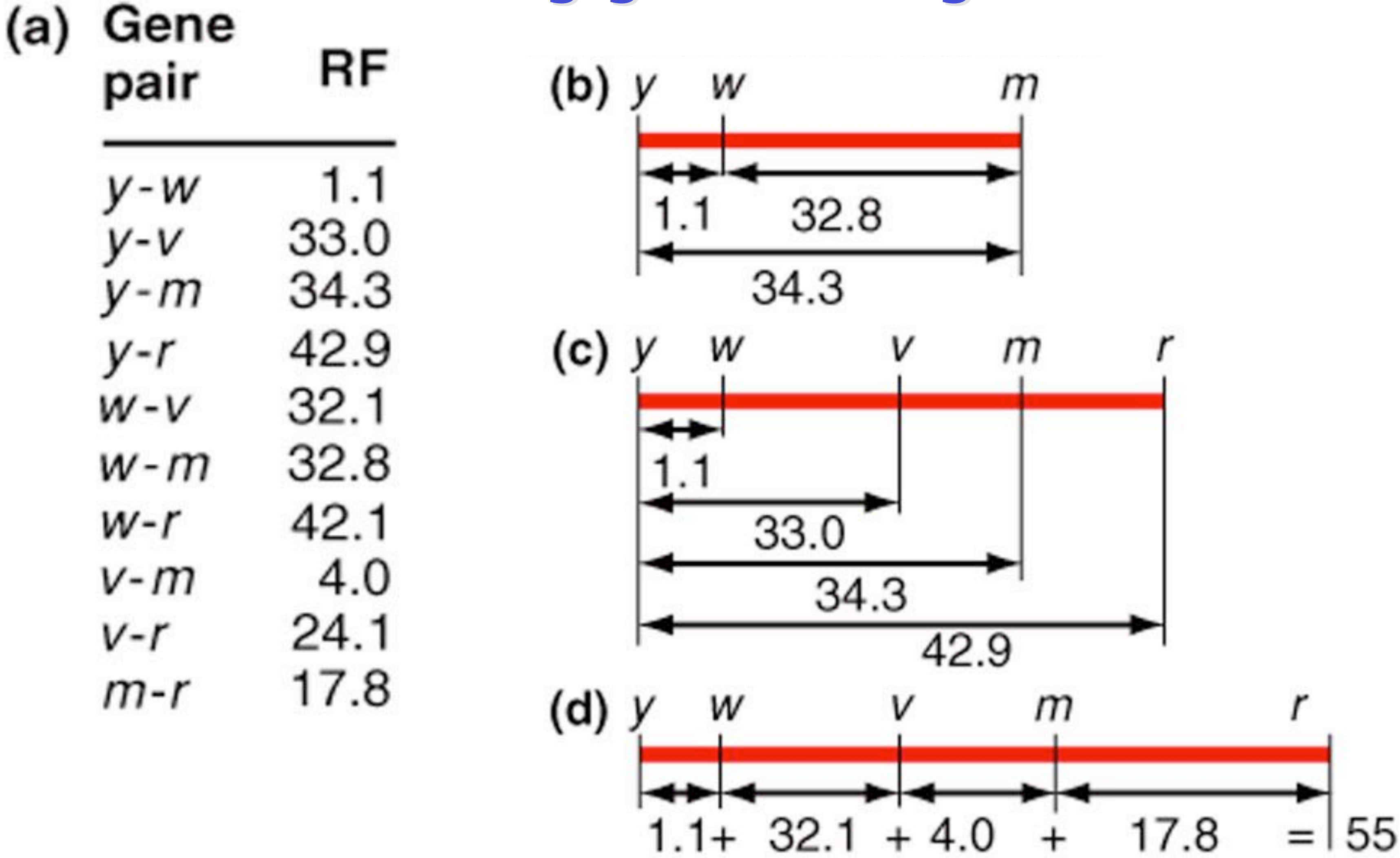
(a)

Gene pair	RF
y-w	1.1
y-v	33.0
y-m	34.3
y-r	42.9
w-v	32.1
w-m	32.8
w-r	42.1
v-m	4.0
v-r	24.1
m-r	17.8

- Two-point crosses: comparisons help establish relative gene positions
- Genes are arranged in a line along a chromosome

Fig. 5.11

Mapping: Locating genes along a chromosome



(a)

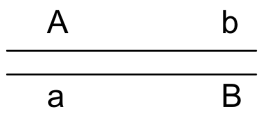
Gene pair	RF
y-*w*	1.1
y-*v*	33.0
y-*m*	34.3
y-*r*	42.9
w-*v*	32.1
w-*m*	32.8
w-*r*	42.1
v-*m*	4.0
v-*r*	24.1
m-*r*	17.8

Genes are arranged in a line along a chromosome

Example problem

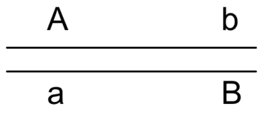
- An individual has the genotype Aa/Bb. Gene loci (*A*) and (*B*) are 15 cM apart. Indicate all the possible gametes this individual can produce, and the proportions of expected progeny genotypes if a testcross is performed on this individual.

- What do we know?



Example problem

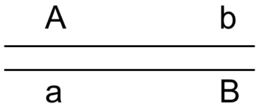
- An individual has the genotype Ab/aB. Gene loci (*A*) and (*B*) are 15 cM apart. Indicate all the possible gametes this individual can produce, and the proportions of expected progeny genotypes if a testcross is performed on this individual.
- What do we know?
  - 15% recombination frequency
  - Means 15% recombinant progeny, 85% parental progeny
  - Testcross is Ab/aB X abab
  - Results
    - Ab = 42.5% (half of 85% parentals)
    - aB = 42.5% (half of 85% parentals)
    - AB = 7.5% (half of 15% recombinants)
    - ab = 7.5% (half of 15% recombinants)



Question

What if we artificially blocked crossing over?

- An individual has the genotype Ab/aB. Gene loci (*A*) and (*B*) are 15 cM apart. Indicate the proportions of expected progeny genotypes if a testcross is performed on this individual.
- What do we know?



  - 0% recombination frequency
  - Testcross is Ab/aB X ab/ab
  - 1)
    - Ab/ab = 42.5% (half of 85% parentals)
    - aB/ab = 42.5% (half of 85% parentals)
    - AB/ab = 7.5% (half of 15% recombinants)
    - Ab/ab = 7.5% (half of 15% recombinants)
  - 2)
    - Ab/ab = 50%
    - aB/ab = 50%
  - 3)
    - AB/ab = 50%
    - Ab/ab = 50%

Limitations of two point crosses

- Difficult to determine gene order if two genes are close together
- Actual distances between genes do not always add up
- Pairwise crosses are time and labor consuming

Homework Problems

- Chapter 6
- # 2, 3, 4, 6, 7, 8, 10, 13