River Valley High School
2025 JC1 H2 Biology

Lecture Topic 16: Inheritance

Name: _____ ( ) Class: 25J___ Date: _____

References

Title	Authors
Biology (8th Edition)	Campbell and Reece
Biological Science 1 and 2 (3rd Edition)	Green, Stout and Taylor
Principles of Genetics	Gardner, Simmons, Snustad
An Introduction to Genetic Analysis (7th Edition)	Griffiths, Miller, Suzuki, Lewontin and Gelbart

Websites

URL	Description
https://knowgenetics.org/mendelian-genetics/	Mendelian Genetics
http://www.dnaftb.org/dnaftb/1/concept/	Animation on Mendel's experiment

H2 Biology Syllabus 9477 (2025)

Candidates should be able to use the knowledge gained in the following section(s) in new situations or to solve related problems.

Related Topics	Content
The Cell Cycle	• Replication and division of nuclei and cells • Understanding of chromosome number and variation • Effect of meiosis on chromosome number and variation
The Structure of Nucleic Acids and Gene Expression	• Central Dogma - DNA to RNA, RNA to protein

Learning Outcomes

2F. Inheritance

a. Explain the terms: *locus, allele, dominant, recessive, codominant, homozygous, heterozygous, phenotype, genotype* and *linkage.*

b. Explain how genes are inherited from one generation to the next via the germ cells or gametes.

c. Explain how genotype is linked to phenotype.

d. Use genetic diagrams to solve problems in dihybrid crosses, including those involving codominance, multiple alleles, sex linkage, autosomal linkage and epistasis.

e. Use genetic diagrams to solve problems involving test crosses.

f. Explain the meaning of the terms linkage and crossing-over and explain the effect of linkage and crossing-over on the phenotypic ratios from dihybrid crosses.

g. Describe the interaction between loci (epistasis) and predict phenotypic ratios in problems involving epistasis. (Knowledge of the expected ratio for various types of epistasis is not required; focus of this section is on problem solving.)

h. Explain how the environment may affect the phenotype, using examples including the effect of diet on differentiation of honeybees.

i. Explain the difference between genetic variation that is continuous (many, additive genes control a characteristic) and genetic variation that is discontinuous (one or a few genes control a characteristic).

j. Use the chi-squared test to test the significance of differences between observed and expected results.

Lecture Outline

I. Introduction
- A. Terminology
- B. Inheriting genes in sexual reproduction
- C. How genotype is linked to phenotype?
- D. Mendel's experiment

II. Monohybrid Crosses and Principle of Segregation
- A. Representing monohybrid cross in symbols
- B. Explanation of monohybrid cross in term of probability
- C. Testcross
- D. Monohybrid inheritance in humans

III. Extending Mendelian genetics
- A. Co-dominance
- B. Multiple alleles
- C. Lethal genes

IV. Sex Determination
- A. Sex linkage
- B. Reciprocal cross
- C. Human pedigree

V. Dihybrid Crosses and Principle of Independent Assortment
- A. Representing dihybrid cross in symbols
- B. Explanation of dihybrid cross in term of probability
- C. Testcross

VI. Linkage
- A. Morgan's *Drosophila* experiment
- B. Types of linkage
- C. Gene mapping and crossover values

VII. Gene Interaction
- A. Gene interaction producing new phenotype
- B. Epistasis
  - B1. Dominant epistasis
  - B2. Recessive epistasis
  - B3. Duplicate recessive

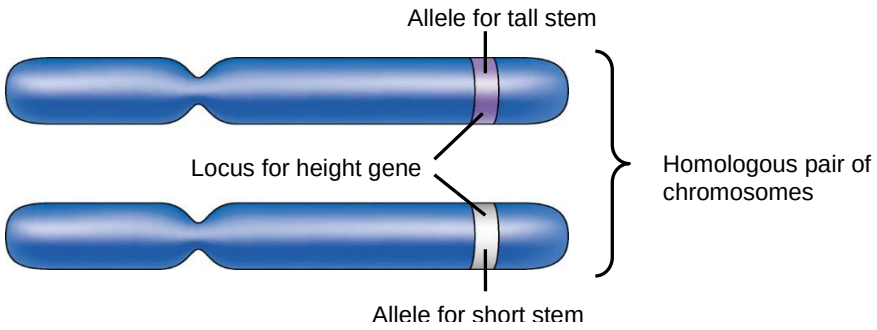
VIII. Variation
- A. Continuous variation
- B. Discontinuous variation
- C. Influence of environment
- D. Sources of variation

IX. Statistical Tests

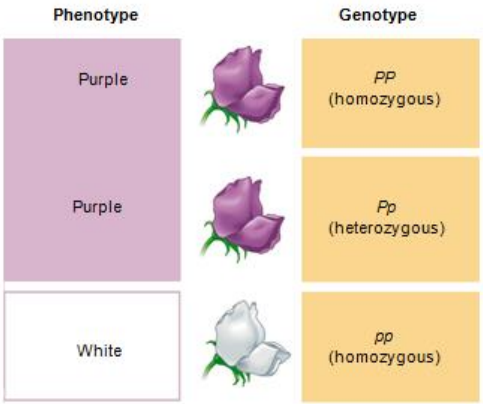
I. Introduction

- Genetics is the study of inheritance of characteristics by offspring from their parents.
- In the study of genetics, subtle but recognizable differences exist between individuals of the same species (even between parents and offspring) which geneticists term as **variation**.

A. Terminology

Gene	• A basic hereditary unit located on a specific locus of a chromosome. • A segment of DNA with unique nucleotide (base) sequence coding for a particular RNA molecule / polypeptide chain. • An unit of inheritance determining a specific phenotype of an individual.
Locus	• position of a gene/allele on a chromosome or within a DNA molecule
Allele	• alternative form of the same gene and responsible for determining the contrasting characteristics of a gene. • For instance, there is a gene responsible for the stem height of pea plant; however, there are 2 forms of it: ⇨ allele for *tall* stem ⇨ allele for *short* stem • Each allele has a unique nucleotide sequence, which may result in different phenotypes. * Alleles are found to be identical in most of their sequences and differ only at one or a few nucleotides of the thousands of nucleotides that make up the gene. * The following diagram represents the DNA of two alleles of one gene and their difference in the nucleotide sequence. Allele 1 AACCG Allele 2 ATTGG • Alleles occupy the same locus of a pair of homologous chromosomes.  ***Fig. 1 Alleles, alternative versions of a gene.*** *Source: Biology (8th Edition) pp. 265*

Dominant allele	• The allele which influences the appearance of the phenotype even in the presence of an alternative allele, i.e. in homozygous or heterozygous condition • encodes a functional protein • represented by a capital letter in a genetic cross. E.g **TT** or **T**t
Recessive allele	• The allele which influences the appearance of the phenotype only in the presence of another identical recessive allele, i.e. in the homozygous condition • Encodes for a non-functional protein or lack of protein • represented by a lower-case letter in a genetic cross. E.g. T**t** or **tt**
Homozygous	• When both alleles at a given locus are identical in diploid condition, may be either homozygous dominant (E.g. TT) or homozygous recessive (E.g. tt). • Homozygous organisms are referred to as homozygotes. • Homozygous individual breeds true (true breeding/pure breeding) i.e., consistently produce the same characteristic when the plants were self-fertilised because its entire offspring receive the same allele from this parent.
Heterozygous	• When different alleles are present in diploid condition. • Dominant form of the allele is expressed, masking the presence of the recessive allele. • Heterozygous organisms are referred to as heterozygotes. They do not breed true. Molecular nature of alleles * In the heterozygote, the protein produced by the functional allele is enough for the normal needs of the cell; so the functional allele acts as a dominant allele.
Co-dominance	• Both alleles are equally expressed in the phenotype of the heterozygote.
Genotype	• genetic makeup or genetic constituents. (**Fig. 2**)
Phenotype	• observable characteristics that are the expression of its genotype. (**Fig. 2**) *** Character** - a heritable feature, such as flower colour, that varies among individuals. *** Trait** - each variant for a character, such as purple or white colour for flowers



There are actually two categories of purple-flowered plants: **PP** (homozygous) and **Pp** (heterozygous).

Fig. 2 Phenotype vs genotype

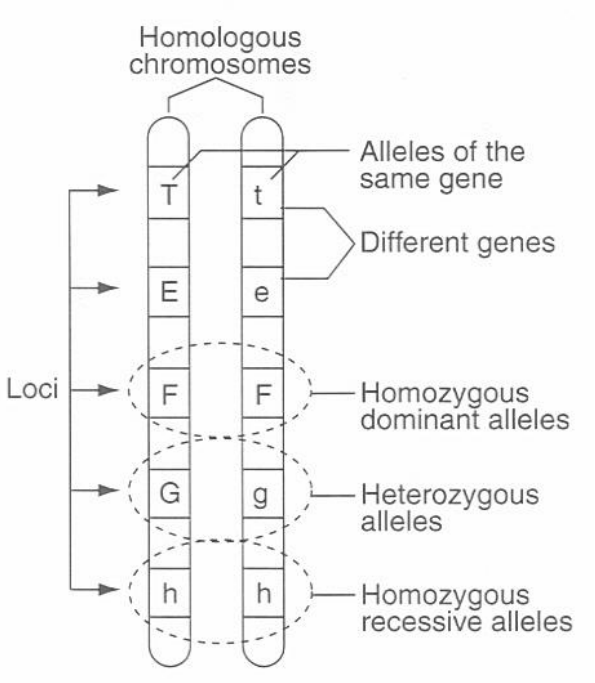


Fig. 3 Summary of terms used in Genetics

B. Inheriting genes in sexual reproduction

- In sexual reproduction, haploid gametes are formed, by meiosis, from the nuclei of diploid cells in the gonads. So, each gamete contains only one copy of each gene.

- At fertilization, male and female gametes fuse to form a zygote. Consequently, the zygote is a diploid cell with two sets of chromosomes (known as homologous pairs) one from each parent.

- Thus there are two copies of each gene. These lie in the same position, or loci, on the two homologous chromosomes. There are at least two alleles of every gene.

- The genes control the different characteristics of organisms. The alternative versions of genes (different alleles) account for variation in inherited characters.

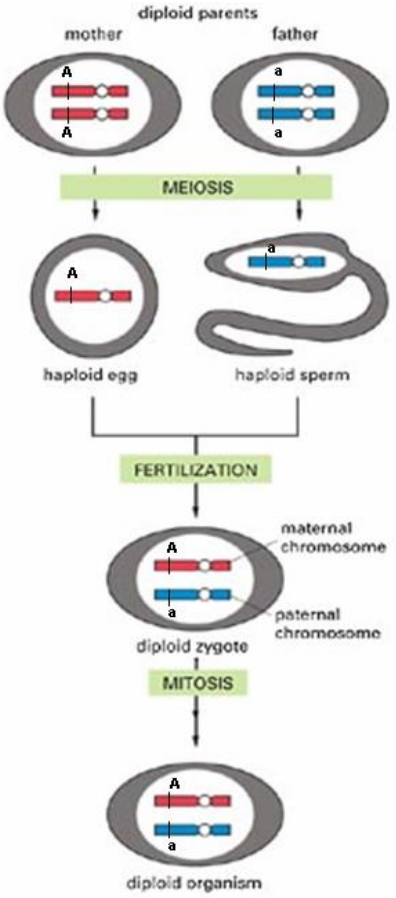


Fig. 4 Genetic inheritance during meiosis, fertilization and mitosis *Source:*
www.edu.pe.ca/rural/class_webs/biology/biology_6...art_2.htm

C. How genotype is linked to phenotype?

- **Central dogma**: **DNA** via transcription to **RNA** via translation to **protein**.

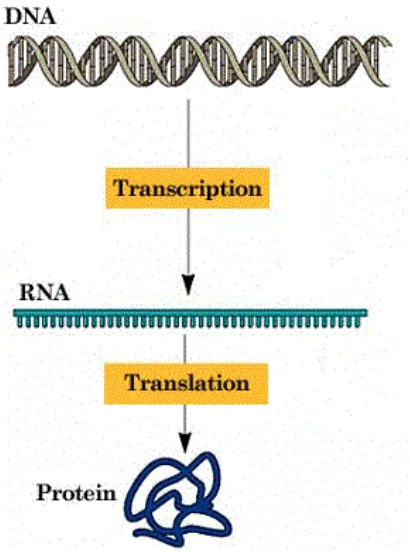


Fig. 5 Central Dogma: Transcription and translation

D. Mendel's Experiment

1. Mendel discovered the basic principles of heredity through hybridization (cross-pollination) experiments with *Pisum sativum* (garden peas).

> The advantages of using peas are:
>
> * Short generation time
> * Large number of offspring from each mating
> * Distinct characteristics, i.e. no intermediate between 2 varieties

2. He observed seven characters, each of which occurred in two alternative forms:

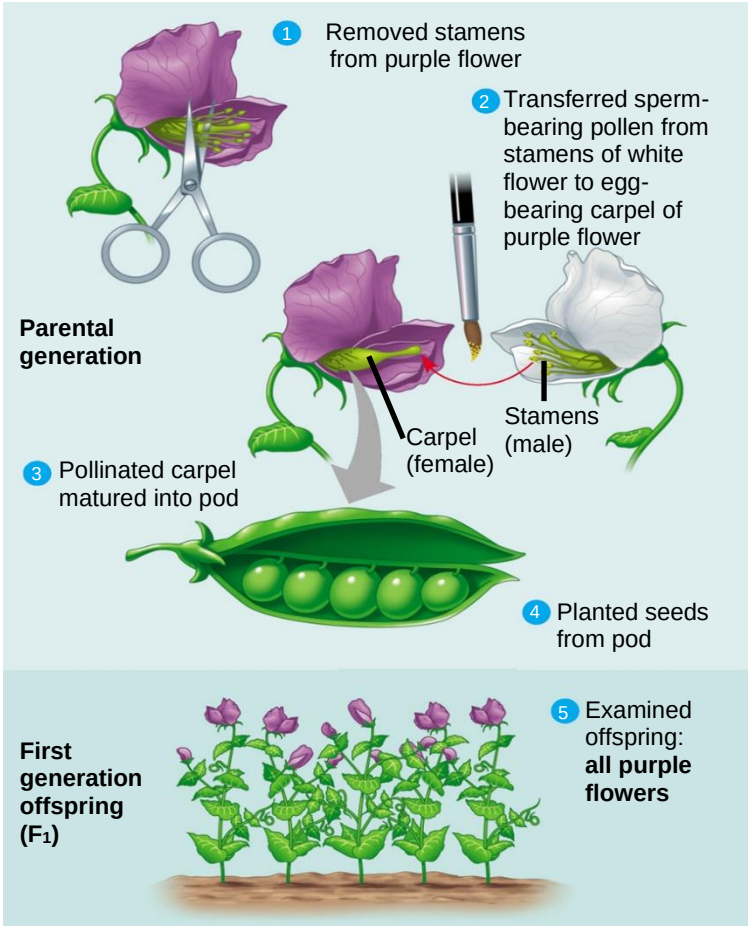
    1. flower color (purple or white)
    2. flower position (axil or terminal)
    3. stem length (long or short)
    4. seed shape (round or wrinkled)
    5. seed color (yellow or green)
    6. pod shape (inflated or constricted)
    7. pod color (yellow or green)

3. To study how a particular character would be transmitted from the parent plant to the next generation, Mendel started his experiments with true-breeding plant varieties with contrasting traits (**Fig. 4**). The true-breeding parental plants are called the Parental generation.

> * True-breeding/ pure breeding - always producing offspring with the same *traits* as the parents when the parents are self-fertilized/self-pollinated. Individuals are either homozygous dominant or homozygous recessive.

4. Following cross-fertilization, the seeds were collected and germinated. The resulting hybrid offspring comprised the F_1 generation (first filial generation, the word *filial* from the Latin word for "son").

5. F_1 generation plants self-pollinate/self-fertilise to produce the next generation, the F_2 generation (second filial generation).



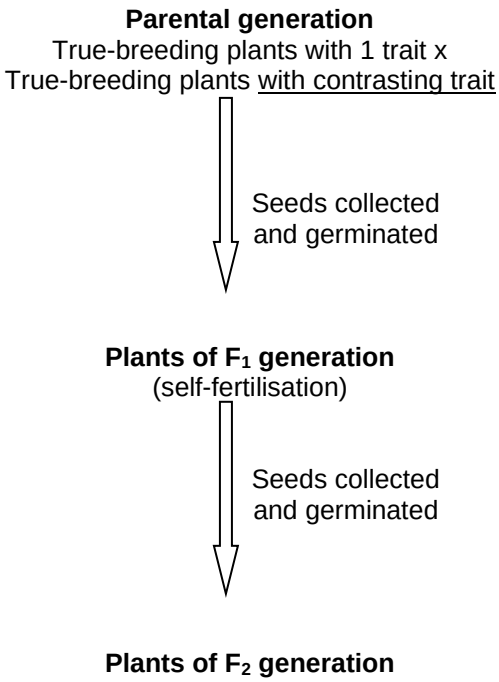


Fig. 6 Crossing pea plants

Source: Biology (8th Edition) pp. 263

6. Mendel observed the transmission of selected traits for at least three generations and arrived at two principles of heredity that are now known as the law of segregation and the law of independent assortment.

II. Monohybrid Cross and the Principle of Segregation

Monohybrid crosses

- Mendel restricted his first series of investigations to the inheritance of a **single pair** of contrasted characteristics is an example of a monohybrid cross.
- The character is controlled by a single gene - a pair of alleles on a single gene locus.

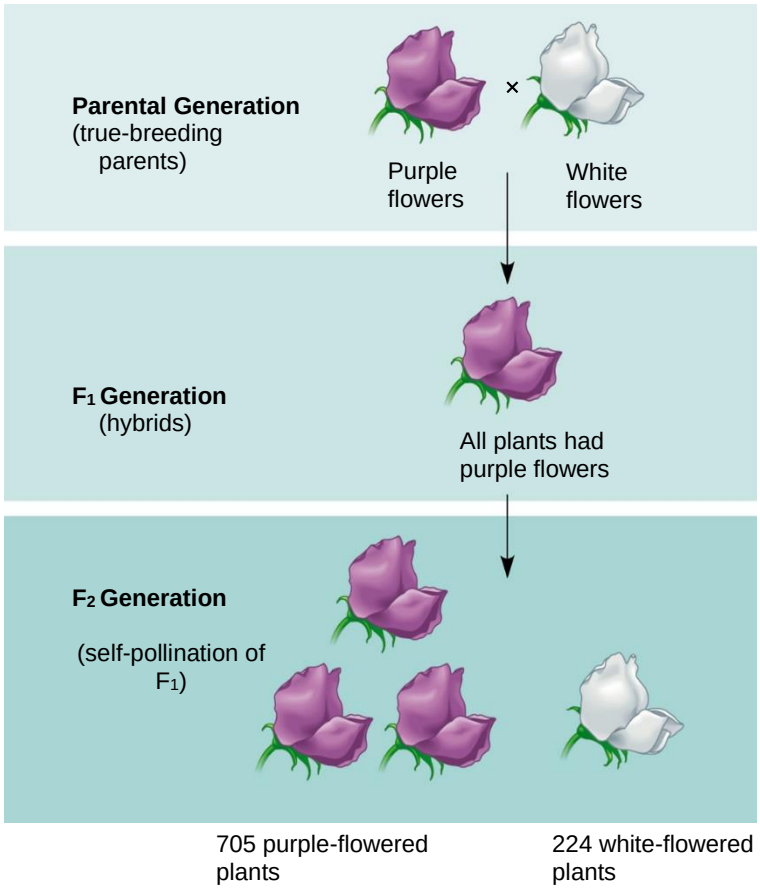
a) Mendel's experiment:

- True-breeding purple-flowered pea plants and white-flowered pea plants were crossed (symbolized by ×). The resulting F_1 hybrids were allowed to self-pollinate or were cross-pollinated with other F_1 hybrids. Flower color was then observed in the F_2 generation.

b) Mendel's observations:

Results

- All the F_1 offspring were purple.
- When he crossed the F_1 plants (by enclosing the flowers in bags to ensure self-pollination), many of the plants had purple flowers, but some had white flowers.
- 705 of the F_2 plants had purple flowers, and 224 had white flowers, a ratio of about 3 purple : 1 white.



- All the F_1 hybrids exhibited only one of the parental traits (purple flowers).
- Plants with white flowers, are non-existent in the F_1 generation, but re-appear in the F_2 generation.
- In both F_1 and F_2 generations, there was no intermediate phenotype type observed.
- The phenotypic ratio of F_2 plants exhibiting two different traits always approximately to **3:1**. The observations were true for all the monohybrid crosses performed on the various characters (Table 14.1).

Table 14.1 The Results of Mendel's F_1 Crosses for Seven Characters in Pea Plants

Character	Dominant Trait	×	Recessive Trait	F_2 Generation Dominant:Recessive	Ratio
Flower color	Purple 	×	White 	705:224	3.15:1
Flower position	Axial 	×	Terminal 	651:207	3.14:1
Seed color	Yellow 	×	Green 	6022:2001	3.01:1
Seed shape	Round 	×	Wrinkled 	5474:1850	2.96:1
Pod shape	Inflated 	×	Constricted 	882:299	2.95:1
Pod color	Green 	×	Yellow 	428:152	2.82:1
Stem length	Tall 	×	Dwarf 	787:277	2.84:1

Fig. 7 Results of Mendel's F_1 crosses for all seven characteristics in pea plants

Source: Biology (8th Edition) pp. 264 - 265

c) Mendel's Conclusion:

1. Since parents were true-breeding, the purple-flowered variety must have possessed 2 purple-flower *factors* and white-flowered variety 2 white-flower *factors*.

   * Mendel's factors are now called **genes**.

2. The heritable factor for white flowers was not lost in the F_1 generation, it must have been masked by the presence of the purple-flower factor.

   ⇨ Purple flower is a dominant **trait** and white flower is a recessive **trait**.

   ⇨ The F_1 possessed one factor from each .parent through the gametes.

   ⇨ The factors do not blend in F_1 but retain their individuality.

The Principle of Segregation

The separation of the pair of parental factors, so that one factor is present in each gamete, became known as Mendel's First Law or the Principle of Segregation which states that:

The characteristics of a diploid organism are determined by alleles which occur in pairs. The alleles of a gene pair segregate during anaphase I of meiosis. Only one can be carried in a single gamete.

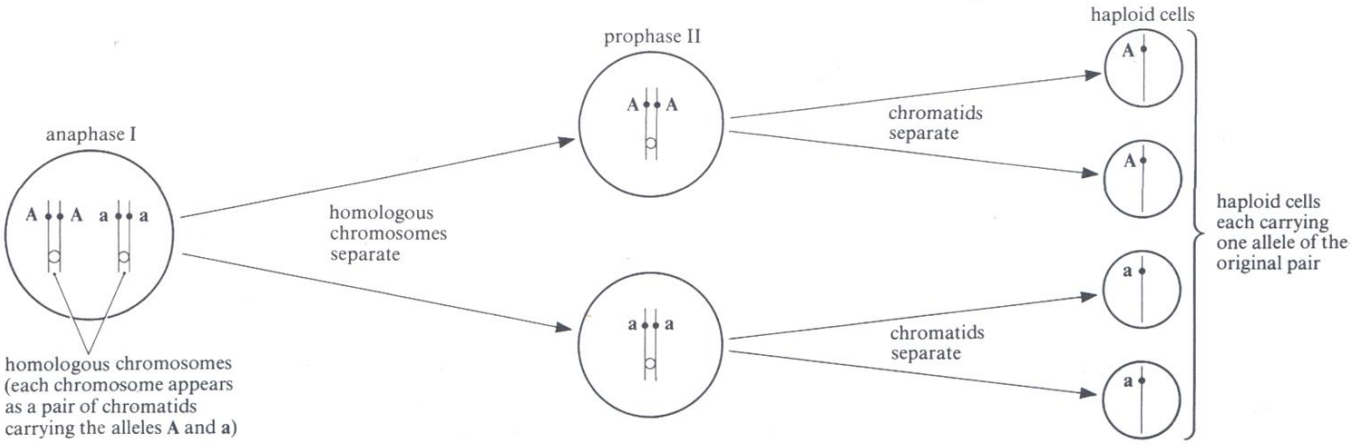


Fig. 8 Mendel's principal of segregation of alleles in terms of separation of homologous chromosomes during meiosis*. Source: Biological Science 2 pp. 815*

A. Representing Monohybrid Cross in Symbols

The investigation of the inheritance of a single pair of contrasted characteristics may be represented in terms of symbols:

Let **P** represent the allele for purple flower (dominant)
p represent the allele for white flower (recessive)

Parental phenotypes: Purple flowers x White flowers

Parental genotypes: x

Gametes produced by parents:

F_1 genotypes:

F_1 phenotypes:

Selfing F_1 generation:

Gametes produced by F_1 generation:

Punnett Square to show the fusion of gametes produced by the F_1 generation:

B. Explanation of the 3:1 Mendelian monohybrid ratio in terms of probability

- Mendel's conclusions regarding the transfer of a single characteristic by each gamete and the genotype appearance can be demonstrated by mathematical probability.
- The probability of a gamete cell from a heterozygous F_1 parent containing either the dominant allele **P** or the recessive allele **p** is 50% or ½.
- If each gamete is represented by ½, the number of possible combinations of F_2 genotype is represented by ½ x ½ = ¼. Hence there are four possible F_2 genotypes.
- As a results of dominance the phenotypic appearance will be 3 dominant phenotypes : 1 recessive phenotypes.

Therefore, the probability of the alleles **P** and **p** appearing in the gamete:

P = ½
p = ½

Using these values the probability of each genotype and phenotype appearing in the F_2 generation can be demonstrated as shown below:

F_1 genotypes:		**Pp**	X		**Pp**	
Gametes:	**P**	**p**	X	**P**		**p**
(In terms of probability)	½	½	X	½		½

Punnett Square to show the fusion of gametes produced by the F_1 generation:

	½ **P**	½ **p**
½ **P**	(½ x ½) ¼ **PP**	(½ x ½) ¼ **Pp**
½ **p**	(½ x ½) ¼ **Pp**	(½ x ½) ¼ **pp**

F_2 genotypes: ¼ **PP** ¼ **Pp** ¼ **Pp** ¼ **pp**

F_2 phenotypic ratio: *¾ dominant* : *¼ recessive*
i.e. 3 dominant : 1 recessive

C. Testcross (Monohybrid)

- If an organism shows the dominant characteristics (for example, a purple flower) then it may be either homozygous for a dominant allele (**PP**) or heterozygous for the dominant allele (**Pp**). In other words, **PP** and **Pp** look alike.
- **Test cross** are used by most breeders to find out the genotype. In a test cross, an organism showing the dominant characteristic is crossed with another organism that is homozygous recessive.

  → If the heterozygous offspring is crossed with its homozygous recessive parent, the test cross can be known as backcross
- For example, to find out whether a purple-flowered plant is homozygous (**PP**) or heterozygous (**Pp**), it is crossed with a white-flowered plant (**pp**).
  - ⇨ All the offspring are purple-flowered, the unknown genotype is homozygous dominant
  - ⇨ A ratio of 1 purple-flowered offspring : 1 white-flowered offspring, the unknown genotype is heterozygous
- A full genetic explanation on how to determine the genotype of an organism with dominant trait:

Let **P** represent the allele for purple flower (dominant)

p represent the allele for white flower (recessive)

Scenario 1: Homozygous purple-flowered plant (parent)

Parental phenotypes: purple flowers (homozygous) x white flowers

Parental genotypes: **PP** x **pp**

Gametes: **P** **p**

Offspring genotypes: **Pp**

Offspring phenotypes: all purple flowers (heterozygous)

Scenario 2: Heterozygous purple-flowered plant (parent)

Parental phenotypes: purple flowers (heterozygous) x white flowers

Parental genotypes: **Pp** x **pp**

Gametes: **P** **p** x **p**

Punnett Square to show the fusion of gametes:

	P	**p**
p	**Pp** purple flower	**pp** white flower

Offspring phenotypic ratio: 1 purple flower : 1 white flowers

D. Monohybrid inheritance in humans

- Examples of monohybrid inheritance in humans include the following conditions:
  - ⇨ Albinism
  - ⇨ Cystic fibrosis
  - ⇨ Haemophilia
  - ⇨ Huntington's disease
  - ⇨ Phenylketonuria (PKU)
  - ⇨ Rhesus blood groups
  - ⇨ Sickle-cell anaemia
- Huntington's disease is caused by a dominant allele; the others are recessive traits.
- All except haemophilia are inherited in a simple Mendelian manner. Haemophilia is sex-linked.

Albinism

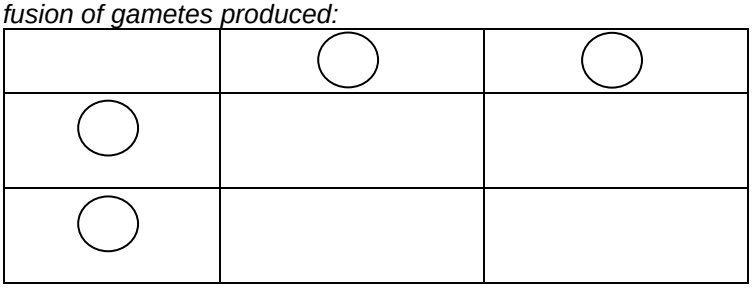
- Albinism is a condition in which the skin is pink and fails to tan, the hair white and the iris pink.
- The reason is that albinos are <u>unable to make the black pigment melanin</u> because they lack an enzyme (tyrosinase) required for its synthesis. They therefore lack the protection from ultraviolet light which melanin normally confers on the skin.
- The allele for albinism is recessive (**a**) and so only exerts its effect in the homozygous state (**aa**). The allele for enzyme involved in melanin production (**A**) is dominant. The genotype of a person with normal pigmentation is therefore **AA** or **Aa**.
- Suppose a couple each with normal pigmentation has an albino child. For this to happen, the child must have the genotype **aa**. Therefore, aside from the possibility of a rare mutation, each parent must be heterozygous (**Aa**). In other words the parents, though not themselves albino, carry the albino allele, for which reason they may be described as carriers.
- If this happened in practice, the couple would probably want to know the likelihood or probability of their next child also being an albino. What would be the likelihood of their next child being an albino?

Parental phenotypes: Carrier father x Carrier mother

Parental genotypes: x

Gametes:  x

Punnett Square to show the fusion of gametes produced:



Offspring phenotypic ratio:

Q: What would be the likelihood of their next child being an albino?

III. Extending Mendelian Genetics

- The patterns of inheritance described by Mendel have a simple genetic basis: each character studied is determined by a gene, for which there are only two alleles, one completely dominant to the other.

- When the allele is completely dominant over another, it will express the trait that it determines, even in the presence of the other allele. As a result, the heterozygous will shows the trait determined by the dominant allele and will be phenotypically indistinguishable from the homozygous dominant. Consequently, the recessive allele can only express its phenotype in the absence of the dominant allele; homozygous recessive.

- Therefore, the F_1 offspring of Mendel's classic pea crosses always looked liked one of the two parental varieties because of the complete dominance of one allele over another.

- But these conditions are not met by all heritable characters, not even in garden peas. The relationship between genotype and phenotype is rarely so simple.

- In this section, we will extend Mendelian genetics to hereditary patterns that were not reported by Mendel.

A. Codominance

- The term codominance is used to describe a situation in which both alleles are equally expressed in the phenotype of the heterozygote.

- The heterozygotes have a phenotype that is different from either of the homozygotes, but not necessarily intermediate between the two different homozygotes.

Example: human MN blood groups

Genetic Basis

- In human, allele **M** for M-type blood is codominant with its allele **N** for N-type blood.

- A homozygous M-type person produces **M antigen** on the red blood cells (phenotype M); a homozygous N-type person produces **N antigen** on the red blood cells (phenotype N).

- The heterozygotes express the characteristics of both **M** and **N antigens** (MN-type blood), i.e. possess red blood cells that carry both **M** and **N antigens** on their surface.

Let **M** represent the allele for the production of protein M (codominant)

N represent the allele for the production of protein N (codominant)

Parental phenotypes:	blood group M	x	blood group N
Parental genotypes:	**MM**	x	**NN**
Gametes produced by parents:	**M**	x	**N**
F_1 genotype:	**MN**		
F_1 phenotype:	All of blood group MN		

Biochemical basis

- Both alleles are expressed equally and they code for functional products. The two products are the same with respect to function but different in exact amino acid sequence. Both products appear in the heterozygote.

Examples of codominance:

Characteristics	Parental phenotype	Heterozygous phenotype
Short-horn cattle	Red x white	Roan (even mixture of white and pigmented hairs)
A-B-O blood group	A-type blood group x B-type blood group	AB-type blood group

Difference in Inheritance pattern between Incomplete Dominance & Codominance with Complete dominance

Incomplete dominance & Codominance	Complete dominance
• Heterozygotes have a phenotype different from either of the homozygotes • Phenotypic ratio in a cross between two heterozygotes is 1:2:1	• Heterozygotes have a phenotype similar to either of the homozygotes • Phenotypic ratio in a cross between two heterozygotes is 3:1

B. Multiple Alleles

- There may be more than two alleles of the same gene present in a population but the maximum number of alleles for each gene that any individual can possess is two, because he has only two copies of each gene
- Alleles are called **multiple alleles** if a gene controlling a characteristic has ≥ 3 alleles

Genetic Basis

- In the **A-B-O** blood series, 4 blood types are generally recognized: **A**, **B**, **AB** and **O**.
- The inheritance of the **A-B-O** groups involves three alleles, here designated $\mathbf{I^A}$, $\mathbf{I^B}$ and $\mathbf{I^O}$.
- Both $\mathbf{I^A}$ and $\mathbf{I^B}$ are dominant over $\mathbf{I^O}$ but $\mathbf{I^A}$ or $\mathbf{I^B}$ are codominant.
- The $\mathbf{I^A}$ allele is responsible for the production of type **A** antigens in the cell membrane of red blood cells and the $\mathbf{I^B}$ allele for the production of type **B** antigens. The third allele in the series, $\mathbf{I^O}$, produces neither antigen
- Any one time, only two of the three alleles can be present in an individual
- Gene for the ABO blood group system is conventionally represented by the symbol **I**

Fig. 10 Antigens and genotypes of the A-B-O blood types

Blood type	Blood contains cellular antigens	Genotype
O	None 	I^OI^O
A	**A** 	I^AI^A, I^AI^O
B	**B** 	I^BI^B, I^BI^O
AB	**A** and **B** 	I^AI^B

- **Application:** Blood Transfusion

  * A person produces antibodies against foreign blood factors, causing clumping of RBCs if a transfusion is performed with incompatible blood

    E.g. C's blood group is A (I^AI^A)

    Antigen A found on RBC surface

    Antibody in serum is Anti-B

  * If the donor's blood has an antigen that is foreign to the recipient, specific proteins called <u>antibodies</u> produced by the recipient bind to the foreign molecules and cause the donor's blood cells to agglutinate (clump together). Recipient may die.

***Fig. 11* Results of mixing various blood groups**
Source: Biology (7th Edition) pp. 257

Let I^A represent the allele for production of type A antigen (codominant)

I^B represent the allele for production of type B antigen (codominant)

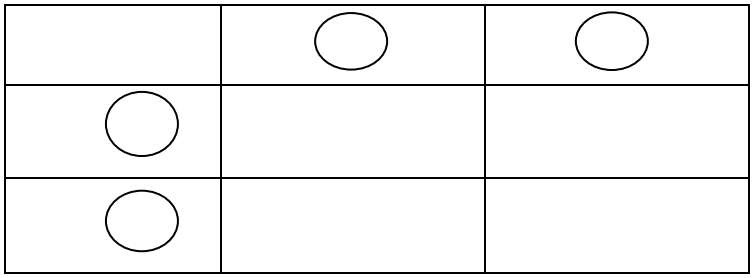
I^O represent the allele that produces neither antigen (recessive)

Parental phenotypes: blood group A x blood group B

Parental genotypes: I^AI^O x I^BI^O

Gametes produced by parents: I^A I^O **x** I^B I^O

Punnett Square to show the fusion of gametes:



F_1 phenotypic ratio: 1 blood group AB : 1 blood group A : 1 blood group B : 1 blood group O

Multiple alleles: Offspring may have genotypes differ from both parents

C. Lethal genes

- A gene that leads to the death of an individual; these can be either dominant or recessive in nature.
- The effects of a lethal gene are clearly illustrated by the inheritance of fur colour in mice: If two yellow mice are crossed with each other, the offspring produced is always in the ratio 2 yellow : 1 agouti fur.
- These results can be explained on the basis that yellow is dominant to agouti and that all the yellow coat mice are heterozygous.
- A cross between two heterozyotes would be expected to yield a monohybrid genotypic ratio of 1: 2: 1.

- However, if all the mice in one of the homozygous classes died before birth, the live births would then show a 2: 1 ratio of heterozygotes to the surviving homozygotes, i.e. fetal death of homozygous yellow coat (**YY**) mice.

Source:

http://sites.duke.edu/dukeresearch/2011/10/

Fig. 12 A yellow coat mouse and an agouti coat mouse

Let **Y** represent the allele for yellow fur (dominant)

y represent the allele for agouti fur (recessive)

Parental phenotypes: Yellow x Yellow

Parental genotypes: **Yy** x **Yy**

Gametes: (**Y**) (**y**) x (**Y**) (**y**)

Punnett Square to show the fusion of gametes:

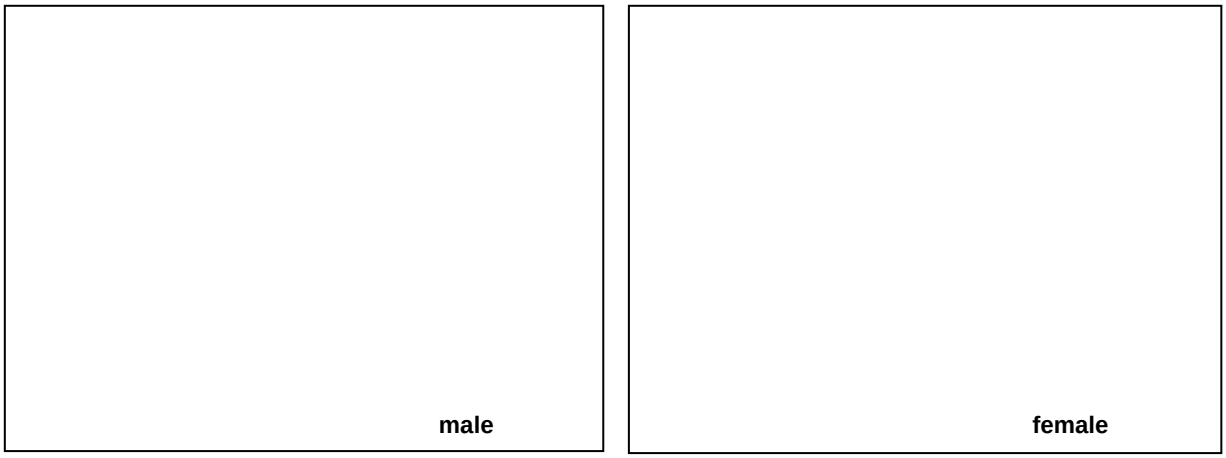
F_1 phenotypic ratio: 2 yellow fur : 1 agouti fur

IV. Sex Determination

- In humans there are twenty-three pairs of chromosomes.
- Of these, twenty-two pairs are identical in both sexes.
- The twenty-third pair, however, is different in the male from the female.

- The twenty-two identical pairs are called **autosomes** whereas the twenty-third pairs are referred to as **sex chromosomes** or **heterosomes**.

Human sex chromosomes



- In females, the two sex chromosomes are identical and are called **X chromosomes**.
- In males, an X chromosome is also present, but the other of the pair is smaller in size and called the **Y chromosome**.
- Unlike other features of an organism, chromosomes rather than genes determine sex.

Production of gametes in humans

As sexual reproduction can only occur between a male and a female, there is only one possible genetic cross:

- It can be seen that in humans the female produces gametes which all contain an X chromosome and are therefore the same. She is called the **homogametic sex** ('same gametes').
- The male, however, produces gametes of two genetic types: one which contains an X chromosome, the other a Y chromosome[1]. The male is called the **heterogametic sex** ('different gametes').

Sex determination in humans

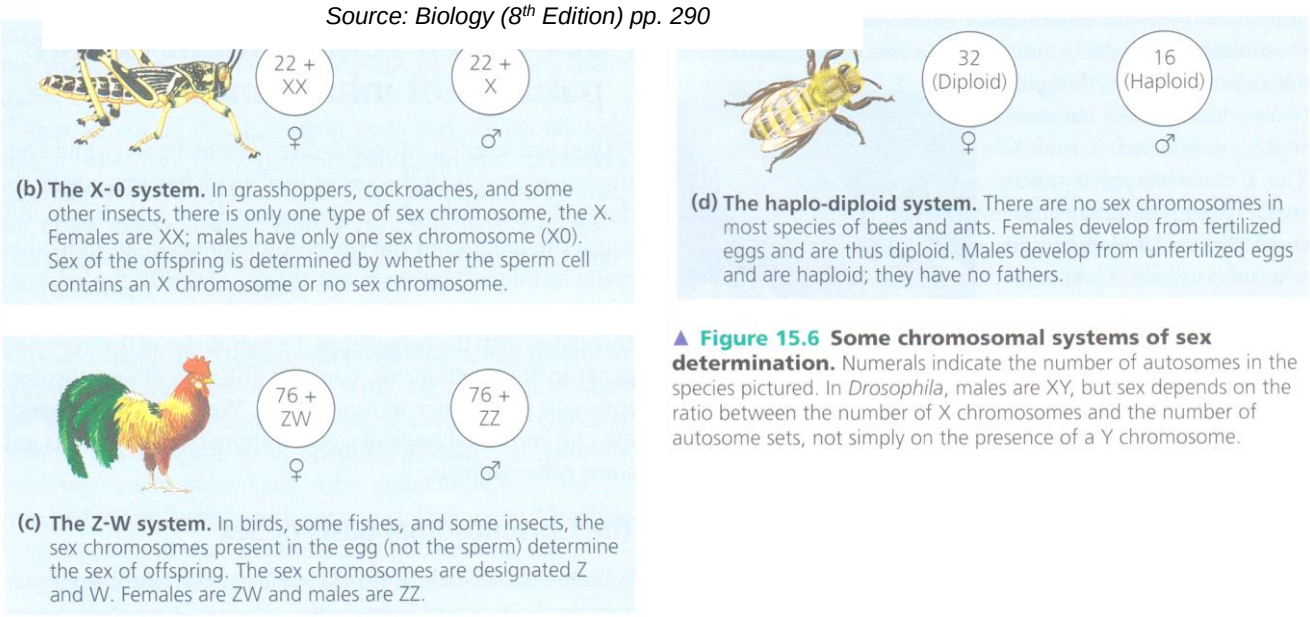
The sex of an offspring is determined by the sperm that fertilises the egg cell

Fig. 23 Process of fertilization and the possible types of zygotes

which codes for a product that acts on the undifferentiated gonads of the young embryo & causes them to differentiate into the testes. The undifferentiated gonads develop into ovaries in the absence of the product.

Fig. 24 Some chromosomal systems of sex determination

Source: Biology (8th Edition) pp. 290



▲ **Figure 15.6 Some chromosomal systems of sex determination.** Numerals indicate the number of autosomes in the species pictured. In *Drosophila*, males are XY, but sex depends on the ratio between the number of X chromosomes and the number of autosome sets, not simply on the presence of a Y chromosome.

A. Sex Linkage

- Sex linkage refers to the carrying of genes on the sex chromosomes.
- These genes determine body characteristics & have nothing to do with sex.
- The X chromosome carries many such genes, the Y chromosome has very few.
- Features linked on the Y chromosome will only arise in the heterogametic (XY) sex.
- Features linked on the X chromosome may arise in either sex.

Sex Linkage in X chromosome

- If the sex-linked condition is linked to the X chromosome, females will require the double recessive state for the condition to arise in them.
- As the recessive gene is relatively rare in the population, this is unlikely to occur.
- In females, the recessive gene is normally masked by the appropriate dominant gene which occurs on the other X chromosome.
- These heterozygous females are not themselves affected but are capable of passing the recessive gene to their offspring. Hence, such females are called **carriers.**
- However, any male receiving the recessive allele from his mother will express the trait.

Sex Linkage in Y chromosome

- When the recessive gene on the Y chromosome occurs in males, it expresses the trait.

1. Haemophilia

- Haemophilia refers to the reduced ability of blood to clot, due to deficiency of one of the blood clotting factors. Haemophilia is a sex-linked character and it is carried on the X chromosome.

Let **X^H** represent the X chromosome with the allele for normal blood clotting (dominant)
X^h represent the X chromosome with the allele for haemophilia (recessive)
Y represent the Y chromosome with neither allele

Scenario 1: Father is a haemophiliac

Parental phenotypes: normal female x haemophiliac male

Parental genotypes: **X^HX^H** x **X^hY**

Gametes produced by parents: ◯ x ◯ ◯

Punnett Square to show the fusion of gametes:

	◯	◯
◯ **X^H**	**X^H X^h** Normal carrier female	**X^HY** normal male

F_1 phenotypic ratio: 1 normal carrier female : 1 normal male

* All daughters will be carriers
* Sons will not have haemophilia

Scenario 2: Mother is a carrier

Parental phenotypes: carrier female x normal male

Parental genotypes: **X^HX^h** x **X^HY**

Gametes produced by parents: ◯ ◯ x ◯ ◯

Punnett Square to show the fusion of gametes:

	◯	◯
◯		
◯		

F_1 phenotypic ratio:

1 normal female : 1 normal carrier female : 1 normal male : 1 haemophiliac male

* 50% chance of each daughter carrying the haemophilia gene
* 50% chance of each son having haemophilia

	X^HX^H	**X^HX^h**	**X^hY**

Characteristics	normal blood clotting	(carrier) shows normal blood clotting but can transmit the allele for haemophilia to the offspring. X^h can be masked by X^H	X^h is sufficient to produce the disease because the Y chromosome does not carry an allele for normal blood clotting to mask the recessive allele
Occurrence	More common in males than in females		
Reason	Males need only 1 copy of X^h to suffer from haemophilia whereas females require 2 copies of the defective allele to be a sufferer. Female haemophiliac normally die of bleeding when they reach puberty		

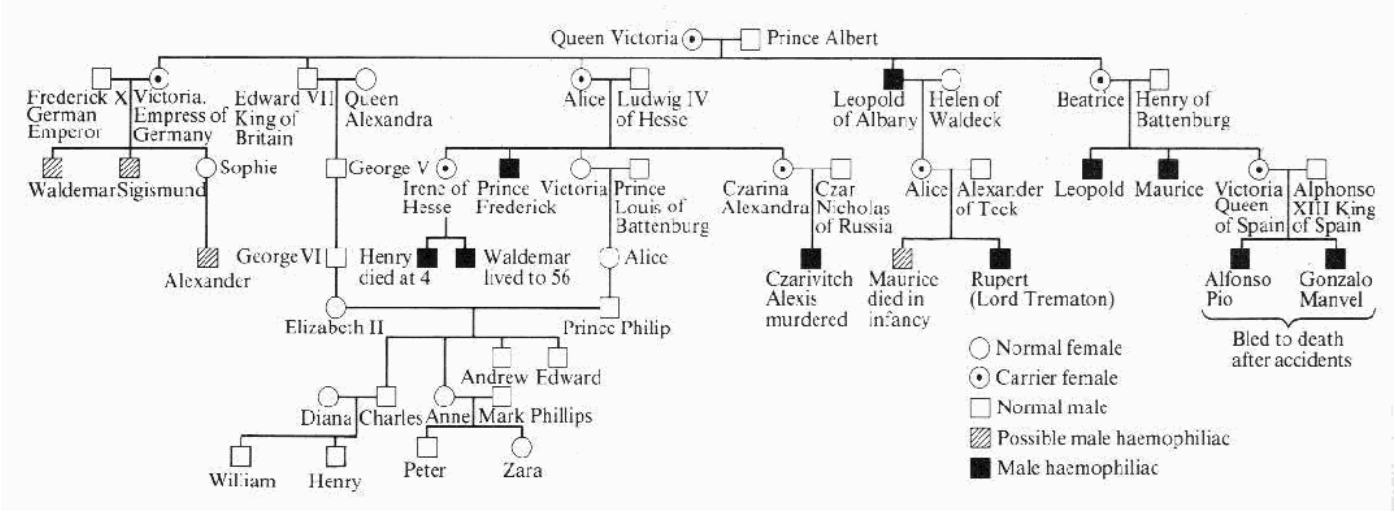


Fig. 25 Transmission of haemophilia in the descendants of Queen Victoria

In the diagram only those descendants involved in the transmission and appearance of haemophilia have been shown. The ancestry of the British Royal Family has been given to show why haemophilia is absent from seven generations of Queen Victoria's descendants. *Source: Biological Science 2 pp. 823*

2. Red-green colour blindness in humans

- Red-green colour blindness is the inability to distinguish between the colours red & green.

Let X^B represent the X chromosome with the allele for normal vision (dominant)
X^b represent the X chromosome with the allele for colour blindness (recessive)
Y represent the Y chromosome with neither allele

Parental phenotypes: normal female x colour blind male

Parental genotypes: X^BX^B x X^bY

Gametes produced by parents: (**X^B**) x (**X^b**) (**Y**)

Punnett Square to show the fusion of gametes:

	X^b	Y
X^B	$X^B X^b$ Normal carrier female	X^BY Normal male

F_1 phenotypic ratio: 1 normal carrier female : 1 normal male

The allele for colour blindness is passed from one sex to the other at each generation.

The father passes it to his daughters who thus become carriers.

The daughters may pass it to their sons who will thus be colour blind.

B. Reciprocal Cross

- Used to determine whether a characteristic is X-linked
- A cross where the same genetic features are used but the sexes are reversed. The results of the 2 crosses will be different.

Example: cross a red-eyed female [wild-type] with a white-eyed male and cross a red-eyed male [wild-type] with a white-eyed female

Investigation of sex-linked character in *Drosophila*

- To determine if the locus for eye colour in *Drosophila* is on the X chromosome, Morgan mated red-eyed female with a white-eyed male.

(a) Crossing a red-eyed female with a white-eyed male, followed by sibling mating of the F_1 generation

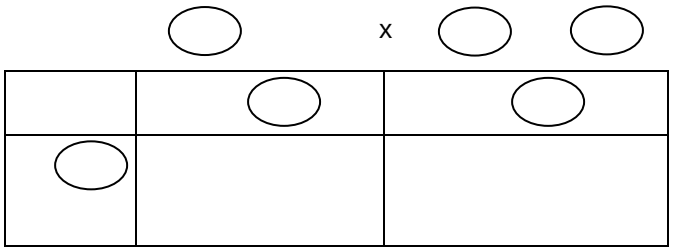
Let X^R represent the X chromosome with the allele for red eye (dominant)

X^r represent the X chromosome with the allele for white eye (recessive)

Y represent the Y chromosome with neither allele

Parental phenotypes: red-eyed female x white-eyed male

Parental genotypes: X^RX^R x X^rY

Gametes produced by parents: 

Crossing F_1 generation:

F_1 phenotypes: red-eyed female x red-eyed male

F_1 geneotype: X^RX^r x $X^R Y$

Gametes produced by F_1 generation: ( ) ( ) x ( ) ( )

	( )	( )
( )		
( )		

F_2 Phenotypic ratio: 2 red-eyed females : 1 red-eyed male : 1 white-eyed male

Conclusion:

- All F_1 offspring had red eyes, so the red eye trait was dominant over the white eye trait.
- The recessive trait – white eyes – was expressed only in males in the F_2 generation, the eye colour gene is located on the X chromosome and that there is no corresponding locus on the Y chromosome.
- If the eye colour gene were unrelated to sex, one would have expected half of the white-eyed flies to be male and half female.
- To verify his explanation and conclusions, a reciprocal cross was carried out where he mated red-eyed male with a white-eyed female:

(b) Crossing a red-eyed male with a white-eyed female, followed by sibling mating of the F_1 generation

Parental phenotypes: white-eyed female x red-eyed male

Parental genotypes: X^rX^r x X^RY

Gametes produced by parents: (X^r) x (X^R) (Y)

	(X^R)	(Y)
(X^r)	$X^R X^r$ red-eyed female	X^rY white-eyed male

Crossing F_1 generation:

F_1 phenotypes: red-eyed female x white-eyed male

F_1 genotype: X^RX^r x $X^r Y$

Gametes produced by F_1 generation: (X^R) (X^r) x (X^r) (Y)

	(X^r)	(Y)
(X^R)	$X^R X^r$ red-eyed female	X^RY red-eyed male

X^r	$X^r X^r$ white-eyed female	X^rY white-eyed male

F_2 Phenotypic ratio: 1 red-eyed female : 1 white-eyed female : 1 red-eyed male : 1 white-eyed male

Conclusion:

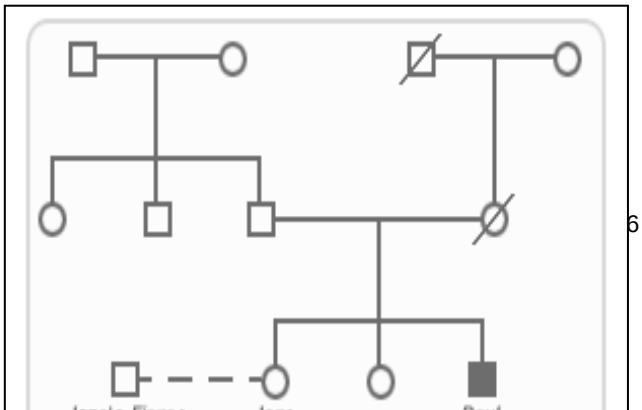
- The F_2 offspring included red-eyed and white-eyed males and females and the results for the original and the reciprocal cross are <u>different</u>.
- Morgan rightly concluded that only the X chromosome carries the gene for eye colour.
- This is because if the inheritance were not sex-linked, the sex of the parents would not have an effect on how the traits were inherited.

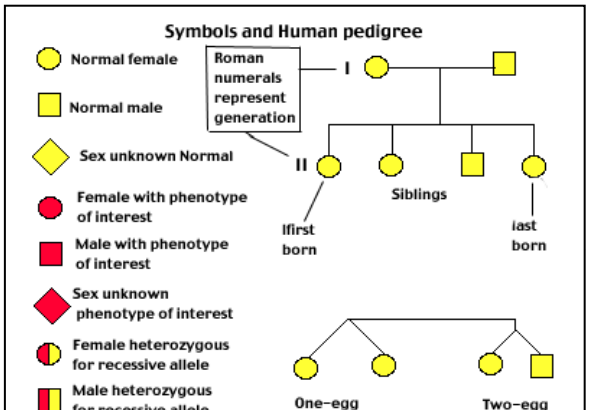
C. Human inheritance investigated by pedigree chart

- Pedigree is a chart of the ancestral history of a group of related individuals.
- Geneticist can use the information in the pedigree to deduce a model for the mode of inheritance of the trait.

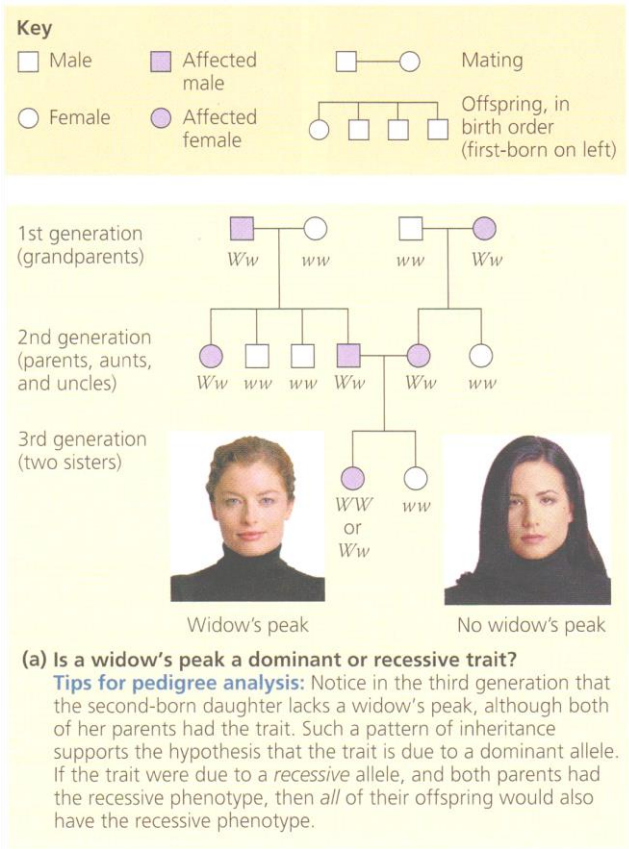
Autosomal dominant	Autosomal recessive	X-linked recessive	X-linked dominant
- Trait appears in every generation - Every affected individual has an affected parent - When one generation does not express the trait, the trait is lost and does not appear in future generations	- Trait is rare - Skipping of generations - Affected individual can have unaffected parents	- More males than females are affected - All the sons of an affected female will be affected - None of the sons of an affected male will be affected. But all his daughters will carry the allele, so ½ of their sons should be affected	- Traits does not skip generation - All daughter, but none of the sons, of an affected father are affected - Affected males must come from affected mother - Approximately half of the children of the affected heterozygote females are affected - Affected females come from affected mothers or fathers.

- Symbols used:





- Fig 24a shows three-generation pedigree that traces the occurrence of a pointed contour of the hairline on the forehead.
- Fig 24b is a pedigree of the same family, but this time we focus on a recessive trait, attached earlobes.



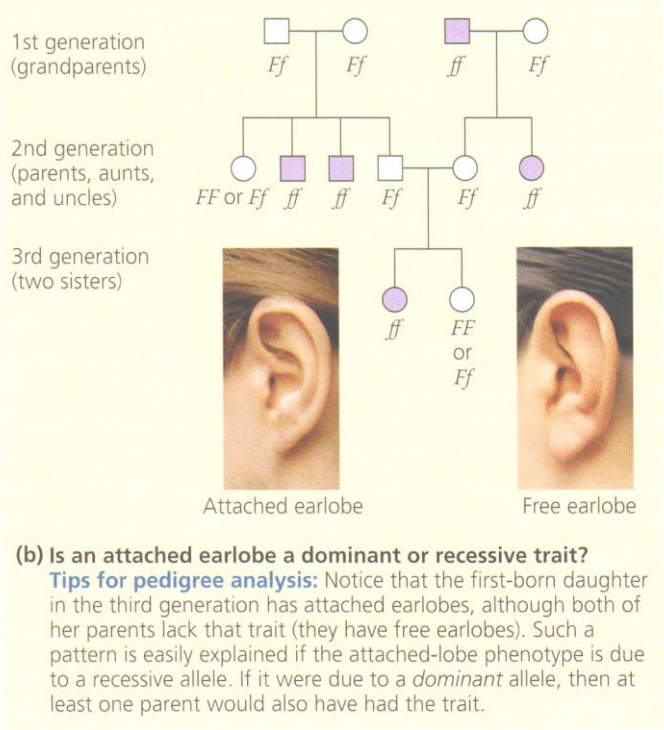


Fig. 26 Pedigree analysis

Source: Biology (8th Edition) pp. 276

V. Dihybrid Cross and the Principle of Independent Assortment

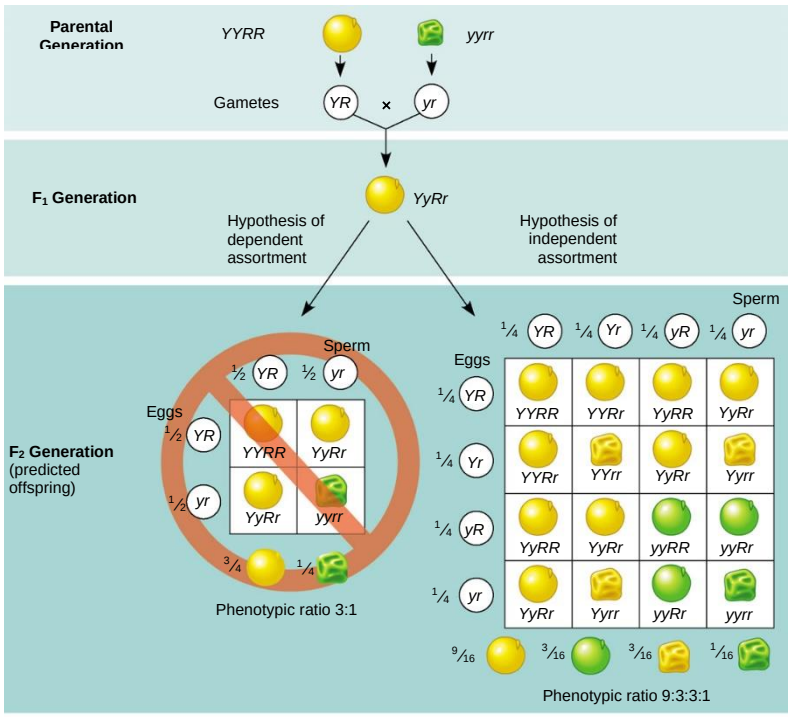
Dihybrid crosses

- Mendel also investigated the simultaneous inheritance of two pairs of contrasted characteristics in the garden pea. These investigations he called dihybrid crosses.
- Dihybrid cross is a cross between individuals involving alleles on two gene loci, each controlling a single character.

a) Mendel's experiment:

- Two true-breeding pea plants — one with yellow-round seeds and the other with green-wrinkled seeds — were crossed, producing dihybrid F_1 plants. Self-pollination of the F_1 dihybrids, which are heterozygous for both characters, produced the F_2 generation.

b) Mendel's observations:



315 108 101 32 Phenotypic ratio approximately 9:3:3:1

Results

- All the F_1 offspring had yellow and round seeds.
- When he crossed the F_1 plants, four different phenotypes were observed.

Yellow-round seeds	315
Yellow-wrinkled seeds	101
Green-round seeds	108
Green-wrinkled seeds	32

Fig. 13 Crossing yellow-round seed plant with green-wrinkled seed plant
Source: Biology (8th Edition) pp.268

- This gave an approximate ratio of 9:3:3:1.

- Mendel also noticed that two new combinations, not represented in the parents, appeared in the progeny: wrinkled and yellow, and round and green. Thus the two pairs of factors were inherited independently.

c) Mendel's Conclusion:

1. All the F_1 plants possess yellow and round, hence round shapes are dominant to wrinkled shapes & yellow colours are dominant to green colours.

2. All other phenotypes which disappear in the F_1 generation but reappear in the F_2 generation are recessive.

3. It can be seen from the Punnett square that there are 16 possible fusions. The observed ratio can be accounted for if all the possible fusions occur with equal likelihood.

4. The alleles of the 2 genes are transmitted independently of each other from parents to offspring & therefore assort freely.

   * i.e. **Y** and **R** alleles do not inherit together.

The Principle of independent assortment

On the basis of these results, Mendel formulated his Second Law or the Principle of Independent Assortment which states that:

During metaphase I, the orientation of the homologous chromosomes is random and independent of one another. During gamete formation, two alleles of homologous chromosomes are segregate into different gametes independently.

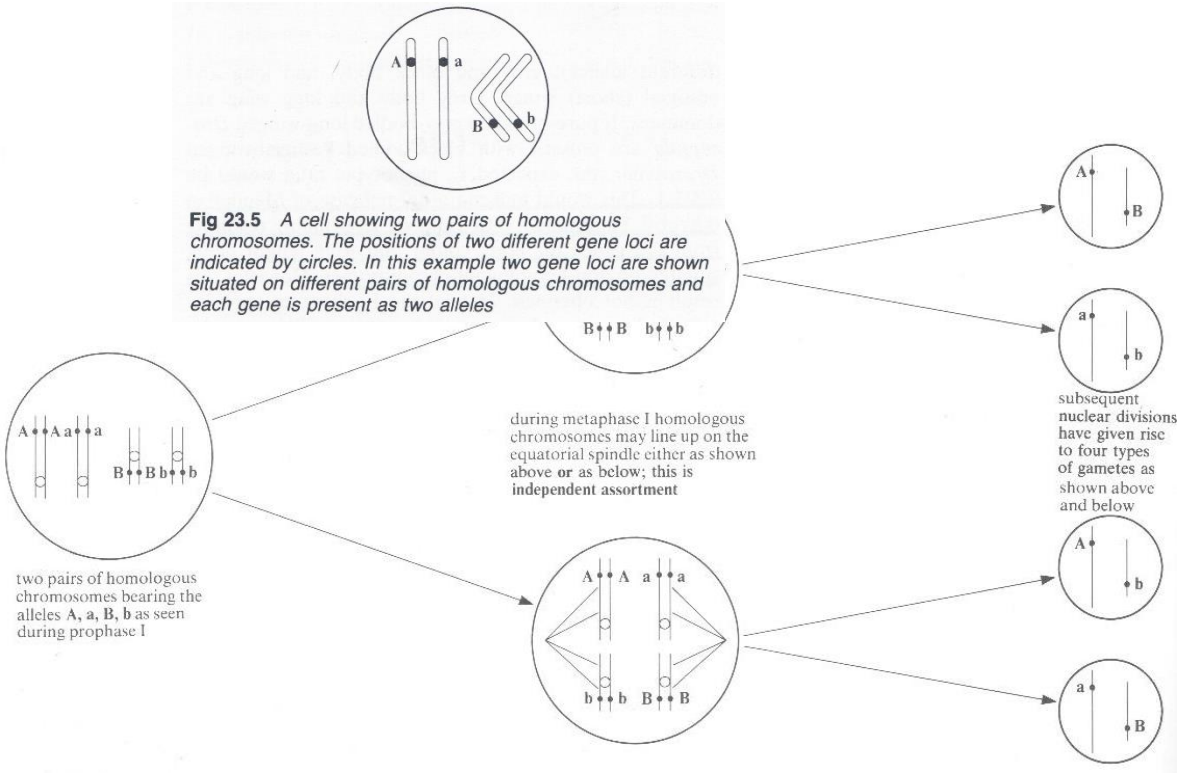


Fig. 14 Mendel's principal of independent assortment of alleles in terms of separation of homologous chromosomes during meiosis*. Source: Biological Science 2 pp. 815*

- Independent assortment requires that the genes concerned are carried on different chromosomes (i.e. genes are unlinked): alleles of the gene for seed colour are located on 1 pair of chromosomes & the alleles of the gene for seed shape on another pair of chromosomes
- Different pairs of homologous chromosomes behave independently of each other; the way 1 pair of homologous chromosomes arrange themselves on the spindle & subsequently separate has no effect whatsoever on the behaviour of any other pair of chromosomes
- The consequence of the independent behaviour of non-homologous chromosomes in meiosis is that 4 different types of gametes (**RY**, **Ry**, **rY**, **ry**) can be formed from a plant that is heterozygous for seed colour & shape (**RrYy**)

A. Representing Dihybrid Cross in Symbols

The investigation of the inheritance of two pairs of contrasted characteristics may be represented in terms of symbols:

Let **Y** represent the allele for yellow colour (dominant)

y represent the allele for green colour (recessive)

R represent the allele for round shape (dominant)

r represent the allele for wrinkled shape (recessive)

Parental phenotypes: yellow-round seed x green-wrinkled seed

Parental genotypes: **YYRR** x **yyrr**

Gametes produced by parents: (**YR**) x (**yr**)

F_1 genotypes: **YyRr**

F_1 phenotypes: yellow-round seeds

Selfing F_1 generation: **YyRr** x **YyRr**

Gametes produced by F_1 generation:

(**YR**) (**Yr**) x (**YR**) (**Yr**)

(**yR**) (**yr**) (**yR**) (**yr**)

Punnett Square to show the fusion of gametes produced by the F_1 generation:

	(**YR**)	(**Yr**)	(**yR**)	(**yr**)
(**YR**)	**YYRR**	**YYRr**	**YyRR**	**YyRr**

	Yellow-round	Yellow-round	Yellow-round	Yellow-round
Yr	**YYRr** Yellow-round	**YYrr** Yellow-wrinkled	**YyRr** Yellow-round	**Yyrr** Yellow-wrinkled
yR	**YyRR** Yellow-round	**YyRr** Yellow-round	**yyRR** Green-round	**yyRr** Green-round
yr	**YyRr** Yellow-round	**Yyrr** Yellow-wrinkled	**yyRr** Green-round	**yyrr** Green-wrinkled

F_2 phenotypic ratio: 9 yellow-round : 3 yellow-wrinkled : 3 green-round : 1 green-wrinkled

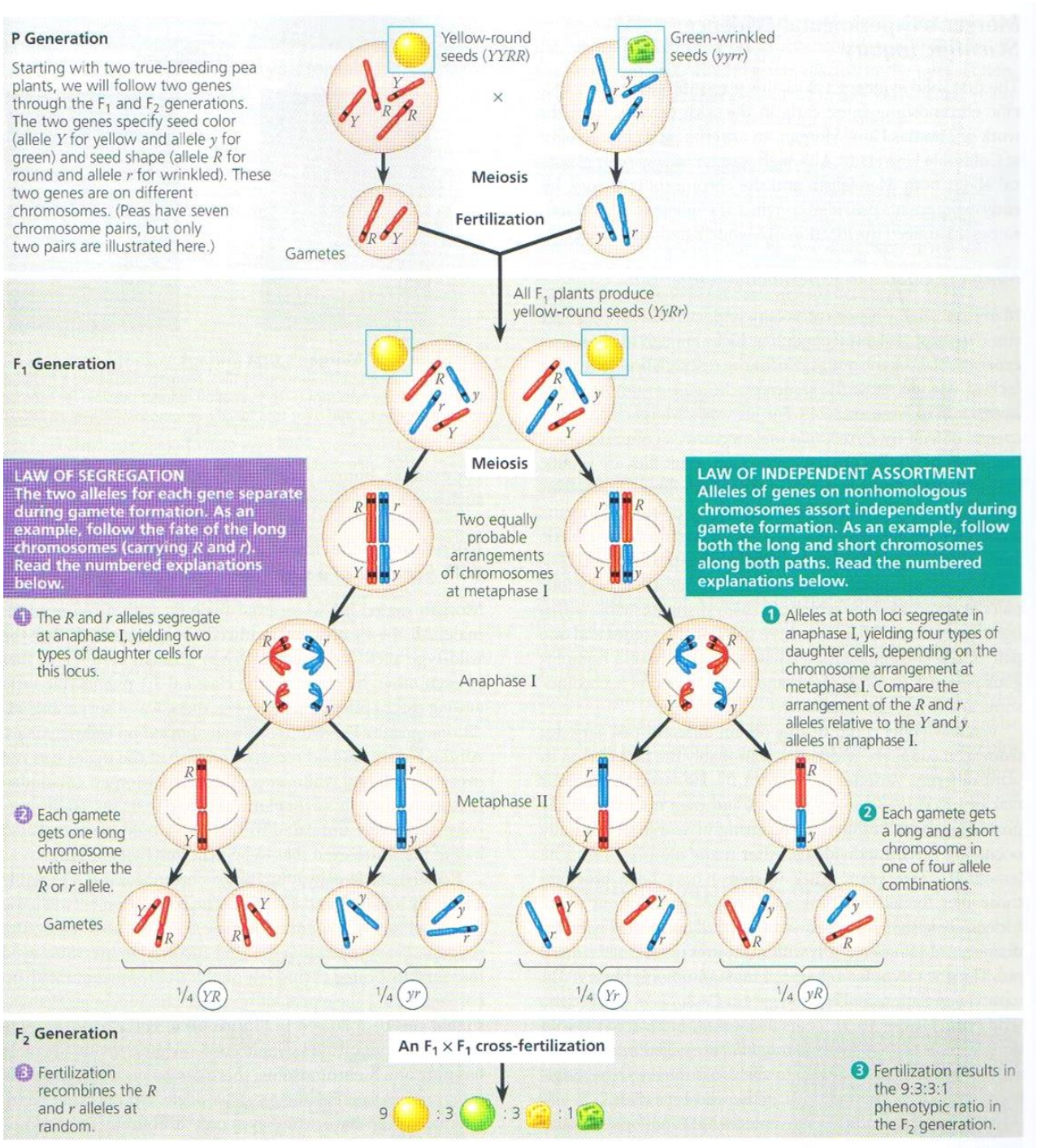


Fig. 15 Chromosomal basis of Mendel's laws *Source: Biology (8th Edition) pp.287*

The alleles for both genes segregate & assort independently because they are carried on separate chromosomes which themselves segregate & assort independently in meiosis.

B. Explanation of the 9:3:3:1 Mendelian dihybrid ratio in terms of probability

- From a consideration of monohybrid inheritance, where ¾ of the F_2 phenotypes show the dominant allele and ¼ the recessive allele, the probability of the four alleles appearing in any F_2 is as follows:

  yellow (dominant) – ¾

  round (dominant) – ¾

  green (recessive) – ¼

  wrinkled (recessive) – ¼

- Transmissions of 2 pairs of alleles are **independent events** & they **assort freely.** Hence the probability of the following combinations of alleles appearing in the F_2 phenotypes is as follows:

  yellow & round = ¾ x ¾ = 9/16

  yellow & wrinkled = ¾ x ¼ = 3/16

  green & round = ¼ x ¾ = 3/16

  green & wrinkled = ¼ x ¼ = 1/16

* Means that the chance of any F_2 seed, **chosen at random**, being both yellow & round is 9 out of 16

⇨ These figures agree with those obtained by the Punnett square method & with the results of Mendel's experiment

Lecture Practice 1

Let T =

t =

P =

p =

Parental phenotypes: Tall plants & purple flowers x Dwarf plants & white flowers

Parental genotypes:

Gametes produced by parents:

F_1 genotypes:

F_1 phenotypes:

Selfing F_1 generation:

Gametes produced by F_1 generation:

Punnett Square to show the fusion of gametes produced by the F_1 generation:

F_2 phenotypic ratio:

C. Testcross (Dihybrid)

- As seen from the Punnett square, the same phenotype may result from several different genotypes. e.g. a **yellow-round seed** may have 1 of 4 possible genotypes: _____

- How to establish the genotype of a yellow-round seed then?

  ➔ **Cross with a homozygous recessive (yyrr) seed**

Outcomes:

1. _____ x yyrr

	( )
yr	**YyRr** Yellow-round

Offspring phenotypic ratio: All yellow-round

2. _____x yyrr

	( )	( )
yr	**YyRr** Yellow-round	**Yyrr** Yellow-wrinkled

Offspring phenotypic ratio: 1 yellow-round : 1 yellow wrinkled

3. _____ x yyrr

	( )	( )
yr	**YyRr** Yellow-round	**yyRr** Green-round

Offspring phenotypic ratio: 1 yellow-round : 1 green-round

4. _____ x yyrr

	( )	( )	( )	( )
yr	**YyRr** Yellow-round	**Yyrr** Yellow-wrinkled	**yyRr** Green-round	**yyrr** Green-wrinkled

Offspring phenotypic ratio: 1 yellow-round: 1 yellow-wrinkled: 1 green-round: 1 green-wrinkled

Summary

Types of inheritance	**Phenotypic ratios in (heterozygote)**	
	F_2 generation	**Test cross on F_1 generation**
Monohybrid cross	3 : 1	1 : 1
Dihybrid cross (unlinked loci)	9 : 3 : 3 : 1	1 : 1 : 1 : 1

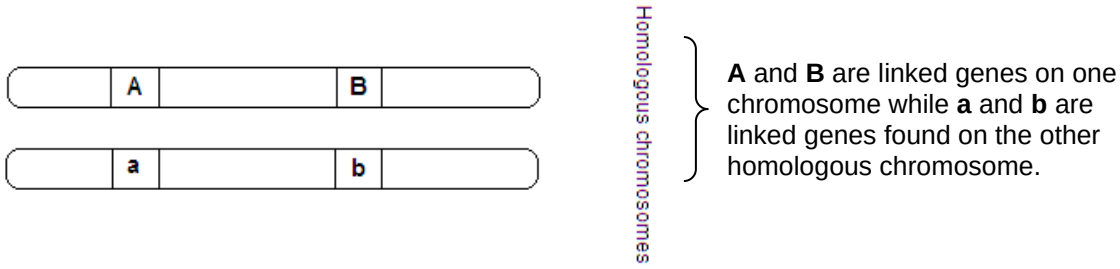
Incomplete dominance	1: 2 : 1	-
Codominance	1: 2 : 1	-

VI. Linkage

(f) Explain the meaning of the terms linkage and crossing-over and explain the effect of linkage and crossing-over on the phenotypic ratios from dihybrid crosses.

- Genes situated on the same chromosome are said to be **linked**.
- All genes on a single chromosome form a linkage group and usually pass into the same gamete and are inherited together.
- As a result of this, genes belonging to the same linkage group usually do not show independent assortment.
- Since these genes do not conform to Mendel's principle of independent assortment they fail to produce the expected F_2 phenotypic ratio of 9:3:3:1 in a breeding situation involving the inheritance of two pairs of contrasted characteristics (dihybrid inheritance).
    - Phenotypes with new gene combinations (recombinants) occur less frequently than the parental phenotypes.

Linked Genes
2 genes are on the same pair of homologous chromosomes



A and **B** are linked genes on one chromosome while **a** and **b** are linked genes found on the other homologous chromosome.

Definition of linkage:

Genes situated on the same chromosome

A. Morgan's *Drosophila* experiment – How linkage affects inheritance

To see how linkage between genes affects the inheritance of two different characters, let's examine Morgan's *Drosophila* experiments.

- In *Drosophila* the genes for body colour and wing length are determined by 2 alleles - grey (b^+) and black body (b); long (vg^+) and vestigial wings (vg).
- Grey body and long wings are dominant over black body and vestigial wings.
- Morgan mated pure-breeding grey-bodied long-winged *Drosophila* ($b^+ b^+ vg^+ vg^+$) with black-bodied vestigial-winged *Drosophila (b b vg vg)* to produce F_1 dihybrid ($b^+ b vg^+ vg$). He then crossed female dihybrid ($b^+ b vg^+ vg$) with males of black, vestigial winged ($b b vg vg$). (**Fig. 16**)

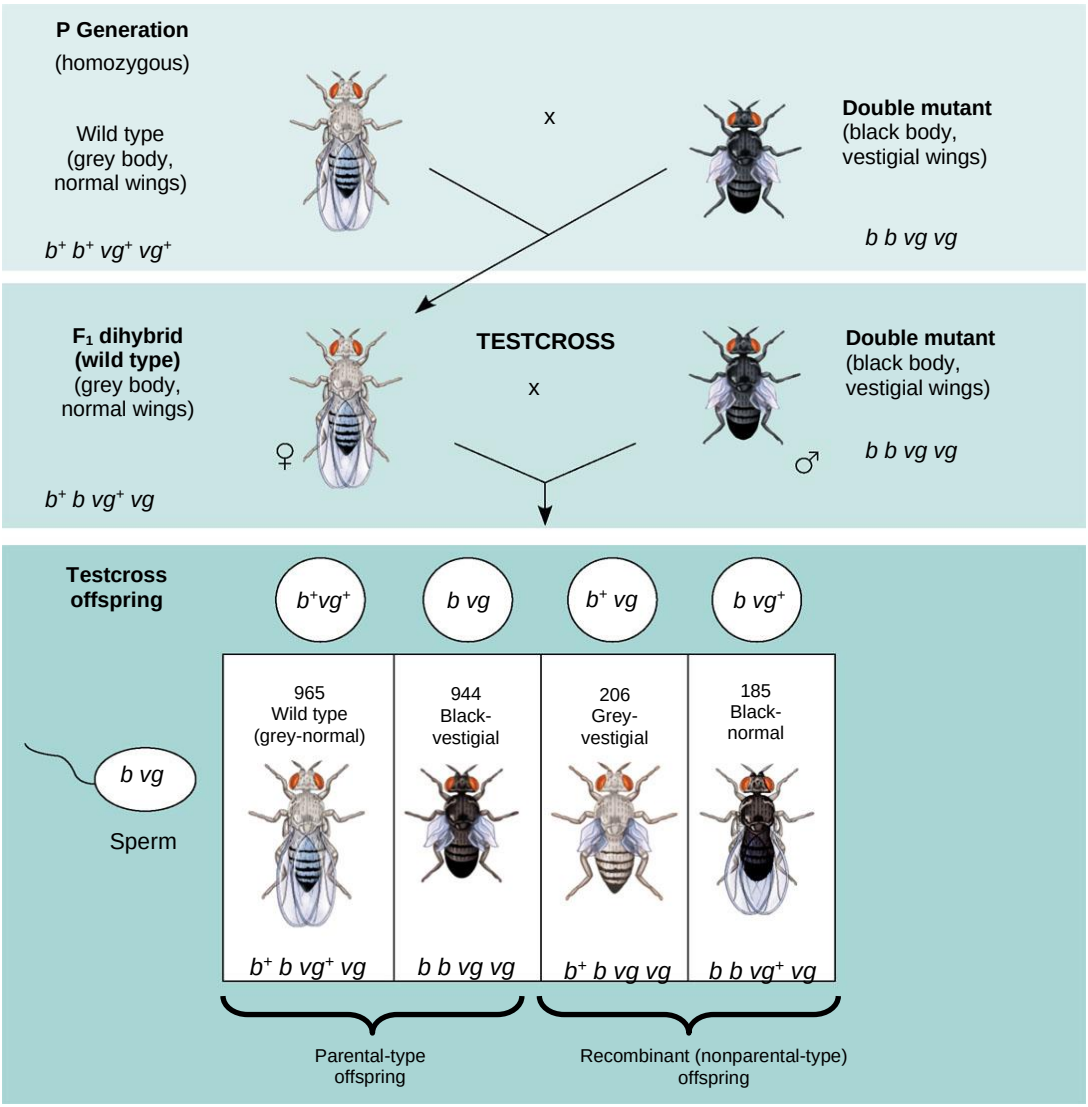


Fig. 20 How does linkage between two genes affect inheritance of characters?

Source: Biology (8th Edition) pp. 293

a) Prediction:

- This corresponds to a testcross. According to Mendel's law of independent assortment, the expected F_2 phenotypic ratio should be 1 grey-long winged : 1 black-vestigial winged : 1 grey-vestigial winged : 1 black-long winged.

b) Results:

- However, this result was not obtained. Instead the F_2 generation showed disproportionately large numbers of grey-long winged and black-vestigial wing flies among the offspring. These two phenotypes correspond to those of the P generation parents and to those of the parents in the testcross.
- The other two phenotypes (grey-vestigial and black normal) numbered fewer than expected based on independent assortment. In other words, the recombinants/ nonparental phenotypes occur less frequently than the parental phenotypes.

c) Explanation:

- Morgan reasoned that body colour and wing shape are usually inherited together in specific combinations because the genes for these characters are found on the same chromosome.
- The production of a relatively small number of offspring with nonparental phenotypes is due to crossing over. In crossing over, exchanges of segment between homologous chromosomes occasionally break the linkage between the two genes.
- **Fig. 21** shows how crossing over in a dihybrid female fly resulted in recombinant eggs and ultimately recombinant offspring in Morgan's testcross.

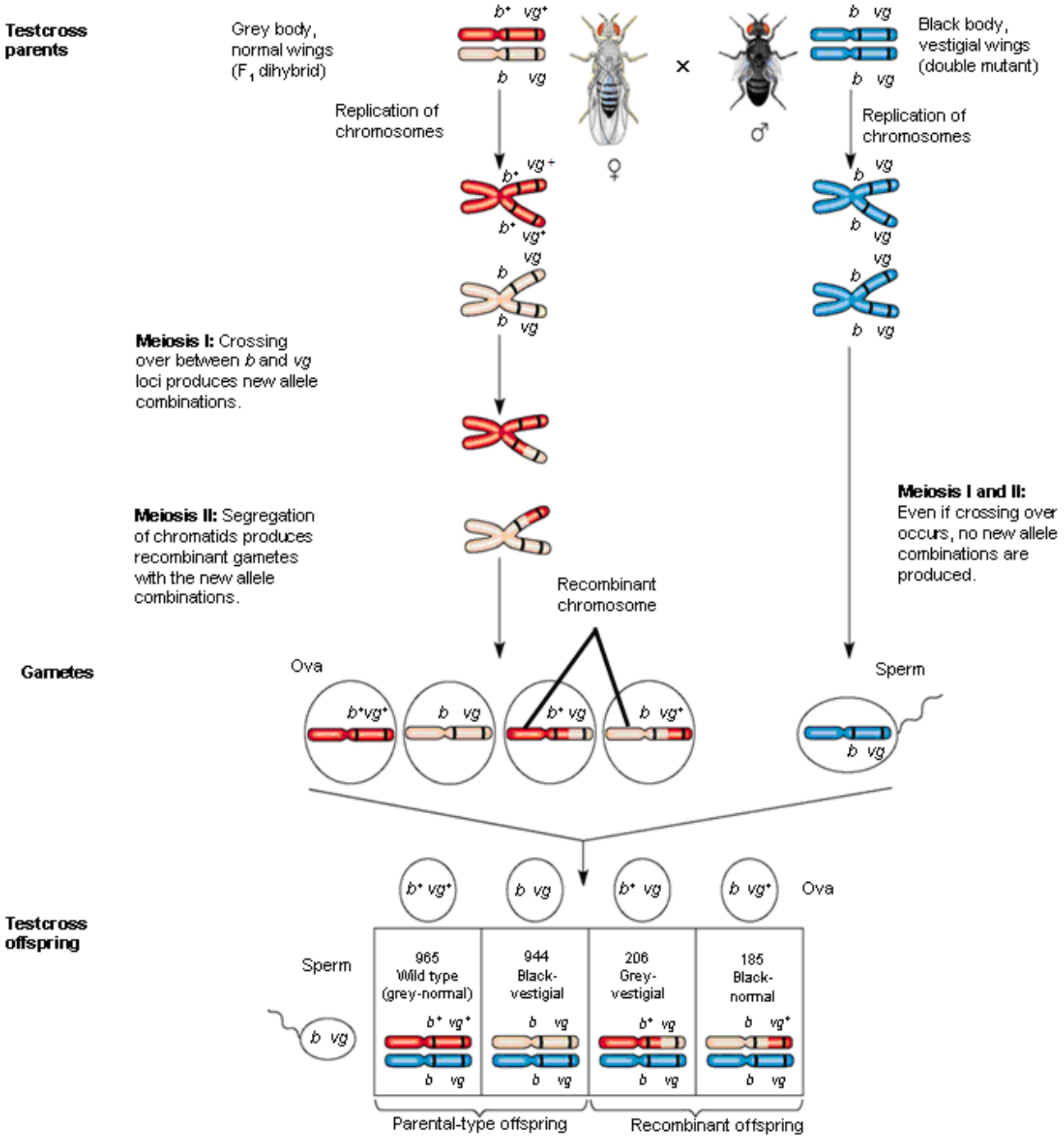


Fig. 21 Chromosomal basis for recombination of linked genes

Source: Biology (8th Edition) pp. 295

Genetic diagram

Let ***b*⁺** represent the allele for grey body (dominant)

b represent the allele for black body (recessive)

***vg*⁺** represent the allele for long wing (dominant)

vg represent the allele for vestigial wing (recessive)

Parental phenotypes: grey body, long wing x black body, vestigial wing

Parental genotypes: $\frac{b^+}{vg^+}\frac{b^+}{vg^+}$ x $\frac{b}{vg}\frac{b}{vg}$

Gametes produced by parents: $b^+ vg^+$ x $b\ vg$

F_1 genotypes: $\frac{b^+}{vg^+}\frac{b}{vg}$

F_1 phenotypes: grey body, long wing

Testcross: $\frac{b^+}{vg^+}\frac{b}{vg}$ x $\frac{b}{vg}\frac{b}{vg}$

Gametes produced: $b^+ vg^+$, $b^+ vg$, $b\ vg^+$, $b\ vg$ x $b\ vg$

Punnett Square to show the fusion of gametes produced:

	$b^+ vg^+$	$b\ vg$	$b^+\ vg$	$b\ vg^+$
$b\ vg$	$\frac{b^+}{vg+}\frac{b}{vg}$ grey body, long wing	$\frac{b}{vg}\frac{b}{vg}$ black body, vestigial wing	$\frac{b^+}{vg}\frac{b}{vg}$ grey body, vestigial wing	$\frac{b}{vg^+}\frac{b}{vg}$ black body, long wing
No. of offfspring	**965**	**944**	**206**	**185**

B. Types of linkage

- The phenomena of linkage is of two kinds:

1. Incomplete linkage

- The linked genes do not always stay together because homologous non-sister chromatids may exchange segments of varying length (which bearing many linked genes) with one another during meiotic prophase, by the process of crossing over. (**Fig. 22 (a) & (b))**
- The linked genes which are widely located in chromosomes and have chances of separation by crossing over are called incompletely linked genes and the phenomenon of their inheritance is called incomplete linkage.
- **Fig 15** is an example of incomplete linkage. The results of testcross showed most of the offspring had parental phenotypes and a small number of recombinants.

2. Complete linkage

- When the linked genes are so closely located in chromosomes that they inherit in same linkage groups for two or more generations in a continuous and regular fashion, then, they are called completely linked genes and the phenomenon of inheritance is called complete linkage. (**Fig. 16 (c)**)

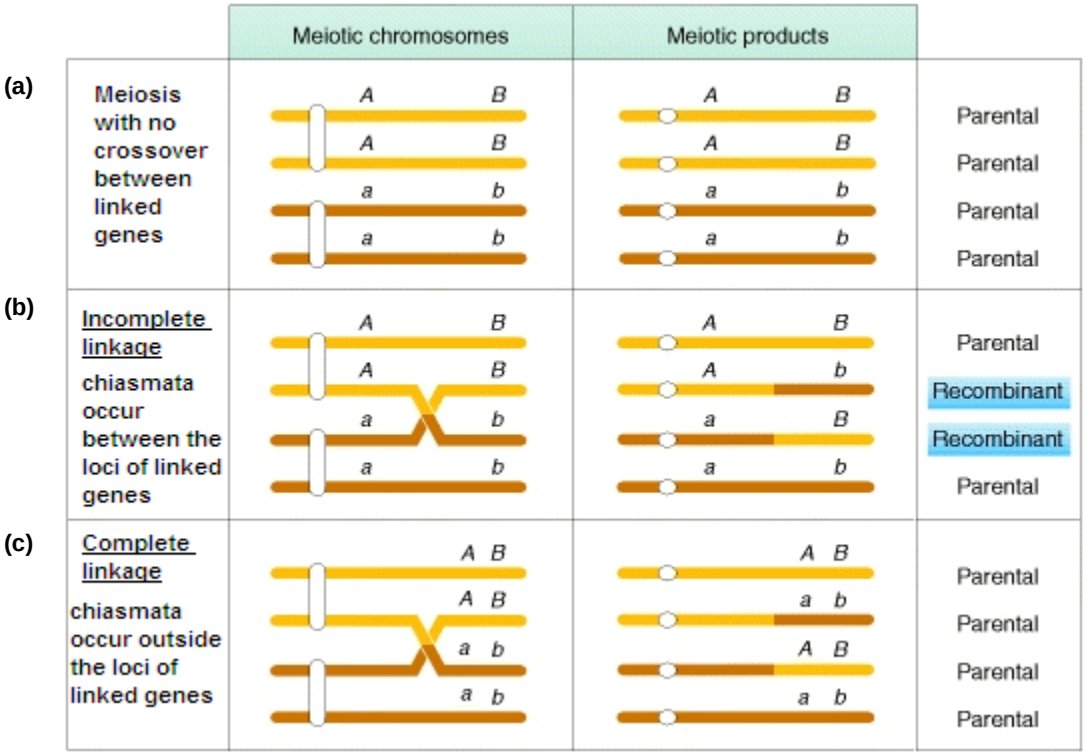


Fig.22 Recombinants arise from meiosis in which non-sister chromatids cross over between the genes under study

Source: Genetic Analysis (7th Ed) pp. 146

Genetic diagram

- If two genes are completely linked then F_2 ratio of 3:1:0:0 should be observed.

Let ***b*⁺** represent the allele for grey body (dominant)
b represent the allele for black body (recessive)
***vg*⁺** represent the allele for long wing (dominant)
vg represent the allele for vestigial wing (recessive)

Parental phenotypes: grey body, long wing x black body, vestigial wing

Parental genotypes: ***b*⁺ *b*⁺ / *vg*⁺ *vg*⁺** x ***b b / vg vg***

Gametes produced by parents: ***b*⁺ *vg*⁺** x ***b vg***

F_1 genotypes: ***b*⁺ *b* / *vg*⁺ *vg***

F_1 phenotypes: grey body, long wing

Interbreeding of F_1: ***b*⁺ *b* / *vg*⁺ *vg*** x ***b*⁺ *b* / *vg*⁺ *vg***

Gametes produced:

Punnett Square to show the fusion of gametes produced:

F_2 phenotypic ratio: 3 grey body, long wing : 1 black body, vestigial wing

Genetic diagram

- If two genes are completely linked, the **backcross** between heterozygous grey-bodied long winged *Drosophila* and homozygous recessive black-bodied vestigial-winged *Drosophila*, ratio of 1:1:0:0 would be produced.

Let b^+ represent the allele for grey body (dominant)

b represent the allele for black body (recessive)

vg^+ represent the allele for long wing (dominant)

vg represent the allele for vestigial wing (recessive)

Parental phenotypes: grey body, long wing x black body, vestigial wing

Parental genotypes: $\frac{b^+}{vg^+}\frac{b^+}{vg^+}$ x $\frac{b}{b}\frac{vg}{vg}$

Gametes produced by parents: b^+ vg^+ x b vg

F_1 genotypes: $\frac{b^+}{vg^+}\frac{b}{vg}$

F_1 phenotypes: grey body, long wing

Testcross: $\frac{b^+}{vg^+}\frac{b}{vg}$ x $\frac{b}{vg}\frac{b}{vg}$

Gametes produced:

Punnett Square to show the fusion of gametes produced:

F_2 phenotypic ratio: 1 grey body, long wing : 1 black body, vestigial wing

C. Genetic mapping and crossover values

- The discovery of linked genes and recombination due to crossing over led to a method for constructing a genetic map - an ordered list of the genetic loci along a particular chromosome.
- The distances between genes on a chromosome can be determined by the recombination frequency or crossover frequency (crossover value (COV)).
- Recombination frequency reflects the relative positions of gene on chromosomes because the further apart linked genes are on the chromosomes, the higher the probability that a crossing-over will occur between them and therefore the higher the recombination frequency.
- Recombination frequency is calculated using the formula:

$$\textbf{Recombination frequency} = \frac{\textbf{Number of individuals showing recombination}}{\textbf{Number of offspring}} \times 100$$

- Using the figures obtained from the cross in Fig 15.10, the recombinant frequency (%) is:

Parental phenotypes	grey body, long wing	965
	black body, vestigial wing	944
Recombinant phenotypes	black body, long wing	206
	grey body, vestigial wing	185

$$\frac{(206+185)}{(965+944)+(206+185)} \times 100$$

$$= \frac{391}{2300} \times 100$$

$$= 17\%$$

- A genetic map is constructed by directly converting the recombinant frequency between genes into hypothetical distances along the chromosome.
  - * A genetic map based on recombination frequencies is called a linkage map.
- A linkage map shows the relative locations of genes along a chromosome.
- By convention, one percent recombinant frequency is equivalent to one map unit or called centimorgans. That means the distance between the genes for body colour and wing length is 17 map unit/centimorgans apart on the same chromosome.
- The recombinant frequency demonstrated that genes are arranged linearly along the chromosome.
  - * The frequency of recombination can have a maximum value of 50%, a result indistinguishable from that for genes on different chromosomes.
- **Fig. 23** below illustrates the method for determining the linear order of genes in a chromosome.

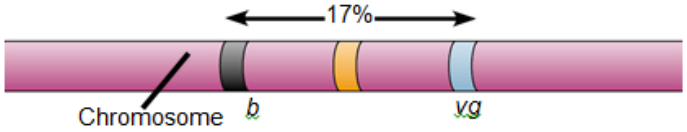


Fig. 23 Constructing a linkage map
Source: Biology (8th Edition) pp. 296

VII. Gene Interaction

- The presence of a pair of alleles occupying a given locus and controlling the production of a single phenotypic characteristic is true in some cases only and exceptional in most organisms.
- Most characteristics are determined by the interaction of several genes. For example, a single characteristic may be controlled by the interaction of two or more genes situated at different loci
- Different types of interactions occur:
  a. Different genes control the same general characteristic, collectively producing a **new phenotype**
  b. One gene masks the expression of others and alters the phenotype – **Epistasis**

A. Gene interaction producing new phenotype

- In the case of the inheritance of the shape of the comb in domestic fowl there are genes at two loci situated on different chromosomes which interact and give rise to four distinct phenotypes, known as pea, rose, walnut and single combs.
- The appearance of pea comb and rose comb are each determined by the presence of their respective dominant allele (**P** or **R**) and the absence of the other dominant allele. Walnut comb results from a modified form of codominance in which at least one dominant allele for pea comb and rose comb is present that is (**P_R_**). Single comb appears only in the homozygous double recessive condition (**pprr**).
- The F_2 genotypes and F_2 phenotypic ratios resulting from crossing a pure-breeding pea-comb hen with a pure-breeding rose-comb cock are shown below.

Let **P** represent presence of pea comb (dominant)

p represent absence of pea comb (recessive)

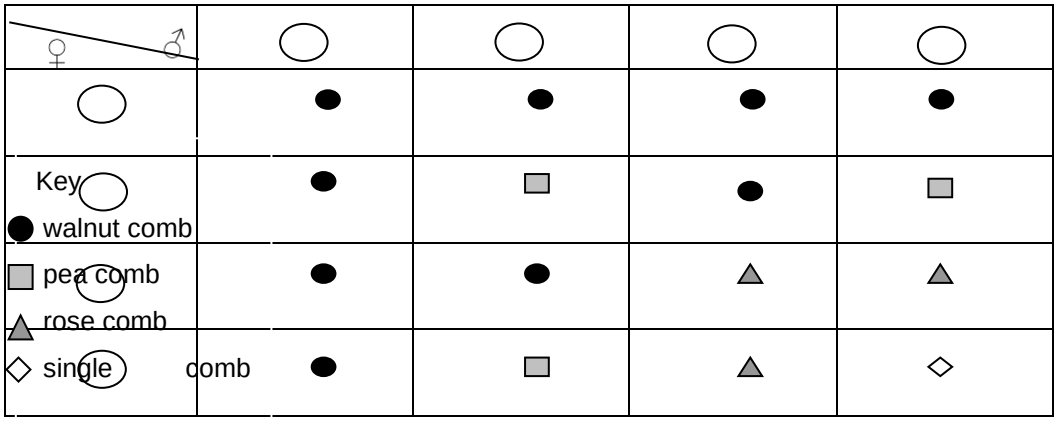
R represent presence of rose comb (dominant)

r represent absence of rose comb (recessive)

Parental phenotypes:	pea comb	x	rose comb
Parental genotypes:	**PPrr**	x	**ppRR**
Gametes produced by parents:	**Pr**	x	**pR**
F_1 genotypes:	**PpRr**		
F_1 phenotypes:	All walnut comb		
Interbreeding F_1 generation:	**PpRr**	x	**PpRr**

Gametes produced by F_1 generation:

Punnett Square to show the fusion of gametes produced:



F_2 phenotypic ratio: 9 walnut comb : 3 pea comb : 3 rose comb : 1 single comb

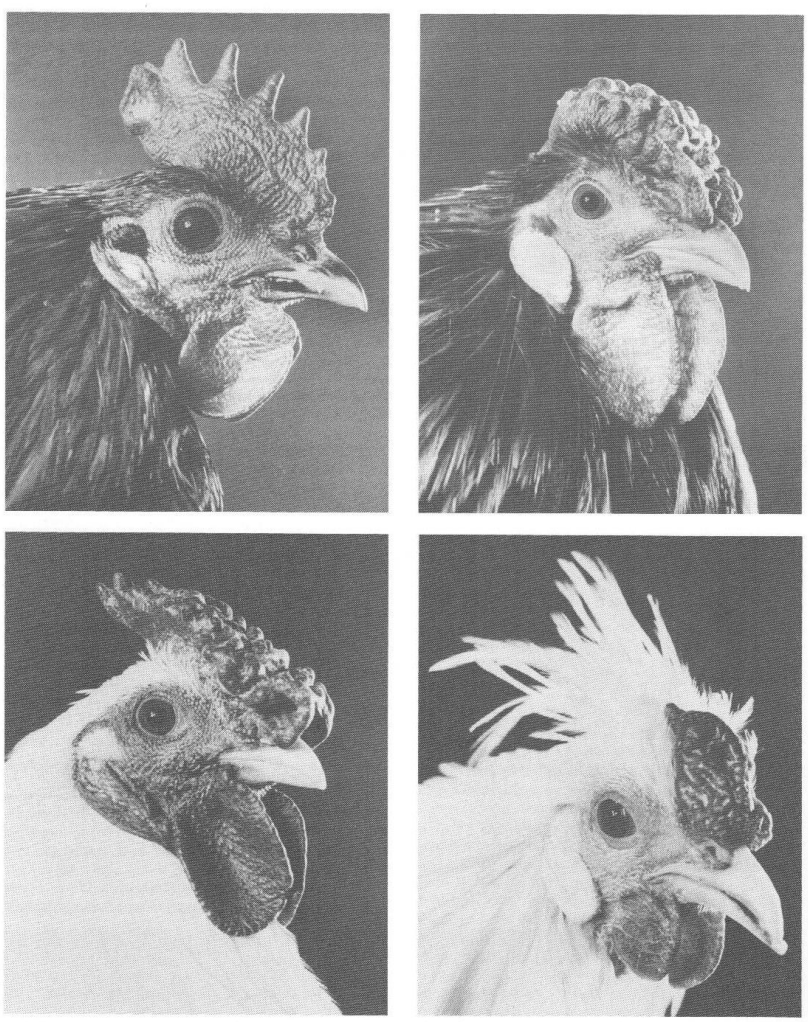


Fig 23.26 *Variation in comb shape in domestic fowl (top left) single comb, (top right) pea comb, (bottom left) rose comb, (bottom right) strawberry comb*

Fig.20 Variation in comb shape of domestic fowl. Top left: single comb, top right: pea comb, bottom left: rose comb, bottom right: strawberry comb *Source: Biological Science 2 (7th Ed) pp. 848*

B. Epistasis

- This is defined as an interaction between two different genes so that an allele of one of them (the **epistatic** gene) "masks" or inhibits the phenotypic expression of the other (the **hypostatic** gene).
    - *Epistasis should not be confused with dominance. Epistasis is the interaction between <u>different genes</u> (non alleles). Dominance is the interaction between <u>different alleles of the same gene</u>.*
- When epistasis is operative between two gene loci, the number of phenotypes appearing in the offspring from dihybrid parents will be <u>less than four</u>. Usually there are only <u>two or three phenotypes</u> resulting from various combinations of the genotypic classes.
- The F_2 ratio is a modification of the 9:3:3:1 ratio, produced by grouping various components of the ratio.

B1. Dominant Epistasis (12:3:1)

- **Dominant epistasis** takes place when a dominant allele hides a phenotype specified by another gene.

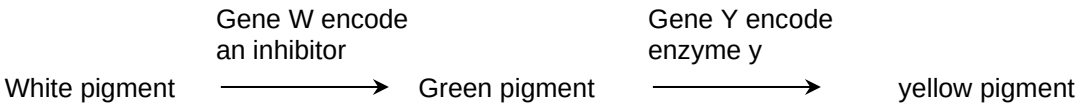
- When the dominant allele at one locus, for example the **A** allele, produces a certain phenotype regardless of the allelic condition of the other locus, then the **A**-locus is said to be epistatic to the **B**-locus. Furthermore, since the dominant allele **A** is able to express itself in the presence of either **B** or **b**, this is a case of dominant epistasis.
- Only when the genotype of the individual is homozygous recessive at the epistatic locus (**aa**) can the alleles of the hypostatic locus (**B** or **b**) be expressed. Thus, the genotypes **A_B_** and **A_bb** produce the same phenotype, whereas **aaB_** and **aabb** produce two additional phenotypes. The classical 9:3:3:1 ratio becomes modified into a 12:3:1 ratio.

Example: Fruit colour

- In summer squash, two genes influence the colour of the fruit.
- With one gene, the dominant allele (**W_**) produces white, while **ww** fruit will be coloured (may be either yellow or green), depending on the genotype of the second gene.
- A second gene's dominant allele (**Y_**) determines yellow while homozygous for the recessive allele (**yy**) are green.
- In the interaction between these two genes, the presence of allele **W** hides the effects of either **Y_** or **yy**, producing white fruit.
- The recessive **w** allele has no effect on fruit colour, the fruit color is determined by the **Yy** gene.
- Thus it is a <u>dominant epistasis</u> because with a single copy of the dominant epistatic **W** allele is sufficient to inhibit pigment production.
- **W_** is thus epistatic to any genotype of the **Yy** gene.
- In a self-cross of white F_1 dihybrids (**WwYy**), the F_2 phenotypic ratio resulting from this type of dominant epistasis is <u>12 white : 3 yellow : 1 green</u>

<u>Biochemical Basis</u>

- The probable biochemical pathway involved:



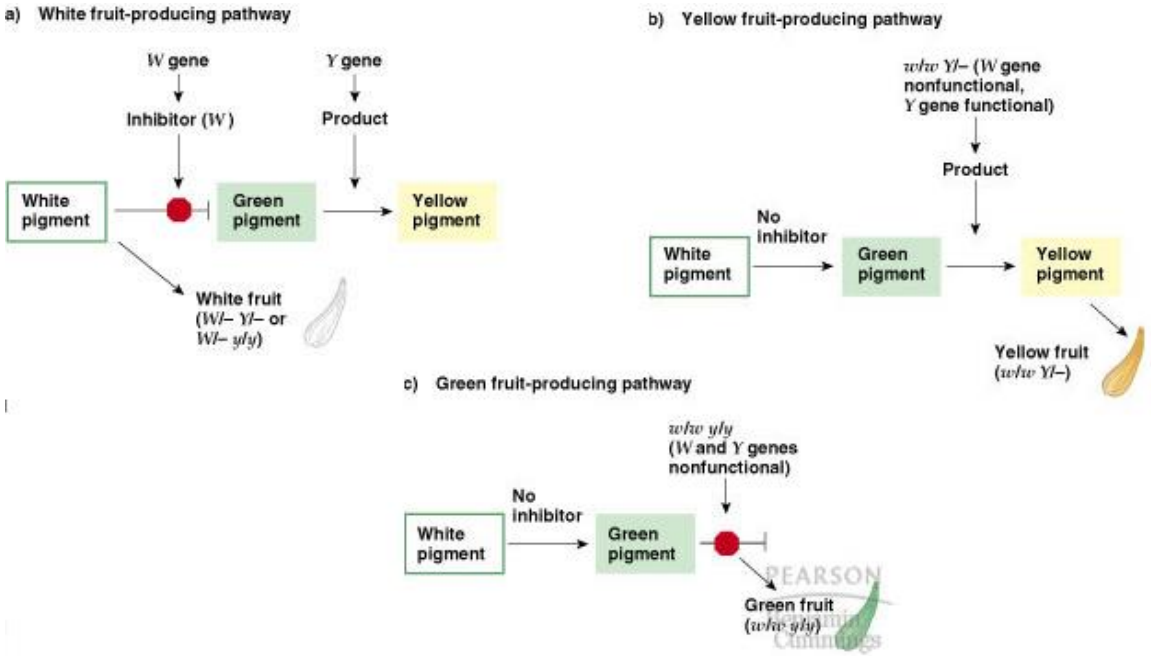


Fig. 21 Three different pathways determining the colour of fruit

Let **W** represent dominant allele that inhibits pigment production (epistatic)

w r**e**present recessive allele for pigment production (epistatic)

Y represent dominant allele for yellow pigment (hypostatic)

y represent recessive allele for green pigment (hypostatic)

Parental phenotypes:	White fruit	x	Green fruit
Parental genotypes:	**WWYY**	x	**wwyy**
Gametes produced by parents:	**(WY)**		**(wy)**
F_1 genotypes:	**WwYy**		
F_1 phenotypes:	All white		
Selfing F_1 generation:	**WwYy**	**x**	**WwYy**
Gametes produced	◯ ◯ ◯ ◯	x	◯ ◯ ◯ ◯

Punnett Square to show the fusion of gametes produced:

Key white fruit yellow fruit green fruit				

F_2 *phenotypic ratio:* 12 white : 3 yellow : 1 green

- In the F_2, 12/16 or ¾ of the F_2 plants produce white fruit and 3/16 + 1/16 = 4/16 = ¼ of the F_2 plants produce coloured fruit. The 3:1 ratio produced by a cross between two heterozygous individuals suggests that a dominant allele at one locus inhibits the production of pigment, resulting in white progeny.
- Among those F_2 plants with coloured fruit, a 3:1 ratio of 3/16 yellow and 1/16 green is observed. This is because a second locus determines the type of pigment produced in the fruit, with yellow (**Y**_) dominant over green (**yy**).

B2. Recessive Epistasis (9:3:4)

- **Recessive epistasis** takes place when a recessive allele masks the expression of other genes.
- If the homozygous recessive genotype is at one locus (e.g. **aa**) suppresses the expression of alleles at the **B**-locus, the **A**-locus is said to exhibit recessive epistasis over the **B**-locus. Only if the dominant allele is present at the **A**-locus can the alleles of the hypostatic B-locus be expressed.

Example 1: Fur colour in mice

- Fur colour in mice is controlled by a pair of genes occupying different loci.
- The epistatic gene determines the presence of colour (the gene is required for the synthesis of hair pigment) and has two alleles, coloured (dominant, **C** allele) and albino/white (recessive, **c** allele).
  - ⇨ Individuals with the genotype **CC** and **Cc** being dominant to non-expression of colour (**cc**)
- The hypostatic gene determines the nature of the colour and its two alleles are agouti (dominant, **A** allele) and black (recessive, **a** allele).
  - ⇨ Individuals with the genotype **AA** or **Aa** are agouti.
  - ⇨ Individuals with the genotype **aa** have black fur.
- The mice may have agouti or black fur depending upon their genotypes, but this will only appear if accompanied by the allele for coloured fur (**C**_).
- The albino condition appears in mice that are homozygous recessive for colour (**cc**) even if the alleles for agouti (**A**_) and black fur (**aa**) are present.
- Thus, it is a <u>recessive epistasis</u> because two copies of the recessive epistatic allele **c** must be present to mask the agouti and black pigments.
- **cc** is epistatic to **A** and **a** alleles
- Three possible phenotypes can occur and they are agouti, black and albino.

<u>Biochemical Basis</u>

- The probable biochemical pathway involved:

Precursor $\xrightarrow{\text{Gene C encode enzyme C}}$ Pigment precursor $\xrightarrow{\text{Gene A encode enzyme A}}$ Grey pigment

(white) (black) (agouti)

What is the expected phenotypic ratio if mating occurs between two agouti mice that are heterozygous for both genes (AaCc)?

Let **C** represent dominant allele for coloured fur (epistatic)
c represent recessive allele for albino fur (epistatic)
A represent dominant allele for agouti fur (hypostatic)
a represent recessive allele for black fur (hypostatic)

Parental phenotypes: Agouti x albino
Parental genotypes: **CCAA** x **ccaa**
Gametes produced by parents: (**CA**) x (**ca**)

F_1 genotypes: **CcAa**

F_1 phenotypes: All agouti

Interbreeding F_1 generation: **CcAa** x **CcAa**
Gametes produced: (**CA**) (**cA**) (**Ca**) (**ca**) x (**CA**) (**cA**) (**Ca**) (**ca**)

Punnett Square to show the fusion of gametes produced:

♀ \ ♂	(**CA**)	(**cA**)	(**Ca**)	(**ca**)
(**CA**)	**CCAA** Agouti ●	**CcAA** Agouti ●	**CCAa** Agouti ●	**CcAa** Agouti ●
(**cA**)	**CcAA** Agouti ●	**ccAA** Albino □	**CcAa** Agouti ●	**ccAa** Albino □
(**Ca**)	**CCAa** Agouti ●	**CcAa** Agouti ●	**CCaa** Black △	**Ccaa** Black △
(**ca**)	**CcAa** Agouti ●	**ccAa** albino □	**Ccaa** Black △	**ccaa** Albino □

Key
● agouti
△ black
□ albino

F_2 phenotypic ratio: 9 Agouti : 3 Black : 4 Albino

Example 2: Flower colour in blue-eyed Mary (*Collinsia parviflora*)

- The pathway for the production of colour pigment in blue-eyed Mary (*Collinsia parviflora*) is as follows:

$$\text{colorless} \xrightarrow{\text{gene } w^+} \text{magenta} \xrightarrow{\text{gene } m^+} \text{blue}$$

- The ***w*** and ***m*** genes are not linked. The ***w*** allele is epistatic on ***m*⁺** and ***m***. conversely, ***m*⁺** and ***m*** can be expressed only in the presence of ***w*⁺**.

- If homozygous white and magenta plants are crossed, the F_1 and F_2 are as follows:

w w m⁺ m⁺ (white) x ***w⁺ w⁺ m m*** (magenta)

↓

F_1 ***w⁺ w m⁺ m*** (blue)

w⁺ w m⁺ m x ***w⁺ w m⁺ m***

↓

F_2	9 ***w⁺_ m⁺_*** (blue)	9
	3 ***w⁺_ m m*** (magenta)	3
	3 ***w w m⁺_*** (white)	4
	1 ***w w m m*** (white)	

Biochemical Basis

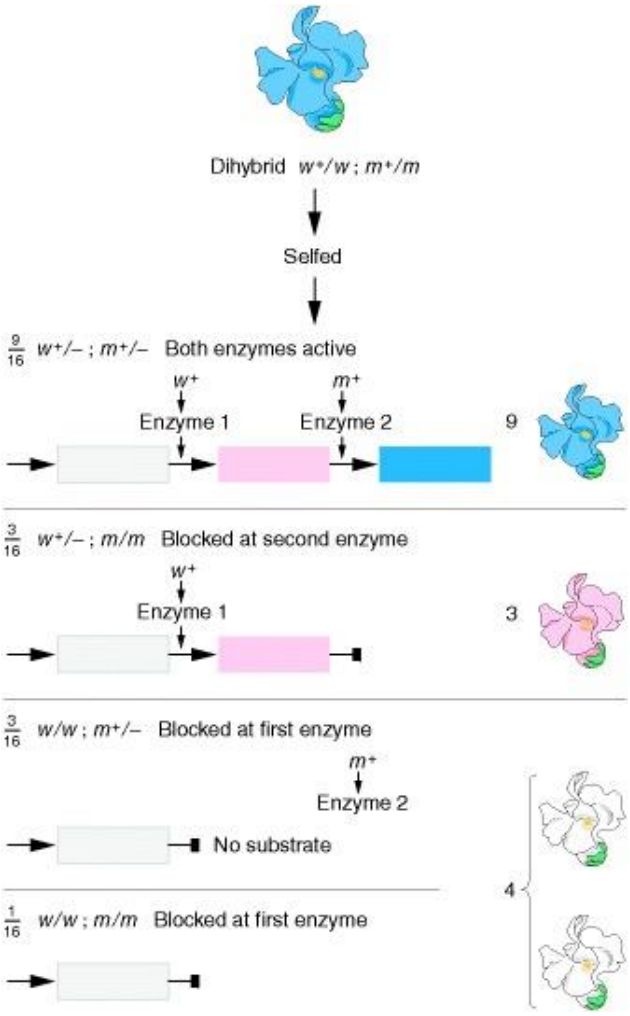


Fig. 22 Different pathways determining the flower colour.

Source: Genetic Analysis (7th Ed) pp. 117

B3. Duplicate Recessive Epistasis (9:7)

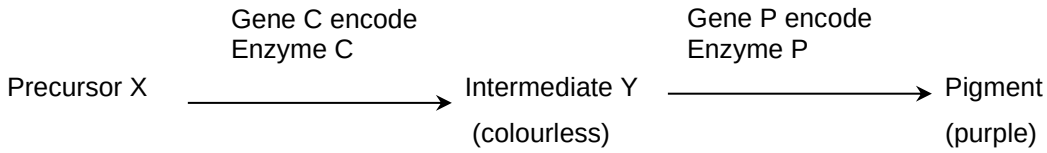
- In the case where identical phenotypes are produced by both homozygous recessive genotypes, the F_2 ratio becomes 9:7. The genotypes **aaB_**, **A_bb** and **aabb** produce one phenotype. Both dominant alleles, when present together, complement each other and produce a different phenotype.

Example: Flower colour in sweet peas

- The colour of the sweet pea flower is controlled by two genes, which are epistatic to each other. Each gene is necessary for the production of an enzyme required for pigment synthesis.
- The recessive allele of either gene encodes a defective enzyme. If any individual is homozygous recessive for either of the two genes, the purple pigment cannot be synthesized. This results in a white phenotype.

Biochemical Basis

- Development of purple flower colour in sweet peas is controlled by two loci (gene C and P). below shows the biochemical pathway involved:



- The interaction of gene products, showing complementary gene action, in the metabolic pathway producing a flower pigment in sweet peas. Functional enzymes encoded by both gene C and gene P are essential for the development of purple flowers.
- The compounds X and Y are both colourless.
- Conversion of X into Y is catalysed by an enzyme C whilst conversion of Y into the purple pigment is dependent on the enzyme P.
- The synthesis of functional enzyme C and P is directed by the alleles **C** and **P** respectively, whilst the alleles **c** and **p** code for the production of non-functional enzyme C and P.
- The plant which has the genotype **CCpp** will be white since it cannot produce functional enzyme P and cannot, therefore, convert Y into the purple pigment.
- Sweet pea which is **ccPP** will also be white since it cannot produce functional enzyme C and hence cannot produce Y which is essential for the synthesis of the purple pigment.

Let **P** represent dominant allele for purple pigment

p represent recessive allele that inhibits pigment production

C represent dominant allele for purple pigment

c represent recessive allele that inhibits pigment production

Parental phenotypes: Purple flower x White flower

Parental genotypes: **CCPP** x **ccpp**

Gametes produced by parents: (**CP**) x (**cp**)

Genotype of F_1 generation: **CcPp**

Phenotype of F_1 generation: All purple

Selfing of F_1 generation: **CcPp** x **CcPp**

Gametes produced: (**CP**) (**Cp**) (**cP**) (**cp**) x (**CP**) (**Cp**) (**cP**) (**cp**)

Punnett Square to show the fusion of gametes produced:

	CP	**Cp**	**cP**	**cp**
CP	**CCPP** Purple	**CCPp** Purple	**CcPP** Purple	**CcPp** Purple
Cp	**CCPp** Purple	**CCpp** White	**CcPp** Purple	**Ccpp** White
cP	**CcPP** Purple	**CcPp** Purple	**ccPP** White	**ccPp** White
cp	**CcPp** Purple	**Ccpp** White	**ccPp** White	**ccpp** White

Key
- purple flower
- white flower

F_2 phenotypic ratio: 9 Purple : 7 White

Summary

No. of gene per characteristic	Type of dominance	Phenotypic ratio in F_2 generation (heterozygote x heterozygote)		Phenotypic ratio in testcross on F_1 generation	Examples
1		Monohybrid			

	Complete dominance	Dihybrid			Flower colour, seed colour and shape
		Linked genes (2 gene loci, dihybrid) Complete linkage			-
		Linked genes (2 gene loci, dihybrid) Incomplete linkage	-		Drosophila body colour and wing shape
	Incomplete dominance			-	Flower colour of carnation
	Co-dominance			-	Human ABO blood group
	Lethal gene			-	Fur colour in mice
2	Non-epistasis/ Gene interaction			-	Comb shape in domestic fowl
	Epistasis			-	Coat colour in mice, flower colour in blue-eyed Mary
					Fruit colour in summer squash
					Flower colour of sweet pea
>2	Polygenic				Height, skin colour

Sex Linkage	Female Parent	Male Parent	Offspring	
			Female	Male
X-linked recessive	Normal	Affected		
	Carrier	Normal		
	Affected	Normal		
X-linked dominant	Normal	Affected		
	Affected (heterozygote)	Normal		
	Affected (homozygote)	Normal		
Y-linked	Only affects males			

VIII. Variation

- The term variation describes the difference in characteristics shown by organisms belonging to the same natural population or species.
- Two forms of variation occur: Continuous and discontinuous.

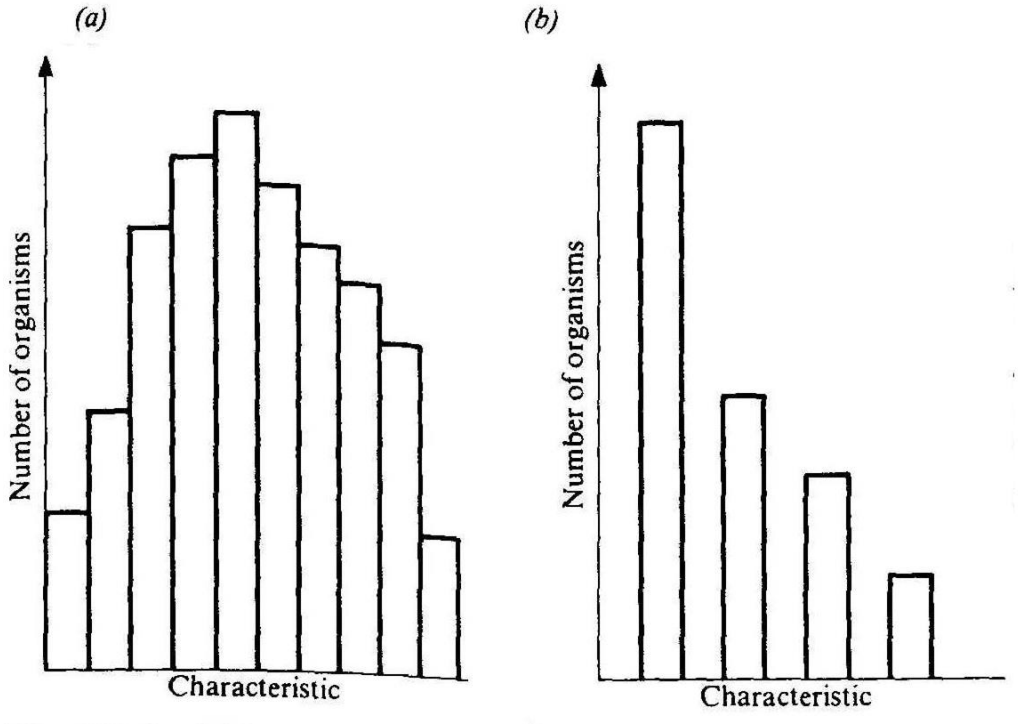


Fig.27 Histograms representing frequency distribution (a) continuous variation (b) discontinuous variation *Source: Biological Science 2. pp. 828*

A. Continuous variation

- Many characteristics in a population show a complete gradation from one extreme to the other without any break.
- This is illustrated most clearly by characteristics such as mass, linear dimension, shape and colour of organs and organisms.
- The frequency distribution for a characteristic exhibiting continuous variation is a normal distribution curve.
- Characteristics exhibiting continuous variation are produced by the combined effects of many genes (polygenes) and environmental factors.
- Polygenic inheritance - when multiple independent pairs of genes have similar and additive effects on the same character.
- Individually each of these genes has little effect on the phenotype but their combined effect is significant.

Example: Skin colour

- Skin pigmentation in humans is controlled by at least three separately inherited genes.
- For each gene, a dark-skin allele (**A**, **B**, **C**) contributing one "unit" of darkness to the phenotype and being incompletely dominant to the other alleles (**a**, **b**, **c**).
- An ***AABBCC*** person would be very dark, while and ***aabbcc*** individual would be very fair.

- An ***AaBbCc*** person would have skin of an intermediate shade. Because the alleles have a <u>cumulative</u> effect, the genotypes ***AaBbCc*** and ***AABbcc*** would make the same genetic contribution (three units) to skin darkness.
- Environmental factors, such as exposure to the sun, also affect the skin-colour phenotype and help make the graph a smooth curve rather than a stairlike histogram.

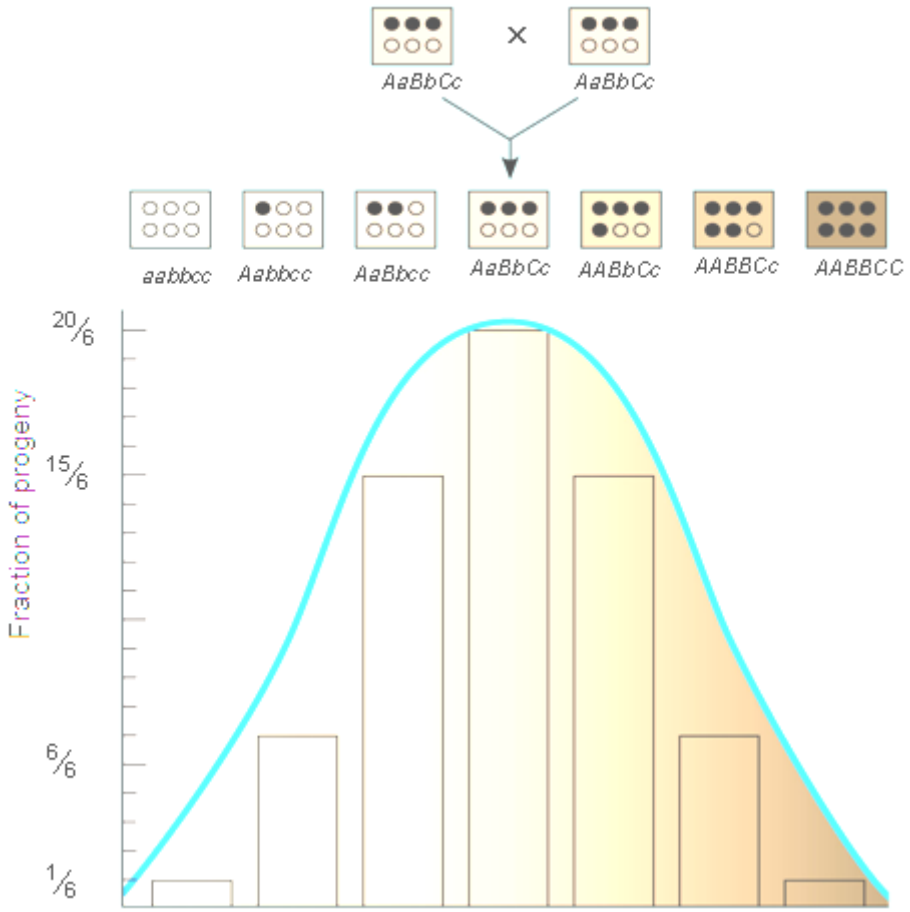


Fig. 28 A simplified model for polygenic inheritance of skin colour

Three separately inherited genes affect the darkness of skin. For each gene, an allele for dark skin (A, B, C) is incompletely dominant to an allele for light skin (a, b, c). Thus, the heterozygous individuals (AaBbCc) represented by the two rectangles at the top of this figure, each carry three "units" of darkness. (Black dots symbolise dark-skin alleles.) Imagine a large number of matings between such heterozygotes. Along the top of the graph are the variations that can occur among offspring. The y axis represents the fractions of these variations among offspring of the matings. The resulting histogram is smoothed into a bell-shaped curve by environmental factors that affect skin colour.

Source: Biology (6th Edition) pp. 259

B. Discontinuous variation

- Variation produces individual showing clear cut differences with no intermediates between them, such as blood groups in humans, wing lengths in *Drosophila* and sex in animals and plants.

- Characteristics showing discontinuous variation are usually controlled by one or two major genes which may have two or more allelic forms and their phenotypic expression is relatively unaffected by environmental conditions.
- Since the phenotypic variation is restricted to certain clear-cut characteristics, this form of variation is alternatively known as qualitative inheritance, as opposed to quantitative inheritance which is characteristic of continuous variation.

A comparison of continuous and discontinuous variation

Continuous	Discontinuous
• Continuous variation giving a range of phenotypes	• Discontinuous variation giving discrete phenotypic classes
• Effect of individual polygenes cannot be observed. Effect of polygenes is additive	• Effect of individual genes can be observed
• The environment has a large effect on the phenotype	• The environment has a small effect on the appearance of the phenotype
• Mechanisms of inheritance investigated using statistical methods	• Mechanisms of inheritance investigated by counting and comparing ratios in the offspring
• Examples: height in humans, skin pigmentation in humans	• Examples: ABO blood group, height of Mendel's pea plant, coat colour in mice, flowe colour in sweet pea

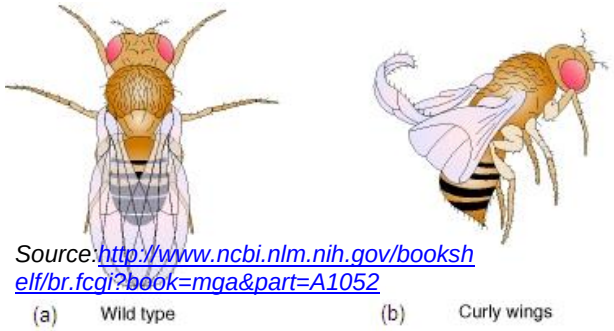
C. Influence of the environment

- The genotype of an individual determines the phenotype.
- However, the degree of expression is greatly influenced by environmental factors during the development of the organism.

1. Temperature

Example: Curly wings in *Drosophila*

- Expression of this trait is affected by the temperature at which the organism has grown.
- At 25^{o}C, pure breeding curly-winged flies produce curly-winged offspring
- At 16^{o}C, straight-winged offspring are produced
- If both sets of offspring are interbred at 25^{o}C, what will the progeny be like?_____



Source: http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mga&part=A1052

Fig. 29 Adult fruit flies (Drosophila melanogaster) with (a) normal-winged (wild-type) and (b) curly-winged phenotypes.

2. Diet

Example: Honeybees

- A bee colony consists of 3 phenotypes: Drones (male developed from unfertilised eggs); Queen (female developed from fertilised egg); Workers (females developed from fertilised eggs).
- Both the queen and workers have similar amount of genetic material but are phenotypically different (workers are smaller, mouthparts are larger and they are sterile).
- In the first 3 days after hatching, all larvae are fed on royal jelly secreted by glands in the head of workers.
- After 3 days, larvae destined to be workers are switched to a diet of honey and pollen while larvae destined to be queen continues with royal jelly.
- The high protein content of royal jelly stimulates the formation and maturation of the female reproductive system i.e. allows expression of genes concerned.

D. Sources of genetic variation in a population

(i) Describe the causes of genetic variation in a population.

- Interaction between discontinuous and continuous variations and the environment results in no two organisms will possess identical phenotypes.
- In sexually reproducing organisms, meiosis and fusion of gametes during fertilisation provide the means of introducing unlimited genetic variation into the population. These may be summarized as follows:

1. <u>Crossing-over</u>

- Crossing over between non-sister chromatids of homologous chromosomes may occur during prophase I of meiosis.
- This produces new combination of alleles and so provides a major source of genetic variation.

2. <u>Independent assortment</u>

- The orientation of the homologous chromosomes (bivalents) on the equatorial plate during metaphase I of meiosis determines the direction in which the pairs of chromatids move during anaphase I. Arrangement of one pair of homologous chromosomes is independent of another, resulting in random assortment of paternal and maternal chromosomes between nuclei of daughter cells
- During metaphase II of meiosis, the orientation of pairs of chromatids is random and determines which chromosomes migrate to opposite poles of the cell during anaphase II.
- These random orientations and the subsequent segregation of the chromosomes give rise to a large number of different chromosome combinations in the gametes.

3. <u>Random fusion of gametes</u>

- A third source of variation occurs during sexual reproduction as a result of the fact the random fusion of male and female gametes.
- Thus, any male gamete is potentially capable of fusing with any female gamete.

X - inactivation in female mammals (Further reading)

- Female mammals, including humans, inherit two X chromosomes. One X chromosomes in each cell in females becomes almost completely inactivated during embryonic development. As a result, the cells of females and males have the same effective dose (one copy) of these genes.
- The inactive X in each cell of a female condenses into a compact object called a Barr body, which lies along the inside of the nuclear envelope. Most of the genes of the X chromosome that forms the Barr body are not expressed. In the ovaries, Barr-body chromosomes are reactivated in the cells that give rise to eggs, so every female gamete has an active X.
- Selection of which X chromosome will form the Barr body occurs randomly and independently in each embryonic cell present at the time of X inactivation. As a consequence, females consist of a mosaic of two types of cells: those with the active X derived from the father and those with the active X derived from the mother. After an X chromosome is inactivated in a particular cell, all mitotic descendants of that cell have the same inactive X. Thus, if a female is heterozygous for a sex-linked trait, about half her cells will express one allele, while the others will express the alternate allele.
- Fig. 35 shows how this mosaicism results in the mottled coloration of a tortoiseshell cat. In humans, mosaicism can be observed in a recessive X-linked mutation that prevents the development of sweat glands. A woman who is heterozygous for this trait has patches of normal skin and patches of skin lacking sweat glands.
- Inactivation of an X chromosome involves modification of the DNA, including attachment of methyl groups ($-CH_3$) to one of the nitrogenous bases of DNA nucleotides.

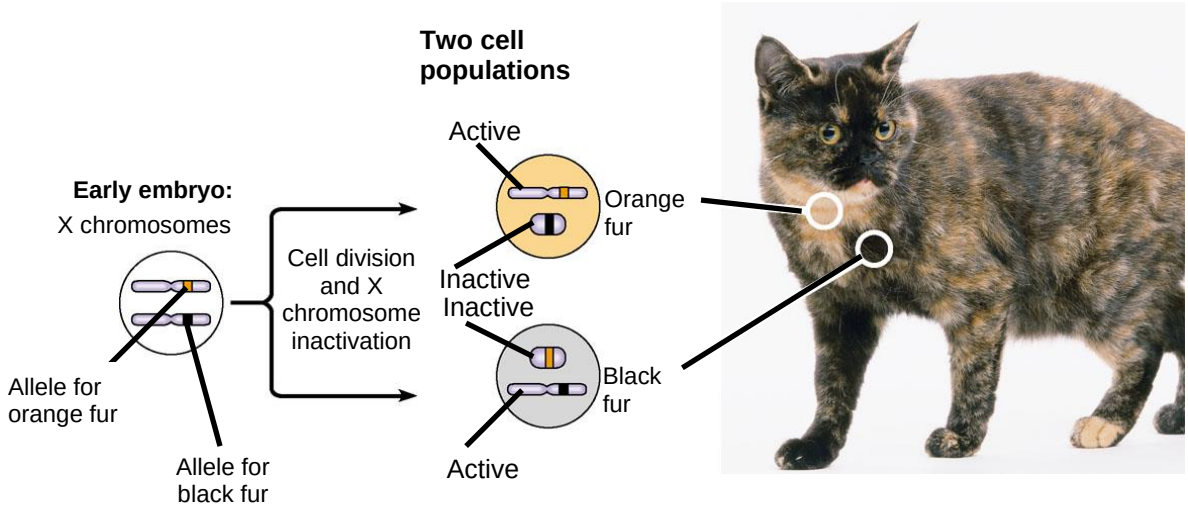


Fig. 35 Inactivation of X chromosome in tortoiseshell cat

Source: Biology (8th Edition) pp. 292

IX. Statistical Tests

- Statistical test are used to determine whether or not differences between the two or more sets of data are likely to be real and not due to chance or sampling error.
- These include the **chi-square test**, and the **t-test**.

A. Chi-square test

- The **chi square test (**or χ^2 **test)** is used to determine if the experimental values (observed values) are significantly different from the expected values.
- The data used must fall into **discrete categories**, for example, colour, size and shape.
- The general formula for the chi square test is

$$\chi^2 = \sum \frac{(O-E)^2}{E} \qquad \nu = c-1$$

where O = observed data in each category

E = expected data in each category based on experimenter's hypothesis

$\sum$ = the sum of

ν = degrees of freedom

c = number of classes

- For example, if the population data fell into two discrete categories, the chi square calculation would be:

$$\chi^2 = \frac{(O_1-E_1)^2}{E_1} + \frac{(O_2-E_2)^2}{E_2}$$

- Let's take a look at the following example:

The cross

- A true-breeding fly with straight wings is crossed to a true-breeding fly with curved wings. The flies of the F_1 generation are then allowed to mate with each other to produce F_2 generation.

  The outcome

  F_1 generation: all offspring have straight wings

  F_2 generation: 262 straight wings

  90 curved wings

  Total : 352

- After carrying out the cross, the question is, does the phenotypic ratio differs significantly from the expected ratio of 3:1 based on Mendel's law of segregation.
- For this analysis we need to come up with two hypotheses which we are going to test. We begin by assuming there is no difference between the observed numbers and the expected numbers. This assumption of no difference is called the null hypothesis (H_0). The alternative hypothesis (H_A) is the opposite of what is stated in the null hypothesis.
- The aim of the test is to prove or disprove the hypothesis.

Step 1

State the null and alternative hypothesis.

- H_o: There is no significant difference between the observed and the expected ratio.
- H_A: There is significant difference between the observed and the expected ratio.

Step 2

Based on the hypothesis, calculate the expected values of each phenotype.

- Firstly, we need to calculate the individual probabilities of each phenotype. According to our hypothesis, there should be a 3:1 ratio in the F_2 generation. Therefore the expected probabilities are

  ¾ = straight wings (dominant trait)

  ¼ = curved wings (recessive trait)

	Observed (O)	Expected (E)	(O-E)	$\frac{(O-E)^2}{E}$
Straight wings	262	264	-2	0.0152
Curved wings	90	88	2	0.0455

Step 3

Apply the chi square formula, using the data for the observed values and the expected values that have been calculated in step 1.

$$\chi^2 = \sum \frac{(O-E)^2}{E} = 0.0152 + 0.0455 = \underline{0.0607}$$

Step 4

Determine degrees of freedom (ν).

- Degrees of freedom can be calculated as the number of categories in the problem minus 1.

  Degree of freedom (ν) = number of categories (c) - 1

  In this example that we have, degree of freedom = 2 - 1 = $\underline{1}$

Step 5

Using a chi square table, interpret the calculated chi square.

- The **p value** listed in the chi square table is the probability that the deviation of the observed from that expected is due to chance alone (no other forces acting).
- By statistical convention, 0.05 probability level is used as the critical value to serve as the basis for accepting or rejecting the hypothesis.
- 2 method of drawing conclusions are as follows:

Method 1

- Look along the row showing one degree of freedom for our calculated χ^2 value of 0.0607. This lies between the 0.00393 and 0.0642 on the table. Looking up this column we see that this corresponds to a probability between 0.95 and 0.80. This means that the probability that chance alone could have produced the deviation is between 0.95 and 0.80.
- Compare the p value of calculated χ^2 value with 0.05 probability:

a) If the p value of the calculated χ^2 value is more than or equal to the p value of 0.05, do not reject your hypothesis. The deviation is not significant and chance alone accounts for it.

b) If the p value of the calculated χ^2 value is less than the p value of 0.05, reject your hypothesis. The deviation is significant and some factor other than chance is affecting the results.

Conclusion:

Given χ^2= 0.0607, ν=1, the probability of the deviation is due to chance is between 0.95 and 0.80 which is greater than 0.05. Hence, do not reject H_o in favour of H_A. There is no significant difference between the observed and the expected ratio. The phenotypic ratio does not differ significantly from the expected ratio of 3:1 based on Mendel's law of segregation.

Method 2

- Look along the row showing one degree of freedom for critical χ^2 value at 0.05 probability. This critical χ^2 value is 3.841. Compare the calculated χ^2 value with the critical χ^2 value at 0.05 probability on a chi-squared table:

a) If the calculated χ^2 value is less than or equal to the critical χ^2 value at 0.05 probability, do not reject your hypothesis. The deviation is not significant and chance alone accounts for it.

b) If the calculated χ^2 value is greater than the critical χ^2 value at 0.05 probability, reject your hypothesis. The deviation is significant and some factor other than chance is affecting the results.

Conclusion:

The calculated χ^2 value of 0.0607 is less than the critical χ^2 value of 3.841 at 0.05 probability. Hence, do not reject H_o in favour of H_A. There is no significant difference between the observed and the expected ratio. The phenotypic ratio does not differ significantly from the expected ratio of 3:1 based on Mendel's law of segregation.

Table 1 Table of chi-squared values

Degrees of Freedom	$P = 0.99$	0.95	0.80	0.50	0.20	**0.05**	**0.01**
1	0.000157	0.00393	0.0642	0.455	1.642	**3.841**	**6.635**
2	0.020	0.103	0.446	1.386	3.219	**5.991**	**9.210**
3	0.115	0.352	1.005	2.366	4.642	**7.815**	**11.345**
4	0.297	0.711	1.649	3.357	5.989	**9.488**	**13.277**
5	0.554	1.145	2.343	4.351	7.289	**11.070**	**15.086**
6	0.872	1.635	3.070	5.348	8.558	**12.592**	**16.812**
7	1.239	2.167	3.822	6.346	9.803	**14.067**	**18.475**
8	1.646	2.733	4.594	7.344	11.030	**15.507**	**20.090**
9	2.088	3.325	5.380	8.343	12.242	**16.919**	**21.666**
10	2.558	3.940	6.179	9.342	13.442	**18.307**	**23.209**
15	5.229	7.261	10.307	14.339	19.311	**24.996**	**30.578**
20	8.260	10.851	14.578	19.337	25.038	**31.410**	**37.566**
25	11.524	14.611	18.940	24.337	30.675	**37.652**	**44.314**
30	14.953	18.493	23.364	29.336	36.250	**43.773**	**50.892**

From Fisher, R. A., and Yates, F. (1943) *Statistical Tables for Biological, Agricultural, and Medical Research*. Oliver and Boyd, London.

Examples

Example 1

Another widespread application of chi square test is in testing for linkage. If the observed results cause rejection of the hypothesis of no linkage, then we can infer linkage.

In an investigation to determine whether the genes for leaf shaped and colour of the stem are linked, pure breeding normal-leafed, purple-stemmed tomato was crossed with a potato-leafed, green-stemmed tomato. All of the F_1 offspring had normal leaves and purple stems. One of these offspring was crossed with a potato-leafed, green-stemmed tomato. The results of the F_2 are given below.

A chi-squared test was carried out to find out if these are two pairs of segregating alleles are at two loci.

H_o: There is no significant difference between the observed and expected numbers/ These are two pairs of segregating alleles at two loci.

H_A: There is significant difference between the observed and expected numbers. These are not two pairs of segregating alleles at two loci.

Appearance/ phenotype	Observed number (O)	Expected number (E)	Difference (O-E)	$(O-E)^2/E$
Normal leaves and purple stems	81	78.25		
Normal leaves and green stems	75	78.25		
Potato leaves and purple stems	72	78.25		
Potato leaves and green stems	85	78.25		

The second cross was a test or back cross, so it was expected that the ratio in the F_2 would be 1:1:1:1. The expected numbers are calculated by adding up the observed numbers and dividing by 4.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Degrees of freedom (c-1) =

Conclusion:

Example 2

A student had read that the smooth periwinkle, *Littorina obstusata*, was more likely to be found on bladder wrack than on other seaweeds. In order to test this preference, the students located 100 smooth periwinkles in a 25 m^2 sample area of rocky shore. For each periwinkle, the students noted the seaweed on which it was found.

H_o: There is no significant difference in the numbers of smooth periwinkles on the different seaweeds

H_A: There is significant difference in the numbers of smooth periwinkles on the different seaweeds

Groups	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2/E$
Spiral wrack	2	25		
Egg wrack	30	25		
Bladder wrack	61	25		
Serrated wrack	7	25		

If the periwinkles were randomly distributed the student would expect to find 25 periwinkles on each of the seaweeds.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Degree of freedom (c-1) =

Conclusion:

Given χ^2=86.96, ν=3, the probability of the deviation is due to chance is greater / less than 0.05. Hence, reject / do not reject H_o in favour of H_A. There is / is no significant difference in the numbers of smooth periwinkles on the different seaweeds. The deviation from the expected is / is not due to chance.

B. *t*-test

- While the chi-squared test can be used to test the statistical significance of discontinuous (discrete) variables the *t*-test is used to test the statistical significance of continuous variables.
- The *t*-test therefore has less application in genetics and far more in other areas of biology, such as ecology.
- The statistical test is mainly used to show whether the difference in the mean could be due to random chance, and is therefore not significant. Alternatively, it cannot be attributed to random chance and is due to experimental treatment alone and is therefore, significant.
- The *t*-test is used when a sample size is relatively small, e.g. under 30 readings/ figures. The mean and standard deviation of these small samples are prone to error since a single 'extreme' reading will have a disproportionate effect. The *t*-test accounts for this error. For the *t*-test to be of use, the data used have to conform to certain conditions, namely:
    1. They must be related to one another.
    2. They must be normally distributed
    3. They must have similar variances.
    4. The sample size must be small.
- The general formula to calculate the value of t is as follow:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

$\nu = n_1 + n_2 - 2$

$$s = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$$

where

n = sample size (number of observations);

$\bar{x}_1$ = mean of the first group;

$\bar{x}_2$ = mean of the second group;

s_1 = standard deviation of the first group

s_2 = standard deviation of the second group

ν = degrees of freedom

Step 1

State the null and alternative hypothesis.

Step 2

Calculate the magnitude of t using the formula above.

Step 3

Determine the degrees of freedom for the experiment from the number of individuals involved.

The **degrees of freedom ($n_1 + n_2 – 2$)** is the sum of the degrees of freedom for each class. The degrees of freedom for a class is (n-1).

e.g. If an experiment included 70 experimental and 80 control plants, (70 +80) – 2 = 148.

Step 4

Using a t-test table, interpret the calculated t value.

- Using the relevant row on the *t*-test table, find the p value of the calculated *t* value. If the degree of freedom of what we have is not found in the table, use the closest value.
- Compare the p value of calculated *t* value with 0.05 probability:

a) If the p value of the calculated *t* value is more than or equal to the p value of 0.05, do not reject your hypothesis. There is no significant difference between the groups. We conclude that the experimental treatment had no effect.

b) If the p value of the calculated *t* value is less than the p value of 0.05, reject your hypothesis. There is significant difference between the groups. We conclude that the experimental treatment did have an effect.

Table 2. Table of t-values

	probability p											
df	.25	.20	.15	.10	.05	.025	.02	.01	.005	.0025	.001	.0005
1	1.000	1.376	1.963	3.078	6.314	12.71	15.89	31.82	63.66	127.3	318.3	636.6
2	.816	1.061	1.386	1.886	2.920	4.303	4.849	6.965	9.925	14.09	22.33	31.60
3	.765	.978	1.250	1.638	2.353	3.182	3.482	4.541	5.841	7.453	10.21	12.92
4	.741	.941	1.190	1.533	2.132	2.776	2.999	3.747	4.604	5.598	7.173	8.610
5	.727	.920	1.156	1.476	2.015	2.571	2.757	3.365	4.032	4.773	5.893	6.869
6	.718	.906	1.134	1.440	1.943	2.447	2.612	3.143	3.707	4.317	5.208	5.959
7	.711	.896	1.119	1.415	1.895	2.365	2.517	2.998	3.499	4.029	4.785	5.408
8	.706	.889	1.108	1.397	1.860	2.306	2.449	2.896	3.355	3.833	4.501	5.041
9	.703	.883	1.100	1.383	1.833	2.262	2.398	2.821	3.250	3.690	4.297	4.781
10	.700	.879	1.093	1.372	1.812	2.228	2.359	2.764	3.169	3.581	4.144	4.587
11	.697	.876	1.088	1.363	1.796	2.201	2.328	2.718	3.106	3.497	4.025	4.437
12	.695	.873	1.083	1.356	1.782	2.179	2.303	2.681	3.055	3.428	3.930	4.318
13	.694	.870	1.079	1.350	1.771	2.160	2.282	2.650	3.012	3.372	3.852	4.221
14	.692	.868	1.076	1.345	1.761	2.145	2.264	2.624	2.977	3.326	3.787	4.140
15	.691	.866	1.074	1.341	1.753	2.131	2.249	2.602	2.947	3.286	3.733	4.073
16	.690	.865	1.071	1.337	1.746	2.120	2.235	2.583	2.921	3.252	3.686	4.015
17	.689	.863	1.069	1.333	1.740	2.110	2.224	2.567	2.898	3.222	3.646	3.965
18	.688	.862	1.067	1.330	1.734	2.101	2.214	2.552	2.878	3.197	3.611	3.922
19	.688	.861	1.066	1.328	1.729	2.093	2.205	2.539	2.861	3.174	3.579	3.883
20	.687	.860	1.064	1.325	1.725	2.086	2.197	2.528	2.845	3.153	3.552	3.850
21	.686	.859	1.063	1.323	1.721	2.080	2.189	2.518	2.831	3.135	3.527	3.819
22	.686	.858	1.061	1.321	1.717	2.074	2.183	2.508	2.819	3.119	3.505	3.792
23	.685	.858	1.060	1.319	1.714	2.069	2.177	2.500	2.807	3.104	3.485	3.768
24	.685	.857	1.059	1.318	1.711	2.064	2.172	2.492	2.797	3.091	3.467	3.745
25	.684	.856	1.058	1.316	1.708	2.060	2.167	2.485	2.787	3.078	3.450	3.725
26	.684	.856	1.058	1.315	1.706	2.056	2.162	2.479	2.779	3.067	3.435	3.707
27	.684	.855	1.057	1.314	1.703	2.052	2.158	2.473	2.771	3.057	3.421	3.690
28	.683	.855	1.056	1.313	1.701	2.048	2.154	2.467	2.763	3.047	3.408	3.674
29	.683	.854	1.055	1.311	1.699	2.045	2.150	2.462	2.756	3.038	3.396	3.659
30	.683	.854	1.055	1.310	1.697	2.042	2.147	2.457	2.750	3.030	3.385	3.646
40	.681	.851	1.050	1.303	1.684	2.021	2.123	2.423	2.704	2.971	3.307	3.551
50	.679	.849	1.047	1.299	1.676	2.009	2.109	2.403	2.678	2.937	3.261	3.496
60	.679	.848	1.045	1.296	1.671	2.000	2.099	2.390	2.660	2.915	3.232	3.460
80	.678	.846	1.043	1.292	1.664	1.990	2.088	2.374	2.639	2.887	3.195	3.416
100	.677	.845	1.042	1.290	1.660	1.984	2.081	2.364	2.626	2.871	3.174	3.390
1000	.675	.842	1.037	1.282	1.646	1.962	2.056	2.330	2.581	2.813	3.098	3.300
∞	.674	.841	1.036	1.282	1.645	1.960	2.054	2.326	2.576	2.807	3.091	3.291
	50%	60%	70%	80%	90%	95%	96%	98%	99%	99.5%	99.8%	99.9%
	Confidence level C											

Example 1

A marker-gardener interested in economy was anxious to know whether there was a significant difference in the production of tomato fruits from flowers which had been pollinated by hand when compared with those which had merely been sprayed with water. His results are as follow:

	Sprayed with water	$(x_1-\bar{x}_1)$	$(x_1-\bar{x}_1)^2$	Sprayed & hand-pollinated	$(x_2-\bar{x}_2)$	$(x_2-\bar{x}_2)^2$
Number of fruits per plant	33	33 – 46.6 = –13.6	184.96	46	46–57.6= –11.6	134.6
	28	28 – 46.6 = –18.6	345.96	42	42–57.6= –15.6	243.36
	56	56 – 46.6 = 9.4	88.36	63	63–57.6= 5.4	29.16
	43	43 – 46.6 = 3.6	12.96	40	40–57.6= –17.6	309.76
	45	45 – 46.6 = – 1.6	2.56	52	52–57.6= – 5.6	31.36
	62	62 – 46.6 = 15.4	237.16	60	60–57.6= 2.4	5.76
	74	74 – 46.6 = 27.4	750.76	82	82–57.6= 24.4	595.36
	45	45 – 46.6 = – 1.6	2.56	74	74–57.6= 16.4	268.96
	32	32 – 46.6 = –14.6	213.16	62	62–57.6= 4.4	19.36
	48			55		

H_O : There is no significant difference in the production of tomato fruits from flowers which had been pollinated by hand with those which had merely been sprayed with water

H_A : There is significant difference in the production of tomato fruits from flowers which had been pollinated by hand with those which had merely been sprayed with water

---

Calculate the mean

$\bar{x}_1$ = 466/10 = 46.6

$\bar{x}_2$ = 576/10 = 57.6

Calculate the Std dev

s_1 =

s_2 =

$$s = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$$

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Conclusion:

Given t = _____, ν =_____, the probability of the deviation due to chance is greater / less than 0.05.

Hence reject / do not reject H_o in favour of H_A.

There is / is no significant difference in the production of tomato fruits from flowers which had been pollinated by hand with those which had merely been sprayed with water. Therefore, it can be concluded that the experimental treatment of the plant _____ effect.